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PHYSIOLOGICAL RESPONSES OF SOME DROUGHT RESISTANT COWPEA GENOTYPES (*VIGNA UNGUICULATA (L.) WALP*) TO WATER STRESS

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ABSTRACT
Drought is an abiotic stress that affects the growth of cowpea genotypes in Sub-saharan Africa One of the first physiological responses to water stress in crops is the functioning of the leaf. The aim of the present study is to determine leaf physiological responses of cowpea to water stress. The study was conducted at International Institute of Agriculture (IITA) Kano state, Nigeria during the period of 17th November to 23rd December 2009. Seven cowpea genotypes differing in drought resistance were evaluated. The experiment was laid out in randomized complete design with three treatment regime, which include unstressed (control), moderate and severe water stress condition. The criteria measured include, Chlorophyll fluorescence parameters, chlorophyll content (SPAD), Water Use Efficiency (WUE) specific leaf area (SLA), shoot and root biomass. The results showed that water stress significantly reduced chlorophyll content (SPAD). 100% reduction was recorded in moderate and severe water stress. The results of photochemical yield (Fv/Fm) indicated that 71% of the genotypes at severe stress had reduction in Fv/Fm, while 42% was recorded in moderate stress. Genotype IT98K-555-1 recorded the highest reduction in Fv/Fm. The result showed a positive correlation between photochemical yield and chlorophyll content (SPAD) at unstressed (r= 0.921), moderate (r=0.903) and severe (r= 0.861) at 5%. Water stress significantly reduced above ground biomass. Lower biomass was recorded more under severe water stress. Reductions in shoot biomass were more significant in IT0K-835-45 and IT98K-555-1. At severe water stress, most of the genotypes recorded lower water use efficiency, except in genotype IT00K-901-5. The results showed a general increase in root biomass in moderate and severe water stress condition, except in IT00K-835-45 and IT96D-610. Increases in the root biomass were recorded more under moderate stress.

Keywords: Water stress, Water Use Efficiency, Leaf physiology, Drought Resistance, Cowpea (Vigna unguiculata (L.) walp)

INTRODUCTION

Drought is a major abiotic stress in the environment that limits crop performance more especially in drier sahelien zone. Soil water is one of the most important factors limiting crop production all over the world, where irrigation is practiced or rain fed crops are grown (Carter, 1989). Drought can significantly influenced plant performance and survival and can lead to major constraints in plant functioning, including a series of morphological, physiological and metabolic changes (Fischer and Turner, 1978; Ludlow and Muchow, 1990). Stomatal conductance, transpiration and photosynthesis are affected due to water stress. Drought affects photosynthesis directly and indirectly and consequently dry matter production, and its allocation to various plant organs (Mayaki *et al*., 1976). Many aspects of plant growth are affected by drought stress (Hsiao, 1973), these include leaf expansion, production and promotes senescence and abscission (Karamanos, (1980).

Water use efficiency is the production of moles of carbon gained in photosynthesis (A) in exchange for water used in transpiration (T), which can be interpreted as the instantaneous water-used efficiency of leaf gas exchange (A/T) (Condon *et al*., 2004). Water use efficiency is an important trait for improving drought tolerance in Cowpea, WUE would help save considerable amount of irrigation water. Further, an improvement in water use efficiency would significantly enhance total biomass production as well as yield at a given level of soil water availability. The evaporation of water during transpiration as well as the CO_2 entry during photosynthesis is controlled by stomatal diffusive factors, and hence total biomass production is often strongly linked to crop canopy transpiration. Water use efficiency can be increased either through a reduction in transpiration rate or an enhancement in the photosynthetic carbon assimilation capacity.

The aim of the study is to evaluate some of the leaf physiological response of cowpea to water stress.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted in the screen house of IITA Kano , Nigeria (Latitude 120 03', Longitude 80 34' and Altitude 486.5m and lies in the Sudan savanna) during the period of 17th November to 23rd December 2009.

Experimental set up

Seven cowpea varieties were used in the experiment; they are arranged in the screen house in a completely randomized design. The experimental materials were subjected to three treatment regimes, which included non stress, moderate and severe water stress condition with three replications.

