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Research Article

Screening for some physiological mechanisms in some drought tolerant genotypes of cowpea (*Vigna unguiculata* (L.) Walp)

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Abstract

Drought is an abiotic stress that affects the growth of cowpea varieties in sub-Saharan Africa and it is one of the most important constraints threatening the food security in the region. The aim of the present study was screening for some physiological mechanisms in some drought tolerant cowpea genotypes (*Vigna ungiculata (L.)* Walp).The research was conducted in the screenhouse of International Institute of Tropical Agriculture (IITA) Kano state Nigeria .Nine genotypes of cowpea differing in drought tolerance were evaluated. The experimental layout used was Randomized Complete Block Design (RCBD) with two treatments (unstressed and water stressed) with three replications. The physiological parameters measured include, shoot and root biomass, plant vigor, Leaf Relative Water Content (LRWC) and Chlorophyll content (SPAD). The result showed that water stress reduces biomass and plant vigor. Lowest shoot biomass and vigor were recorded in IT00K-901-5. Increase in Leaf Relative Water Content was recorded in TVU7778 and IT97K-573-1-1. There was general decrease in chlorophyll content (SPAD) in all the varieties after water stress induction. Different rate of drought tolerance was observed among the nine varieties, IT97K-499-35, IT97K-573-1-1 and TVU7778 are drought tolerance based on some physiological mechanisms they exhibited while IT00K-1263 and IT00K-901-5 were susceptible to water stress conditions.

Keyword: Cowpea, Chlorophyll (SPAD) Drought tolerance, Screening

INTRODUCTION

In the natural environment plants are well adapted to minimize damages which only occur under extreme conditions. In the frame of “physiological window” mild drought induces in plants regulation of water loss and uptake allowing maintenance of their leaf relative water content within the limits where photosynthetic capacity and quantum yield show little or no change. The most severe form of water deficit is desiccation when most of the protoplasmic water is lost and only a very small amount of tightly bound water remains in the cell. According to Larcher (1987), stress contains both destructive and constructive elements and is a selection factor as well as a driving force improving resistance and adaptive evolution. Repair processes lead also to hardening of plants by establishing a new physiological standard, which is an optimum stage of physiology under the changed environmental conditions.

Cowpea (*Vigna unguiculata* L. Walp.) is a grain legume grown in savanna regions of the tropics and subtropics. Its value lies in its high protein content with its ability to fix atmospheric nitrogen, which allows it to grow on, and improve poor soils (Steele, 1972). It is cultivated for its seed (shelled green or dried), pods and leaves, which are consumed in fresh form as green vegetables, while snacks and main meal dishes are prepared from the dried grain. All the plant parts used for food are nutritious, making it extremely valuable, where many people cannot afford protein foods such as meat and fish. The rest of the cowpea plant, after pods are harvested, is also used as a nutritious livestock fodder. Cowpea also has the ability to be intercropped with cereals such as millet and sorghum. Its diversity of uses, nutritive content and storage qualities have made cowpeas an integral part of the farming system in the West African region (Eaglesham *et al*., 1992). However, most of the world's cowpeas are grown primarily in dry regions where drought is prevalent among several yield-reducing factors (Watanabe *et al*., 1997).

Drought is one of the most important constraints threatening the food security of the world (Barters and Nelson, 1994). The economies of most of African nations rely heavily on exports of rain dependent agricultural products, which are often seriously affected during periods of severe drought. This makes drought a serious natural disaster in Africa, as it is associated with many socioeconomic miseries. Drought on the African continent often causes large scale water and food deficits, hunger, famine, exodus of people and animal, diseases, deaths, and many other severe, chronic societal problems (Ogallo, 1993).

