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Original Article

The influence of zinc and selenium on some biochemical responses of *Vigna unguiculata* and *Zea mays* to water deficit condition and rehydration

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ABSTRACT: The influence of zinc and selenium on some biochemical responses (lipid peroxidation, ascorbate, glutathione, growth rate, mineral content, catalase and glutathione peroxidase activities) of cowpea and maize seedlings to water deficit condition and rehydration were investigated. Plants seedlings were exposed to water deficit condition for 14 days. The relative water content in whole plant tissues was reduced from 78.6% (control) to 50.0% (water deficit) and 70.1% (control) to 37.3% (water deficit) for cowpea and maize samples respectively. Selenium-treated samples showed higher RWC values for both normal and water deficit samples. Under water deficit conditions, general increase in the levels of the biochemical parameters was recorded. Effects of water deficit were more pronounced on the maize samples than cowpea. At $P<0.05$, water deficit samples showed significant increase in MDA levels and antioxidant enzymes activities; the activity of selenium in reducing water deficit effects reached statistical significance and was also found remarkable due to its non-accumulation in the plant samples. In this present investigation, water deficit induced 7 times, 10 times and 12 times increases in the Fe, P and N contents respectively of water deficit cowpea samples. The water deficit maize samples also showed 6 times and 5 times levels of P and N respectively. Water deficit cowpea sample treated with selenium once weekly (WOS) returned back to full turgor after 6 days of rehydration with water. Rehydration caused significant reductions in MDA levels and antioxidant enzymes activities. The use of antioxidant chemicals particularly selenium is therefore recommended in this study to farmers that reside in the zone of short term drought.

KEYWORDS: selenium, zinc, growth rate, mineral

INTRODUCTION

There are habitats throughout the world that present challenges to crop plant through lack or excess water, salts or toxic substances in the soil (Zhu, 2001). The native flora of any region has morphological and physiological adaptations to the stresses they will encounter. According to Poppy (2000), interest in the study of adaptation of plants to environmental stress was pioneered at the University of Liverpool as part of studies on the evolution of tolerance to heavy metals in plants. This was both a practical opportunity to reclaim land damaged in Britain and an opportunity to understand how plants could adapt rapidly to new environmental conditions. An interest in the response of plants to other abiotic stresses therefore developed, with research extending from whole plant studies through aspects of physiology to molecular studies.

Mowla *et al.*, (2000) defined abiotic stress as the pressure or tension resulting from physical factors or conditions such as drought, water logging, heat, salinity and solar radiation in the environment. Crop plants have been selected by humans for particular characteristics, such as rapid growth and high yields, which can make them perform poorly if subjected to any stress (Uzogara, 2000). A deficit of rainfall over cropped areas during critical periods of the growth cycle can result in destroyed or under developed crops with greatly depleted yields. Drought stress in plants falls into midday water deficit and long term water deficit, stress symptoms from drought vary, depending on intensity and duration of drought and growth stage of the plant. Plants adopt various mechanisms to overcome drought stress; these include escape, avoidance, tolerance and recovery (Ekanayake, 1990).

In Nigeria, the most important grains are cowpea, sorghum, millet, rice, maize and wheat (Wudiri, 1999). Of all these cereals, maize and cowpea remain the most popularly grown and consumed in all ecological zones of the country. Maize has been in the diet of Nigerians for centuries. It started as a subsistence crop and has gradually become more important crop. Maize has now risen to a commercial crop on which many agro-based industries depend on as raw materials (Iken & Amusa, 2004). Some poorer countries depend primarily on maize, barley, sorghum or millet for their staple food (Obatolu, 1998). Cowpea (*Vigna unguiculata*) is a leguminous grown throughout the African continent as well as in some parts of Southeast Asia and Latin America. Though native to West Africa, this legume has become a part of the diet of about 110 million people throughout the world. Having a protein content of about 25 percent, this legume is a cheap source of protein for the poor.

Though the Nigerian government have invested in the development of thee crops, there has been a downswing in production as a result of reduced interest from the farmers. However, climatic adaptation and dry season are far more fundamental reasons that account for the collapsing production (Ajiboso, 2009). Therefore, the goal of this present study is to determine the influence of zinc and selenium on some biochemical responses of *Vigna unguiculata* and *Zea mays* seedlings to mild water deficit condition and rehydration.