Pot Preparation

Sixty three Pots each 17cm diameter and 18cm height with a perforated opening at their basal parts were used for the experiment. The pots were filled with 3kg of top soil (mixture of sandy and loamy) up to level of 2 litre of the pot. The pots were irrigated with water to field capacity. Basal applications of NPK fertilizer at 0.18g were imposed in each pot.

Planting/sowing

Four seeds were sown at the depth of 2cm; planting was done at 2cm spacing between the holes. Labels containing pot number, variety name and treatment type were attached to all pots. At 7 days after sowing all pots were thin to two plants per plot.

Water Stress Treatment

The crop received equal amount of water at two days interval for establishment. Watering treatments were introduced 14 days after sowing. The pots surface was covered with polyvinyl beads to minimize evaporation from the soil. Three levels of water regimes were imposed and this include; treatment 1 (T1) as unstressed; T2, moderate water stress and T3, severe water stress. Before the stress imposition all the pots were irrigated to field capacity. At the unstressed pots, continuous watering was maintained by weighing the pots every day and at the sane time adding the amounts of water that equal to the loss in weight from the pot. Under moderate water stress pots, watering was done at haft of the amount of water loss in weight from the pots. At severe water stress, complete withdraw of water was imposed. Pots were weighed at 10.00am in the morning of each day for the period of 22 days to obtained daily cumulative water transpired.

Data collection

Leaf Area measurement

Sample of leaves from the initial and final harvest were estimated using Scion image software. The leaves were scanned using HP Scanner, the preliminary images were converted to from color to grayscale (selected from the output type menu). The final version was saved as a TIFF file. The leaf area were calculated from the TIFF file using a public domain software (Scion image) as suggested by O'Neal *et al*. (2002).

Specific Leaf Area (SLA)

The specific lea area was calculated using the formula (Nagesware Roa *et al*., 2001)

SLA = Leaf area (cm2)/ Leaf weight (g)

Dry Weight

Leaves after scanning were oven dried in a ventilated oven for 48hrs at 80^0C together with the stem samples at initial and final harvest, weights of the samples were recorded with a sensitive balance at resolution ± 0.000g.

Root dry weight

Root samples were obtained by washing all the pots with mesh to remove tiny roots from the soil. Samples of the roots were washed and oven dried at 80^oC for 48hrs. Weights of the dry roots were measured using a sensitive balance at resolution ± 0.00g.

Determination of Total Transpiration and Water Use Efficiency

Cumulative water transpired during the experimental period was determined gravimetrically, the pots were initially weighed to obtain the pots initial weights before stress induction. . Pots were weighed at 10.00am in the morning of each day for the period of 22 days to obtained daily cumulative water transpired in all the experimental pots.

The total water transpired daily was computed and cumulative water transpired was reported in grams.

Water Use Efficiency was calculated as suggested by (Impa *et al*., 2005)

$WUE = (BM_{final} – BM_{initial}) /CWT$

Where,

$BM_{initial}$= Biomass at 0 days after water stress induction

BM_{final} = Biomass at 22 days after water stress induction

CWT = cumulative water transpired during the same experimental period.

Chlorophyll fluorescence

Chlorophyll fluorescence was measured using $0S\text{-}30_P$ chlorophyll flourometer. The following parameters were recorded;

Fo = minimal level when all the QA are in oxidized state (open reaction centres)

Fm= maximum fluorescence when QA are reduced (close reaction centres)

Fv= (Fm-Fo), refers to as photochemical fluorescence quenching

Fv/Fm = maximum quantum efficiency of photosystem II (PSII)

Measurements were taken in the morning from 10.00am and leaves were dark adapted for 30 minutes using dark adapted clips. Two readings were taken in each fully expanded leaves.

RESULTS AND DISCUSSION

Photochemical yield (Fv/m)

The result from Table 1 showed that, 42% of the genotypes at moderate water stress had reduced their photochemical yield (Fv/Fm) while 71% of the genotypes at severe water stress recorded reduction in the Fv/Fm. Genotypes recording higher Fv/m under moderate water stress include IT00K-835-45, IT00K-901-5 and IT99K-377-1. The genotype highest genotype with reduced photochemical yield (Fv/Fm) is recorded by IT98K-555-1 with 26%. At severe drought condition, genotypes exhibiting higher photochemical yield include IT00K-835-45 and IT00K-901-5. The lowest was recorded by IT98K-555-1 (10%). The results showed a positive correlation between chlorophyll content and maximum quantum, efficiency of photo system (PS II) (Figs 1, 2 and 3). The present study showed that water stress reduced photochemical apparatus (PSII) at severe water stress. This finding concurs with that of Hamidou *et al*. (2007) in cowpea.