When plants are subjected to drought stress, a number of physiological and morphological responses have been observed and the magnitude of the response varies among species and between varieties within a crop species (Kramer, 1980). Morphological and physiological traits that might enhance drought tolerance have been proposed, but only a few of these mechanisms have been demonstrated in the expression of tolerance under field conditions (Ludlow and Muchow, 1990). In some cultivated cereals, osmotic adjustment has been found to be one of the most effective physiological mechanisms underlying plant resistance to water deficit. Osmotic adjustment, as a process of active accumulation of compatible osmolytes in plant cells exposed to water deficit may enable (1) a continuation of leaf elongation, though at reduced rates (Turner, 1986); (2) stomatal and photosynthetic adjustments (Morgan, 1984); (3) delayed leaf senescence (Hsiao *et al*., 1984); (4) better dry matter accumulation and yield production for crops in stressful environments (Boyer, 1982). A better understanding of both the morphological, physiological and biochemical mechanisms involved in plant response to water deficit could therefore help improve cowpea productivity in dry land areas (Blum and Ebercon, 1981). The research was aimed at screening for some physiological mechanisms of drought tolerance in some selected genotypes of cowpea (*Vigna unguiculata*).

MATERIALS AND METHODS

The experiment was conducted in a rain protective screenhouse at the International Institute of Tropical Agriculture (IITA) located at 12^0 03N latitude and 8^0 32E longitude, Kano, Nigeria. Nine cowpea genotypes were used in the study (IT00K-1263, ITOOK-901-5, IT97K-499-35, IT97K-568-19, IT97K-573-1-1, IT98D-1399, IT98K-503-1, IT99K-7-21-2-2 and TVU7778) and they are arranged in complete randomized block design with two treatment regime of water stress (unstressed and stressed) in three replications.

Fifty four (54) plastic pots of size 18.6cm were filled with top soil, arranged on the iron benches in the rain protective screenhouse, each pot was pegged with a plastic peg indicating the cowpea variety to be planted in the pot and watered for 3 days. A specially made woody guide was pushed into the soil to make holes uniformly at 2cm depth and 4 holes were made per pot. Two finger picked 2 seeds (which was collected from IITA) and sown in each hole. After one week (7days) of germination the plants in the pots were thinned to 2 plants per pot and 7days later the pots were thinned to 1 plant per pot. The pots were watered daily using a small watering can for two weeks (14 days) after which drought was imposed on pots by withdrawal of irrigation in the stress pots.

DATA COLLECTION

Chlorophyll Content

The total chlorophyll content was measured using Minolta chlorophyll SPAD 502 meter and SPAD readings were taken every week for 4 weeks (28 days) (Ferus and Arkosiva, 2001).

Leaf Relative Water Content

Appropriate water status in terms of physiological consequence of cellular water deficit was measured (Schonfeld *et al*., 1999). Leaf relative water content estimated using the formula below.

LRWC (%) = (FW-DW/TW-DW) X 100

Fresh leaves were detached from the shoot of the plant in each pot and were put in to labeled envelops. The fresh leaves were weighed immediately using electric weighing scale and recorded.

Weighed fresh leaves were put in to small transparent nylon bags labeled using marker, distilled water was added on the leaves in the nylon bags which were tied and placed in a cooler containing ice block for 2 hours. After 2 hours the leaves were removed from distilled water, dried with tissue paper, weighed on electric weighing scale and the weights w

Turgid weighed leaves were put back into the labeled envelops and kept in the glass house for one week (7 days) arranged in a box. The leaf biomass was taken using electric weighing scale and recorded (Schonfeld *et al*., 1999).

Shoot Biomass

The plant shoot in each pot was cut using a razor blade from their roots. Each shoot was put into small size brown labeled envelops which were arranged into a box and kept in the glass house for a week (7 days) then the dried shoots were weighed using electric weighing scale and the shoots biomass were recorded (Schonfeld *et al*., 1999).

Root Biomass

Root biomass was determined by washing the pots using sieve and water. After washing each pot, the roots were removed and put into labeled envelops. The envelopes were arranged in a box and kept in the glass house for one week (7 days). Dried roots were weighed on electric weighing scale and the dry weights were recorded (Schonfeld *et al*., 1999).