MATERIALS AND METHODS

Collection of Maize and Cowpea seeds

Certified healthy seeds of maize (local white) and cowpea (Ife brown) were obtained from National Cereals Research Institute (NCRI) Badeggi, Niger State, Nigeria, West Africa.

Chemicals and Reagents

All chemicals and reagents used were of analytical grade, and obtained from BDH Poole, England.

Growth conditions

Mature glasshouse grown plants (local white and Ife brown varieties of maize and cowpea respectively) were used for all the experiments. Ten seeds of each variety according to the method described by Pattanagul & Madore (1999) were potted in loamy soil and kept in a screen house under natural conditions (12 hours day and 12 hours night) and approximately 30 ^{o}C and 20 ^{o}C day/night temperatures. Plants were watered daily and grown under these conditions for 3 weeks, by which time they had reached the desired height, canopy diameter, and have abundant mature source leaves. The experimental design was of four (4) different groups and these groups were labeled as A, B, C and D to indicate the plants watered always (A or WAC), plants watered once a week (B or WOC), plants watered once a week plus Zinc (C or WOZ), and plants watered once a week plus Selenium (E or WOS) respectively. For the water deficit condition, plants were put under stress by light-watering once at the start of the week, and then depriving them of water for the rest of the week. This process was repeated weekly for a 2-week period, and the described experiments were carried out till the end of week 2. For group A, plants were watered daily throughout 14-day period. Potable water was used to wet the plants; the volume of water used was at the consumptive level of daily requirements. The growth procedure was also repeated for cowpea. The groups that were exposed to Selenium (WOS) and Zinc (WOZ) were watered with water containing Selenium (0.2 mg/l) (Wedekind et al., 2004) or Zinc (40 mg/l) (Pandolfini et al., 1992). Potable water was used to wet the plants; the volume of water used was at the consumptive level of daily requirements.

Measurement of growth rate

The viability test for survival rate described by Oka *et al.* (2003) was used to measure the growth rate. The total number of leaves on each plant was noted, the number of leaves fully germinated was also considered as the survival rate. Also, each plant height was measured on the first phase of the experiment prior to treatment and this measurement was repeated on the last day of the experiment i.e. after treatment, in order to consider effects of treatment on plant growth.

Determination of Relative Water Content (RWC)

For each group, 3 plants were randomly harvested after exposure to water deficit conditions, their roots were quickly rinsed in water and gently blotted dry. Each plant was separately weighed for fresh weight determination. Dry weight was determined after 2-day of incubation in an oven (PHL, E811181, England) at 70 ^{o}C (Pattanagul and Madore, 1999). The relative water content was calculated using the equation:

$$\text{Relative Water Content} = \frac{Fresh\ weight - Dried\ weight}{Fresh\ weight} \times 100$$

Determination of Mineral content

Mineral content of plant and soil samples were determined using the methods described by AOAC (1990) using an Atomic Absorption Spectrophotometer (AAS) (Burk- 2713 England).

Preparation of plant homogenate

Plant homogenates were prepared according to the method described by Gachotte et al. (1995). All operations were carried out at 4 ^{o}C. A sample weighing 5g from 35-day old seedlings was homogenized for 10 seconds in 20 ml of 0.1 M Tris-HCl buffer, pH 7.8, containing 0.5 M Sucrose and 1 mM EDTA. The homogenate of the mixture was centrifuged at 6000 x g for 15 min; the supernatant obtained was kept for further analysis.

Determination of lipid peroxidation (MDA levels)

A modification of the method of Varshney and Kale (1990) was used. Samples (0.2 ml) of the maize and cowpea homogenates in separate test tubes were added to the Tris–KCl buffer, to which 0.75 ml of 30% TCA was added. Then 0.75 ml of 0.75% thiobarbituric acid (TBA) was added and placed on a water bath for 45 minutes at 80 ^{0}C. The absorbance of the clear supernatant was measured against a reference blank of distilled water at 531.87 nm in a spectrophotometer (Model: 210VGP, AAS).

Determination of catalase activity

A modification of the method described by Sinha (1972) was used. Samples were diluted 1:50 with distilled water before carrying out the catalase assay. The control and sample were analyzed using spectrophotometer (Model: 210VGP, AAS) at 570 nm.