The reduction in photochemical activities could be due to the disturbance or damages of photosynthetic apparatus (PS II) (Niinemets, 2002).

Chlorophyll fluorescence parameters are direct indicators of the photosynthetic activity (Lichatenthaler *et al*., 2000). Hamidou *et al*. (2007) asserts that the decrease in photochemical activity under stress condition at vegetative stage was mainly due to stomatal process.

Chlorophyll content (SPAD)

Results for analysis of total chlorophyll content (SPAD) are presented in Table 2. The results showed that at both moderate and severe water stress there was 100% reduction in the chlorophyll content of the stressed genotypes. Under moderate water stress, the genotype recording highest reduction was IT00K-901-5 (24.51%) and the lowest reduction was recorded in IT97K-819-118 (6.64%). Under severe water stress, the reduction in chlorophyll content ranged from 14.82% in IT00K-835-45 to 38.21% by IT99K-377-1. Cowpea has the ability to reduce its chlorophyll content, change position of leaflets under drought (drought avoidance mechanism) (Agbicodo *et al.*, 2009). They become paraheliotropic and oriented parallel to the sun's rays when subjected to soil drought, causing them to be cooler and thus transpire less (Shackel and Hall 1979).

The results for specific leaf area at final harvest showed that, increases in SLA under both moderate and severe water stress were recorded in IT00K-835-45 and IT98K-819-118.The highest reduction was recorded in IT98K-555-1 under moderate and severe water stress (Table 2). Similar findings were reported by Samson and Helmut (2007) in cowpea that water deficit reduced significantly the total leaf area and total dry matter.

Shoot and Root Biomass

The shoot and root biomass are presented in Figs 4 and 5 respectively, water stress significantly reduced above ground biomass, lower biomass was recorded in severe water stress genotypes, than moderate water stress. Reductions in shoot biomass were more significant in IT0K-835-45 and IT98K-555-1. Genotype IT97K-819-118 recorded low reduction in biomass under severe stress. Under moderate stress, significant reductions in biomass were recorded in all the genotypes. Genotypes, IT99K-377-1 and IT97K-819-118 recorded lower reduction in their biomass (Fig.4)

The results showed a general increase in root biomass in moderate and severe water stress condition, except in IT00K-835-45 and IT96D-610. Increases in the root biomass were recorded more under moderate stress condition (Fig. 5). These results concur with that of Alyemeny (1998) *in Vigna ambacensis* L. that water stress results in significant reduction in stem dry weight and increased root length. Increase in root biomass in water stressed genotypes may be due to ability of the cowpea to divert assimilates to enhance the growth of the roots so as to exploit deeper parts of the soil water. Similar reports were reported for other legumes by Turk and Hall (1980). The study reported reduction in SLA under severe water stress, this adaptive mechanism of cowpea to water stress helps in reducing water loss from the evaporative surfaces. It has been reported that smaller leaf area may be ascribed to acceleration of leaf senescence and abscission (Constable and Hearn, 1978) or to the sensitivity of leaf expansion to water stress (Boyer, 1970; Whiteman and Wilson, 1965).

Water Use Efficiency

The result for Water use efficiency of cowpea genotypes (Fig 6) showed that, genotypes exhibiting higher water use efficiency were recorded more at moderate stress conditions and this was recorded in IT99K-377-1, IT97K-819-118 and IT98K-205-8. Genotypes showing lower WUE include IT98K-555-1. At severe water stress, most of the genotypes recorded lower water use efficiency, except in genotype IT00K-901-5.