Visual Scale

Visual Scale for plant vigor was determined using plant vigor score (1-5) whereby

1 = very poor, 2 = poor, 3 = average, 4 = good and 5 = very good (Amede *et al*., 2004).

Data analysis

Data was subjected to analysis of variance (ANOVA) using a Gen stat statistical software to determine whether the treatments effects were significant. The treatment and variety means were separated using the least significant differences (LSD) test.

RESULTS

Table 1 present the result for initial and final shoot biomass. Based on the statistical analysis there is no significant difference in the treatment, varieties and treatment by varieties interactions in initial shoot biomass while in final shoot biomass there is significant difference in treatment ($p<0.0001$) and varieties but no significant difference was found in treatment by varieties interactions.

At 21 days after water stress induction there was general decrease in final shoot biomass in almost all varieties except in TVU7778 that showed (0.02g) increase in final shoot biomass than the initial shoot biomass. Variety IT98K-503-1 indicated (2.47) highest decrease in final shoot biomass than the initial shoot biomass.

The result for vigor of some cowpea varieties are presented in Table 2. The result of the statistical analysis indicate that there is significant difference in the treatment and varieties ($p<0.0001$) while treatment by varieties interactions showed there is no significant difference at vegetative stage. At 14 days after imposing drought variety IT97K-499-35 and variety IT97K-573-1-1 recorded highest visual score (3) while variety IT00K-901-5 have the least visual score (1.67).

The result for root biomass of some cowpea varieties are presented in table 3. The statistical analysis indicate that there is no significant difference between treatment, varieties and treatment by varieties interaction. It was founded that root biomass increased in variety IT97K-499-35, variety IT99K-7-21-2-2, variety and TVU7778, variety IT98D-1399. There was reduction of root biomass in variety IT00K-1263, variety IT00K-901-5, variety IT97K-573-1-1, variety IT97K-568-19 and variety IT99K-503-1.

Based on the result obtained from Table 4 for percentage leaf relative water content of some cowpea varieties, the statistical analysis indicate that there is no significant difference in treatment, varieties and treatment by varieties interactions in initial. At 21days after water stress, the result showed that there is significant difference in the treatment.

The leaf relative water content in variety IT97K-568-19 (10.7%), variety TVU7778 (6%) and variety IT97K-573-1-1 (5.3%) recorded the highest percentage increase in relative water content while variety IT99K-7-21-2-2 (38%) showed the highest percentage in decreasing relative water content.

The result for chlorophyll content of some cowpea varieties is presented in Table 5. The result of the statistical

Table 1. Leaf Relative Water Content (LRWC) (%) of some cowpea varieties at initial and 21 days after water stress

	Initial LRWC		Final LRWC(21 days)	
Varieties	Unstressed	water stressed	Unstressed	water stressed
IT00K-1263	79.4	75.8	77.5	48.7
IT00K-901-5	73.0	78.5	80.6	54.1
IT97K-499-35	67.8	64.1	70.4	50.8
IT97K-568-19	78.4	75.6	44.4	51.5
IT97K-573-1-1	69.9	79.8	57.1	62.4
IT98D-1399	74.0	77.4	65.6	52.9
IT98K-503-1	77.8	71.2	70.3	60.4
IT99K-7-21-2-2	70.0	76.5	79.5	41.5
TVU7778	80.7	82	56.2	62.2
Mean	74.6	75.7	66.9	53.8
L.S.D (5%)				
Treatment	4.80NS		11.73**	
Varieties	10.18NS		24.89NS	
Treat by varieties interactions	14.40NS		35.20NS	

Table 2. Chlorophyll content of some cowpea varieties before and after water stress