Determination of glutathione (GSH) level

The assay for GSH was done according to the method described by Griffith (1980). The control and sample were analyzed using spectrophotometer (Model: 210VGP, AAS) at 412 nm.

Determination of ascorbate content

The method of Yijing *et al.* (1999) was used in the determination of ascorbate content of the samples. To 2 ml of sample was added 2 ml Phosphoric acid (2.5%) and 1 ml 2,4-dinitrophenylhydrazine to form the derivative bis-2,4-dinitrophenylhydrazone. The control and sample were analyzed using spectrophotometer (Model: 210VGP, AAS) at 520 nm.

Determination of glutathione peroxidase activity

The activity was measured according to the method of Hopkins and Tudhope (1973), in which 0.5 ml of 0.56 M sodium phosphate buffer pH 7.0, 0.3 ml of 0.5 M EDTA, 0.2 ml of 1 mM $NaNO_3$, 0.5 ml of hydrogen peroxide, and 0.25 ml of 0.2 mM NADPH were added to 1 ml extracted solution (sample). The decrease in absorbance at 340 nm was measured with a spectrophotometer.

Statistical Analysis

The data obtained were analysed using Duncan Multiple Range Test and Minitab statistical tool (SPSS and Minitab Inc. Software).

RESULTS

The malondialdehyde (MDA) levels of normal and water deficit maize and cowpea seedlings are shown in Table 1. There was significant increase ($p<0.05$) in MDA of water deficit samples of both plants compared to normal, with the exception of cowpea samples treated with selenium (WOS). Selenium also induced significant reduction ($p<0.05$) in MDA level of maize than zinc. There was a significant reduction ($p<0.05$) in glutathione levels of water deficit samples when compared to the control, while reduction in glutathione level was more pronounced in selenium treated samples than zinc. The results obtained show significant reduction ($p<0.05$) in ascorbate content of water deficit samples of both plants except WOS samples. Selenium generally increased the ascorbate content of maize samples (WAS & WOS). Zinc showed no effect on ascorbate content of water deficit sample (WOZ) when compared to WOC. Table 1 also shows significant elevation ($p<0.05$) in activities of catalase and glutathione peroxidase of water deficit maize and cowpea samples when compared to normal watered samples (control). Selenium induced higher activities of catalase and glutathione peroxidase than zinc in both water deficit and normal samples.

Table 2 shows the growth rate in term of relative water content (RWC), number of matured leaf per plant and height difference between days 21 and 35. The mean values of relative water content (RWC) of cowpea and maize samples grown under normal and water deficit conditions were shown in Table 2. Zinc and Selenium had no effect on the relative water content (RWC) of both maize and cowpea grown under normal condition. Water deficit was observed to have induced significant effect (reduction) on the relative water content (RWC) of both plants. Zinc also exacerbates the effect of water deficit on both plants while Selenium attenuates the effect of water deficit on the relative water content (RWC) and height difference (growth rate) of cowpea between 21 and 35 days of planting. The results obtained show significant increase ($p<0.05$) in number of leaf per plant of maize and cowpea seedlings grown under normal condition while significant reduction ($p<0.05$) was observed in water deficit samples except WOS seedling that showed higher number of matured leaf than WOZ and WOC seedlings.

Table 3 shows the results of the effect of water deficit on mineral contents of normal and water deficit maize and cowpea seedlings. There was significant increase ($p<0.05$) in the levels of mineral contents of both plants grown under water deficit condition. Fe, N & P significantly increase ($p<0.05$) to about 7, 12 and 10 times and 2, 5 and 6 times in WOC samples of cowpea and maize respectively. However, as shown in Table 4, water deficit induced significant reduction ($p<0.05$) in the level of Fe and significant increase ($p<0.05$) in the levels of N & P of both plants soils samples.

As reflected in Table 5, the results of 6 days rehydration of Cowpea sample watered once weekly with Selenium (WOS) generally showed difference in the values of the assessed parameters, when compared with the values obtained before rehydration. Rehydration induced significant increase ($p<0.05$) in relative water content (RWC) and leaf/plant (growth rate) of water deficit Cowpea plant sample (WOS) while significant reductions were observed in MDA, glutathione level, glutathione peroxidase and catalase activities and ascorbate content of WOS.