CONCLUSION

It may be concluded that, water stress at severe condition reduced the photochemical yield and chlorophyll content of the genotypes. IT98K-555-1 exhibited a higher reduction in all the physiological parameters measured and thus concluded to be susceptible to water stress. General reductions in above ground biomass were exhibited over all water stress conditions. Increases in root biomass were recorded in all the genotypes except in IT00K-835-45, IT96D-610 and IT98K-555-1. Genotypes, IT98K-205-8, IT99K-377-1 and IT97K-819-118 were drought tolerant as they exhibit low reduction in WUE, and photochemical yield (Fv/Fm).

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Table 1: Effect of water stress on Chlorophyll Fluorescence of some cowpea genotypes grown under different water stress conditions

	Fm			Fo			Fv			Fv/m		
Geno	WT	MD	SD	WT	MD	SD	WT	MD	SD	WT	MD	SD
IT00K-835-45	460	512	510	189.3	201.3	170.7	271	310	339	0.584	0.595	0.657
IT00K-901-5	451	451	464	270.3	192.7	118	180	258	346	0.398	0.526	0.574
IT96D-610	517	360	400	288.3	180	206	229	180	194	0.442	0.389	0.415
IT97K-819-118	449	436	448	159	166.7	146.3	290	269	302	0.56	0.618	0.546
IT98K-205-8	497	524	496	175	157.3	198.7	322	366	297	0.638	0.625	0.599
IT98K-555-1	445	392	273	213.3	254.3	112.3	232	138	161	0.484	0.356	0.434
IT99K-377-1	474	491	238	229.3	188.7	166	245	303	72	0.486	0.59	0.298
Mean	471	452	404	217.8	191.6	159.7	253	261	244	0.513	0.528	0.503
SE	55.4	60.8	40	35	34	22.66	63.6	55.9	45.6	0.0898	0.0936	0.0858

Key: SE, standarad error of means; Fm, maximum fluorescence when QA are in reduced state; Fo, minimum fluorescence when QA are in oxidized state; Fv, photochemical fluorescence quenching; Fv/m, maximum quantum efficiency of photosystem II (PSII); WT, wet; MD, moderate water stress,; SD, severe water stress.

Table 2: chlorophyll content (SPAD) and specific leaf area (SLA) of cowpea genotype under different water stress conditions

	SPAD			SLA1			SLA2		
	WT	MD	SD	WT	MD	SD	WT	MD	SD
IT00K-835-45	52.87	42.8	45.03	183.3	257.2	193.7	225	336	309
IT00K-901-5	42.83	32.33	29.8	312.6	296.5	307.3	192	180	243
IT96D-610	43.67	38.7	40	285.1	280.3	249.5	255	323	181
IT97K-819-118	42.13	39.33	38.6	321.8	301	298.9	232	330	262
IT98K-205-8	52.93	43.63	45.17	307.1	295.5	288.7	242	226	293
IT98K-555-1	31.3	26.3	20.8	210.4	282.4	296.2	239	47	97
IT99K-377-1	43.33	33.7	26.77	313.6	309.2	329.2	233	275	221
Mean	44.15	36.69	35.17	276.3	288.9	280.5	231	245	230
SE	1.966	1.969	4.08	51.7	29.9	39.8	70	64.3	50.6

Key: SE, standard error of means; SLA, specific leaf area; WT, wet; MD, Moderate droughts; SD, severe drought

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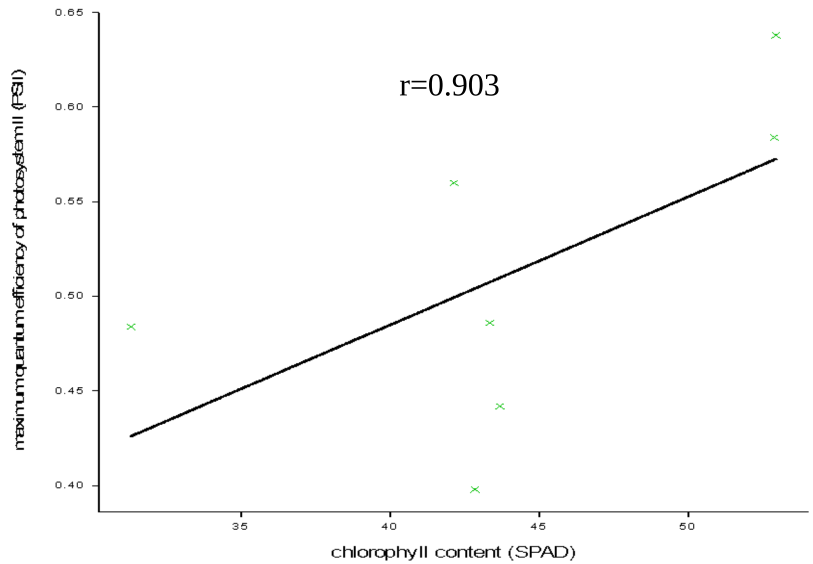