	14 DB		7 DA		14DA	
Varieties	Unstressed	water stressed	Unstressed	water stressed	Unstressed	water stressed
IT00K-1263	44.23	42.13	36.4	32.23	31.9	21.1
IT00K-901-5	51.27	46.73	41.07	33.13	40.2	12.5
IT97K-499-35	54.07	55.93	55.67	42.3	50.7	40.7
IT97K-568-19	55.97	55.67	42.3	50.7	40.7	7.6
IT97K-573-1-1	54.33	47.77	44.4	30.37	35.2	33.8
IT98D-1399	43.8	48.63	34.8	33.03	37	18.9
IT98K-503-1	51.97	53.33	50.03	39.23	41.6	11
IT99K-7-21-22	59.9	60.8	53.53	38.87	45.8	11.1
TVU7778	39.4	40.07	25.23	20.13	34.4	1
Mean	50.55	50.32	43.63	34.55	39.6	17.4
Mean squares						
T		1.821NS		3.217**		5.48**
G		3.863**		6.825**		11.63**
	5.463NS		9.652NS		16.45*	

Table 3. Vigor of some cowpea varieties at 14 days after imposition of water stress

Varieties	Unstressed	Water stressed
IT00K-1263	4.33	1.33
IT00K-901-5	5.00	1.67
IT97K-499-35	4.67	3.00
IT97K-568-19	4.67	1.67
IT97K-573-1-1	4.67	3.00
IT98D-1399	4.33	2.00
IT98K-503-1	4.33	1.67
IT99K-7-21-2-2	4.33	1.33
TVU7778	3	1.00
Mean	4.37	1.74
Mean squares		
Treatment	0.452**	
Varieties	0.952**	
Treat by varieties interactions	1.355NS	

analysis indicate that there is significant difference in varieties (p<.001) but no significant difference in treatment and treatment by varieties interactions for chlorophyll content at 7 days and 14 days after planting before imposing water stress. According to the statistical analysis for chlorophyll content at 7 days and after drought stress there is significant difference in varieties and treatment (pr<.001) but no significant difference in treatment by varieties interactions. At 14 days after drought stress treatment (pr<.001) and varieties are significant while treatment by varieties interaction were significant at. (p<0.004)

The leaves chlorophyll content of the plants reduces every day, under sever water stress there is great difference in

Table 4. Shoot biomass (g/plant) of some cowpea varieties at initial and 21 days after water stress induction

	Initial shoot biomass		Final shoot biomass (21 days after water stress	
Varieties	Unstressed	water stressed	Unstressed	water stressed
IT00K-1263	0.12	0.123	0.457	0.247
IT00K-901-5	0.11	0.127	0.273	0.167
IT97K-499-35	0.143	0.13	0.473	0.273
IT97K-568-19	0.127	0.117	0.367	0.193
IT97K-573-1-1	0.17	0.153	0.407	0.217
IT98D-1399	0.113	0.097	0.357	0.167
IT98K-503-1	0.107	0.107	0.397	0.150
IT99K-7-21-2-2	0.117	0.150	0.330	0.193
TVU7778	0.06	0.363	0.107	0.127
Mean	0.119	0.152	0.352	0.181
Mean squares				
Treatment	0.0612NS		0.0521**	
Varieties	0.1298NS		0.1106**	
Treat by varieties interactions	0.1835NS		0.1564NS	

Table 5. Root Biomass (g/plant) of some cowpea varieties at 21 days after water stress

Varieties	Unstressed	Water stressed
IT00K-1263	0.33	0.25
IT00K-901-5	0.31	0.183
IT97K-499-35	0.373	0.47
IT97K-568-19	0.36	0.207
IT97K-573-1-1	0.313	0.287
IT98D-1399	0.19	0.22
IT98K-503-1	0.607	0.22
IT99K-7-21-2-2	0.177	0.393
TVU7778	0.11	0.193
Mean	0.308	0.269
Mean squares		
Treatment	0.0948**	
Genotypes	0.201**	
T by G interactions	0.2843**	

reduction of chlorophyll content, variety IT99K-7-21-2-2 (34.9%) and variety TVU7778 (33.4%) indicated higher percentage of chlorophyll content reduction while variety IT97K-573-1-1 recorded least chlorophyll reduction at (1.4%).