Table 6 shows the Stay-Green Score (SGS) for the evaluation of the performance of the Maize and Cowpea plants grown under normal and water deficit conditions. All the plants grown under normal condition showed a better performance than the plants grown under water deficit condition in term of number of green leaves retained, grading of greenness of leaves, turgidity or wilting, sensitivity and resistance to water deficit. The WOS sample (sample watered once weekly with Selenium) performed better than other water deficit samples in term of turgidity, sensitivity and resistance to water deficit condition. After 14 days of water deficit under the Screen house growth conditions, water deficit induced stunted growth in both plants; the influence of Selenium and Zinc on the growth rate of both plants under normal and water deficit conditions differs. Zinc induced significant reductions in the height of Cowpea grown both under normal and under water deficit conditions whereas Selenium induced slight increase in the height of Cowpea grown under both conditions.

DISCUSSION

The induction of active oxygen species by water stress in plants have been well established (Brou et al., 2007; Halliwell and Gutteridge, 1989) and these reactive oxygen species are not only generated as by-products of endogenous biological reactions, but also their formation increases during biotic and abiotic stresses (Brou et al., 2007). These species are able to react with DNA, lipids, proteins and almost any other constituent of plant or animal cells

TABLE 1 **Effects of mild water deficit condition on antioxidant activities in maize and cowpea plant seedlings**

	MDA (units/mg protein) X 10^5		GSH (mg/g tissue)		ASC (Molar)		CAT (units/ g tissue) x 10^4		GPx (units/ g tissue) x 10^5	
	Maize	Cowpea	Maize	Cowpea	Maize	Cowpea	Maize	Cowpea	Maize	Cowpea
WAC	5.7±0.1^a	6.4±0.1^a	40.7±0.6^b	36.4±0.1^a	1.8±0.1^b	0.6±0.0^a	52.5±0.3^a	64.3±0.2^b	59.2±0.1^a	65.8±0.1^b
WOC	7.8±0.1^b	7.4±0.0^b	37.1±0.1^a	36.4±0.1^a	0.8±0.0^a	0.7±0.0^a	69.5±0.3^c	52.2±0.2^a	70.7±0.1^c	66.2±0.1^b
WOZ	7.2±0.0	6.1±0.1	25.8±0.1	25.1±0.1	0.8±0.0	0.7±0.1	68.7±0.3	58.3±0.1	72.0±0.1	68.8±0.4
WOS	6.3±0.1	4.9±0.0	17.5±0.5	29.5±0.1	3.8±0.0	2.7±0.0	88.7±0.2	80.0±0.1	81.2±0.1	83.2±0.1

Results are mean values of triplicate determinations and are expressed in Mean± SEM
WAC = Control with water always; WOC = Control with water once weekly
WOZ = Zinc with water once weekly; WOS = Selenium with water once weekly
Different letters (a, b) within the same row are significantly different (P<0.05)

TABLE 2 **Effects of mild water deficit condition on growth rate in maize and cowpea plant seedlings**

	RWC		Leaf/plant		Height difference	
	Maize	Cowpea	Maize	Cowpea	Maize	Cowpea
WAC	70.1±0.1^a	78.6±0.1^b	3.0±0.1^c	5.0±0.1^d	18.0±0.1^c	5.0±0.1^e
WOC	37.3±0.1^c	50.0±0.1^d	2.0±0.1^b	2.0±0.1^b	6.0±0.1^b	3.0±0.1^b
WOZ	18.0±0.3	10.0±0.0	1.0±0.1^a	1.0±0.1^a	2.5±0.1^a	1.3±0.1^a
WOS	7.2±0.0	59.7±1.7	1.0±0.1^a	3.0±0.1^c	1.7±0.1^a	3.2±0.1^c

Results are mean values of triplicate determinations and are expressed in Mean± SEM
WAC = Control with water always; WOC = Control with water once weekly.
WOZ = Zinc with water once weekly; WOS = Selenium with water once weekly
Different letters (a, b) within the same row are significantly different (P<0.05)

TABLE 3 **Mineral content (ppm) of cowpea and maize seedlings grown under normal and water deficit conditions**