Fig 1: Regression relationship between chlorophyll content and maximum quantum efficiency of cowpea at non stress water condition

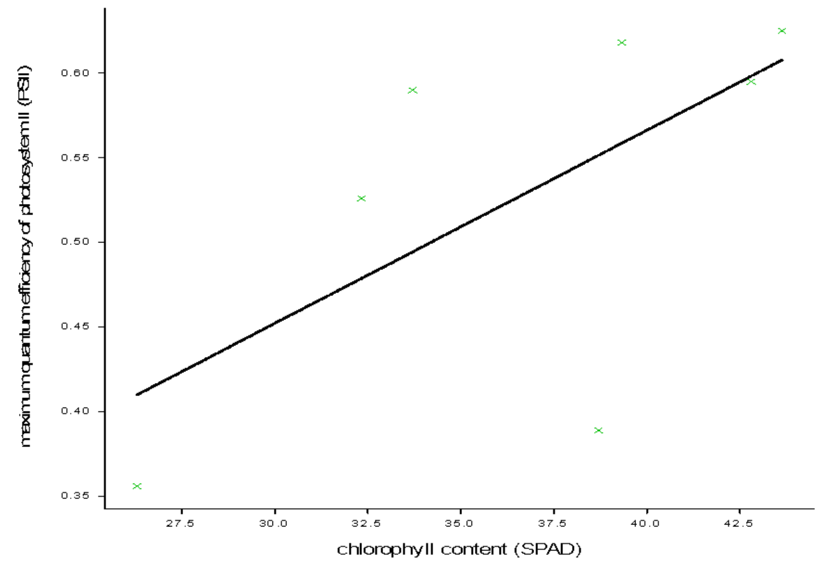

Fig 2: Regression relationship between chlorophyll content and maximum quantum efficiency of cowpea at moderate water stress condition

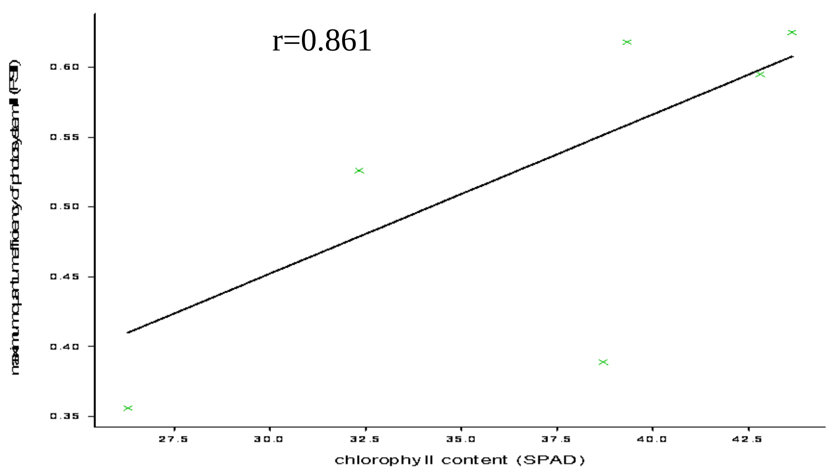

Fig 3: Regression relationship between chlorophyll content and maximum quantum efficiency of cowpea at severe water stress