DISCUSSION

Decline in root and shoot dry weight under water deficit may be attributed to root damage and death thereby reducing the activity of the roots (Munns and Termaat, 1986). There was inhibition of root growth which may be attributed to reduced extensibility of the root tip tissue due to hardening of the expanding cell walls. Reduced root growth impact negatively on plant growth owing to the fact that available surface area for absorption of water and mineral salts is reduced (Neumann *et al*., 1994). In the present study, among the nine cowpea varieties, there was general decrease in final shoot biomass except variety TVU7778 which recorded increase in final shoot biomass than the initial shoot biomass. Varieties with increase in root biomass include: IT97K-499-35, IT99K-7-21-2-2, TVU7778, and IT98D-1399. There was reduction of root biomass in variety IT00K-1263, IT00K-901-5, IT97K-573-1-1 and IT99K-503-1. A reduction in shoot growth coupled with continued root growth would result in an improved plant water status under extreme water deficit conditions. This may be due to tolerance mechanism exhibited by variety IT97K-499-35 and IT99K-7-21-2-2 for survival under drought conditions since increased root surface area allows more water to be absorbed from the soil. Luvaha (2005) reported that in mango seedlings, root growth continues at very low water potentials which are completely inhibitory to shoot growth.

Based on visual scoring for plant vigor, variety IT97K-499-35 (3) and IT97K-573-1-1(3) recorded the highest visual score which may be due to reduced leaf senescence. Opening of the stomata enhance photosynthesis and the extension of root deep in to the soil for absorption of water during water stress. Variety IT00K-901-5 (1.67) recorded least visual score; this may be due to leaf senescence and reduction of leaf surface area as mechanism to reduce transpiration and increase turgor.

Plants regulate their diurnal water status at a favorable level by the control of stomata aperture. Leaf relative water content, in drought stress recorded decreased in varieties IT00K-1263 (29%), IT00K-901-5 (25.5%), IT97K- 499-35 (19.6%), IT98D-1399 (12.7%), IT99K-21-2-2 (38%) and IT98K-503-1 (9.9%). Similar result was reported in beans by korir *et al.(* 2006) that there was decrease in LRWC after water stress induction. Varieties with increased LRWC include IT97K-568-19 (9.1%), IT97K-573-1-1(5.3%) and TVU7778 (6%).Increase may be due to stomata closure , which helps to maintain high Leaf Relative Water Content by decrease in transpiration and the production of non toxic metabolites which increase leaf water potential and consequently increase plant turgor.

Under severe water stress there is higher reduction in chlorophyll content, varieties IT99K-7-21-2-2 (34.9%) and TVU7778 (33.4%) indicated higher percentage of chlorophyll content reduction while variety IT97K-573-1-1 recorded least reduction in chlorophyll content (1.4%). Reduction in photosynthesis (chlorophyll content) in water stressed leaves may be due to stomata closure (Hsiao, 1973). Higher stomata conductance increases carbon dioxide (CO_2) diffusion into the leaf and favors higher photosynthetic rates. Higher photosynthetic rates could in turn favor a higher biomass and higher crop yields. Evapo-transpiration at the leaf surface lowers leaf temperature accompanied with higher stomata conductance which enhances leaf cooling.

CONCLUSION

It may be concluded that, at severe water stress condition varieties IT00K-1263 and IT00K-901-5, exhibited a higher reduction in most of the physiological parameters measured and thus concluded to be susceptible to water stress. Decreases in shoot biomass were recorded in all the varieties except in varieties TVU7778.Variety IT99K-7-21-2-2 and IT98D-1399 which showed increase in root biomass as the only drought tolerance mechanism. Varieties IT97K-499-37, IT97K-573-1-1 and TVU7778 were drought tolerant as they exhibit some adaptive mechanism for drought tolerance. Such as increase in leaf relative water content, shoot and root biomass, leaf senescence and stomata closure.

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