	Cowpea				Maize			
M/S	WAC	WOC	WOZ	WOS	WAC	WOC	WOZ	WOS
Fe	0.8±0.0	6.0±0.2	4.2±0.0*	6.7± 0.2*	0.8±0.0	1.1±0.0	1.1±0.1	1.6± 0.0
N	0.5±0.1	6.1±0.2	4.8± 0.2*	5.6± 0.2*	1.5± 0.0	8.1± 0.2	5.7±1.1*	5.0± 0.4*
P	0.2±0.0	2.0± 0.1	2.1± 0.1*	2.2± 0.1	0.3± 0.0	2.0± 0.0	2.1± 0.0	2.7± 0.3*
Se	2.0±0.0	2.4±0.0	2.4±0.1	2.4±0.1	1.3±0.1	1.2±0.0	1.1±0.0*	2.7± 0.3*
Zn	4.55±0.2	6.5±2.3	6.8± 3.0*	5.0± 0.3*	1.3±0.0	1.3±0.3	2.4±0.3*	1.3± 0.5

Results are mean values of triplicate determinations and are expressed in Mean± SEM
WAC = Control with water always; WOC = Control with water once weekly.
WOZ = Zinc with water once weekly; WOS = Selenium with water once weekly
* Different letters (a, b) within the same row are significantly different (P<0.05)

TABLE 4 **Mineral content (ppm) of normal and water deficit soil samples**

	Cowpea				Maize			
M/S	WAC	WOC	WOZ	WOS	WAC	WOC	WOZ	WOS
Fe	2.0±0.0	1.1±0.0	1.8±0.3	1.3±0.1	1.3±0.0	1.2±0.0	1.1±0.1	1.3±0.0
N	1.2±0.1	1.3±0.0	1.3±0.1	1.3±0.0*	1.5±0.0	1.7±0.1	1.8±0.3	1.8±0.0*
P	1.1±0.0	1.6±0.0	1.3±0.7	1.2±0.0	1.4±0.2	1.8±0.2	2.1±0.0	1.9±0.1*
Se	4.8±0.6	4.2±0.8	6.7±0.3	7.1±0.1	1.7±0.1	5.6±0.8	8.1±0.2	9.2±0.4
Zn	5.7±0.3	1.5±0.1	6.1±0.7	3.5±0.7	1.6±0.2	3.9±0.1	5.2±0.3	4.1±0.1

Results are mean values of triplicate determinations and are expressed in Mean± SEM

WAC = Control with water always; WOC = Control with water once weekly.

WOZ = Zinc with water once weekly; WOS = Selenium with water once weekly

* Different letters (a, b) within the same row are significantly different (P<0.05)

TABLE 5 **Biochemical variables in water deficit sample (WOS) in the presence of selenium before and after rehydration**

Variables	After rehydration	Before rehydration
Leaf/ plant	5.0±1.8	3.0±0.5
Change in height (cm)	2.4 ± 0.2	3.5±0.7
RWC (%)	75.2 ± 6.4	60.7± 3.8

Results are mean values of triplicate determinations and are expressed in Mean± SEM

WAC = Control with water always; WOC = Control with water once weekly.

WOZ = Zinc with water once weekly; WOS = Selenium with water once weekly

Different letters (a, b) within the same row are significantly different (P<0.05)

TABLE 6 **Stay-Green Score (SGS) for normal and water deficit cowpea and maize seedlings**

Observation	Samples			
	Cowpea (normal)	Water deficit	Maize (normal)	Water deficit
Number of green leaves retained	All	All	All	Nil
Grade the greenness of leaves	High	Moderate	High	Brownish
Turgidity, wilting of leaves, complete or partial drying	Turgid	Partial drying with wilted leaves in WOC	Turgid	Complete drying
Sensitivity to water deficit condition	-	Sensitive to prolong water deficit condition	-	Sensitive to early water deficit
Drought resistance (temporal escape, avoidance, tolerance & recovery)	-	Temporal escape was observed in the plants but on prolonged drought will be susceptible	-	None

(Beauchamp & Fridovich, 1971) thereby resulting into plant death or low growth and productivity. However, under water deficit condition reactive oxygen species (ROS) formation is usually exacerbated. ROS (particularly superoxide and hydroxyl radicals) are damaging to essential cellular components such as DNA, proteins and lipids (He et al., 1995). The consequence of the generation of free radicals and ROS is lipid peroxidation (Allessio, 1993). Lipid peroxidation is one of the best known manifestations of oxidative cell injury. It causes loss of the structural and functional integrity of the membrane (Kyle & Farber, 1991).

The results of the present investigation which showed higher levels of MDA in water deficit samples than control samples confirmed the generation of free radicals in water deficit samples. MDA has been well documented as an index for determining lipid peroxidation (Varshney & Kale, 1990). Lipid peroxidation is evident in this study from the increase in MDA levels of water deficit samples. The results of this investigation confirm earlier reports of He et al., (1995) on increased generation of free radicals and ROS in plants grown under water deficit condition. However, the mean values of MDA obtained for water deficit samples watered with antioxidant chemicals mostly selenium are significantly lower than the values obtained for water deficit samples watered without antioxidant chemicals (control) showing the antioxidant properties of selenium and zinc.

The glutathione levels of the water deficit samples when compared to the control (grown under normal condition) were significantly reduced. GSH has been found to be low in many disease states indicating oxidative stress and inadequate antioxidant activity to "mop up" the free radicals (Kyle & Farber, 1991). The results showing reduced glutathione levels in this study indicated cellular damage in plants grown under water deficit condition. However, the water deficit plants showed significant increase in ascorbate level, catalase and glutathione peroxidase activities. The increased catalase and glutathione peroxidase activities in water deficit samples were the results of the consequence of free radicals and reactive oxygen species (ROS). It has been well documented that plants have complex protective mechanisms to prevent the damage initiated by free radicals; these could be enzymatic such as catalase, peroxidases, superoxide dismutase etc. and non-enzymatic such as ascorbic acid, tocopherol, glutathione (Evan, 2000). The increased ascorbate contents of water deficit samples may also be attributed to compensatory change adopted by plants to survive the effects of water deficit condition (Ajiboso & Adeyemo, 2010).

The watered samples that were fortified with selenium were observed to have increased ascorbate levels, as well as elevated catalase and glutathione peroxidase activities. In this investigation, there was a significantly high positive correlation between selenium, ascorbate, catalase and glutathione peroxidase. According to Donald & Donald (1999), selenium in the form of selenocysteine is incorporated at the four active sites of the enzyme glutathione peroxidase. This enzyme assumes a critical role in protecting against free-radical and oxidative damage and thereby initiating the release of other antioxidant substrates. Selenium, ascorbate, catalase and glutathione peroxidase are documented free radical scavengers (Evan, 2000). The positive correlation observed in this study between ascorbate, catalase and glutathione peroxidase activities could be linked to selenoenzyme property of selenium and glutathione peroxidase; since increased in concentration of selenium was associated with infiltration of ascorbate, catalase and glutathione peroxidase into the plant cells (Ajiboso & Adeyemo, 2010). The selenium antioxidant activity was thereby concluded on its selenoenzyme property via glutathione peroxidase. It is therefore not surprising that upon rehydration of cowpea water deficit sample watered with Selenium once weekly (WOS), the plants regained their healthy status within 6 days of rehydration with significant increase and reduction in its RWC value and ascorbate content respectively.

The results of this investigation also show that zinc induced reduction in lipid peroxidation, glutathione and ascorbate levels in watered plants fortified with zinc. Zinc plays critical roles in the defense system of cells against ROS. Zinc is involved in inhibition of apoptosis (programmed cell death) which is preceded by DNA and membrane damage through reactions with ROS (Calmak, 2000). Zinc deficiency causes severe reductions in crop production. A number of physiological impairments in zinc deficient cells cause inhibition of growth, differentiation and development of plants. Zinc interferes with membrane-bound NADPH Oxidase producing ROS. In zinc deficient plants, the iron concentration increases, this potentiates the production of free radicals. Zinc nutritional status of plants influences photoxidative damage to chloroplasts, catalyzed by ROS. Zinc deficient leaves are highly light-sensitive, rapidly becoming chlorotic and necrotic when exposed to high light intensity (Calmak, 2000).

In the mineral contents investigation, excessive uptake of Fe by water deficit samples must have resulted from the water deficit condition. According to Asch et al. (2005), excessive uptake of Fe^{2+} ion by the roots and its translocation into the leaves correlate with formation of toxic oxygen radicals. Exacerbation of toxic oxygen radicals has been reported during water deficit (He et al., 1995). Toxic oxygen radicals have been reported to reduce CO_2 assimilation, photosynthetic rate and stomatal resistance (Finn and Brun, 1980), which result to decreased growth and development or death of plant. It has also been well documented that water content and other mineral levels were all positively correlated with organic nitrogen level (Janssen, 1993). The results of the present investigation on increased Fe, phosphorus and nitrogen levels in relation to water deficit samples confirm this report. Jupp and Newman (1987) reported an increase in phosphorus uptake by plant during mild drought. The results obtained on the significant increase in phosphorus level of water deficit samples (WOC) confirm the earlier report of Jupp and Newman (1987). The uptake of Fe, N and P by water deficit plants showed their relevance and significance in plant growth and development (survival). It is well established that Fe is involved in the formation of chlorophyll, and nitrogen fixation. It is also a component of many enzymes associated with energy transfer while phosphorus is a constituent of nucleic acid, seed ripening, and metabolism of fats, root development, respiration process and efficient functioning and utilization of nitrogen. It is closely concerned with the vital growth process in plants. Therefore, in this present investigation increase in Fe, N and Phosphorus uptake was observed as one of the adaptive mechanisms used by plants to overcome the effects of drought or water deficit condition. The uptake and transport of mineral nutrients occurs in water. Minerals dissolved in soil water move into plant roots and then to the vascular system, more specifically the xylem, for transport throughout the plant. There they combine with proteins to form enzymes that control the biochemical reactions essential to plant health and growth.

In the simplest terms, the driving mechanism for the transport of water through a plant is transpiration or the evaporation of water from plant leaves. Transpiration occurs as long as stomata are open. Transpiration sets up a negative pressure that drives the movement of water from the soil to the plant and through the plant via the xylem. The movement of water from soil to roots to xylem to leaves to air can be viewed as a continuum. Interruption at any stage of the continuum stops or reduces the flow of water, leading to wilting of leaves (Finn and Brun, 1980). Leaves wilting were observed in all the water deficit samples except cowpea watered with selenium solution once weekly (WOS) as shown in the stay green score table.

The difference in the RWC values of maize and cowpea is an indication of the response of monocotyledon (maize) and dicotyledon (cowpea) seeds to moisture conditions prevailing in field environments. It is observed in this study that the minimum seed moisture content required for germination was about 80% for Cowpea and about 70% for Maize. At 32 ^{o}C, the moisture tension or potential not less than 80% for Cowpea and not less than 70% for Maize are water requirements for germination to occur within 5-8 days. The water deficit plants showed lower RWC values than normal plants that were supplied water on daily basis. The high water content of normal plants observed in this study agrees with an earlier study of Ajiboso (2009). Rajagopal et al. (1977) reported variations in term of increased and decreased RWC in plants grown under normal and water deficit conditions respectively.

The physical observation made on maize samples subjected to water deficit condition during stay green score (SGS) evaluation is in agreement with the report of Shaw (1976) on the extreme susceptibility of maize to drought stress at flowering. It was observed that as water withholding began, the third leaf had nearly completed its growth in cowpea WOS samples, adequate observation was made on the number of growing and liquated leaves and plant height at the end of 14 days of water deficit condition.

CONCLUSION

The results of this work have shown that antioxidant nutrients selenium and zinc play important roles in combating the effects of water deficit in plants. Water deficit, if not properly checked, may lead to plant death or low growth and productivity. The peculiar problem of water deficit in agriculture may be solved through the optimum utilization of antioxidant properties of selenium and zinc. In this present study, selenium appears to be more effective than zinc in reversing water deficit plant back to turgor. Unlike zinc, selenium is also safe for use by patent and poor resource farmers since this investigation did not show its accumulation in the plants. This is significant since selenium has a high bioavailability. Based on these conclusions, we recommend the use of antioxidant chemical (selenium) by farmers that work in semi-arid or arid zones, and that further investigation should be carried out on effects of selenium on plant yield for food grains.

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