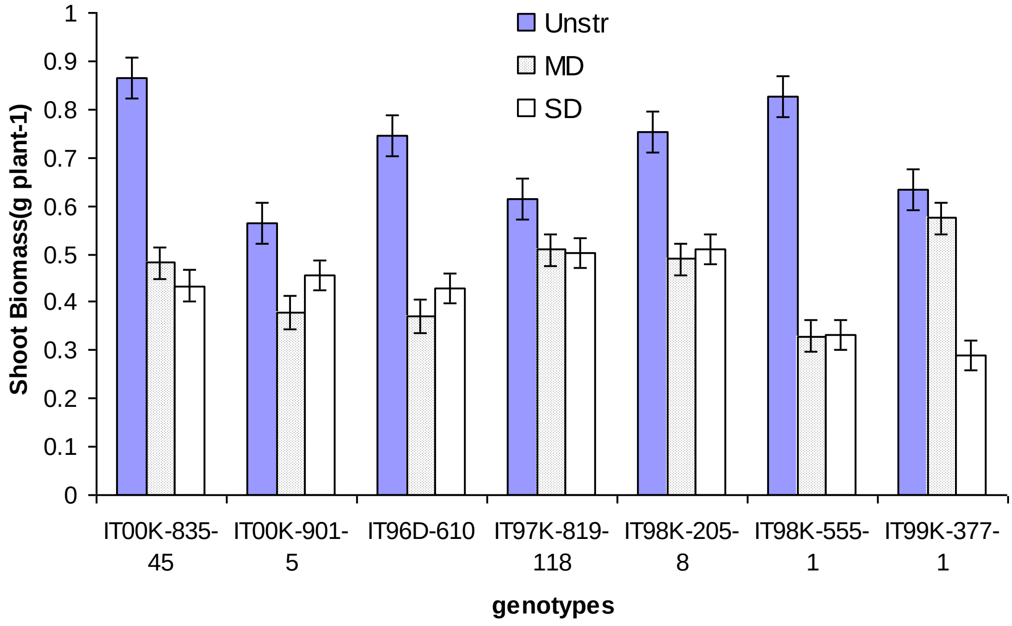

Fig 4: Shoot biomass of cowpea genotypes grown under different water stress condition, vertical bar represent ± standard error of means for three replication

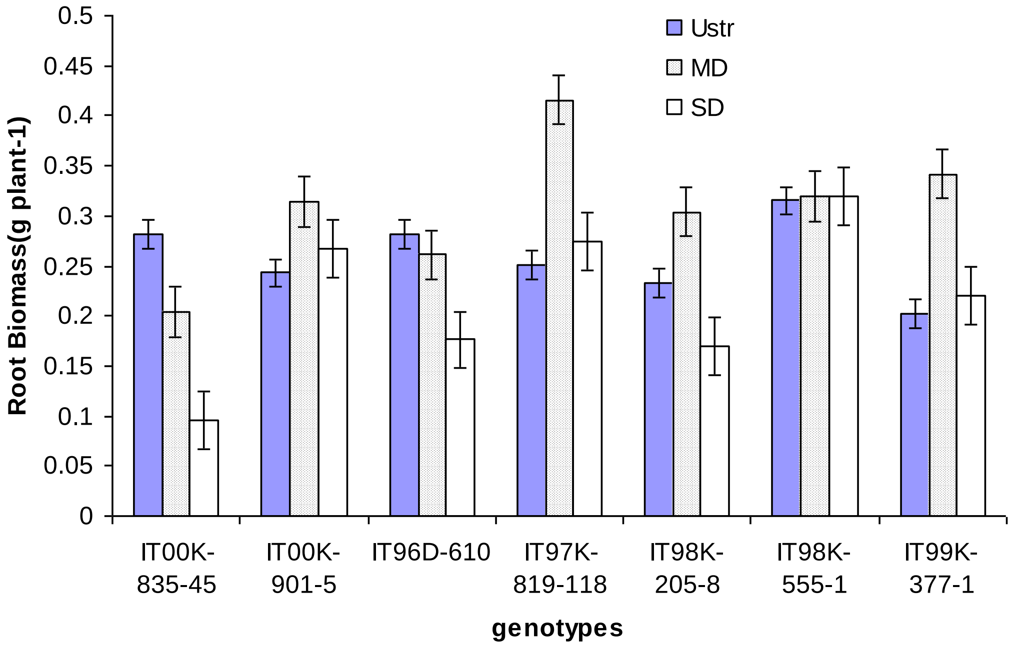

Fig 5: Root biomass of cowpea genotype grown under different water stress condition, vertical bar represent ± standard error of means for three replication

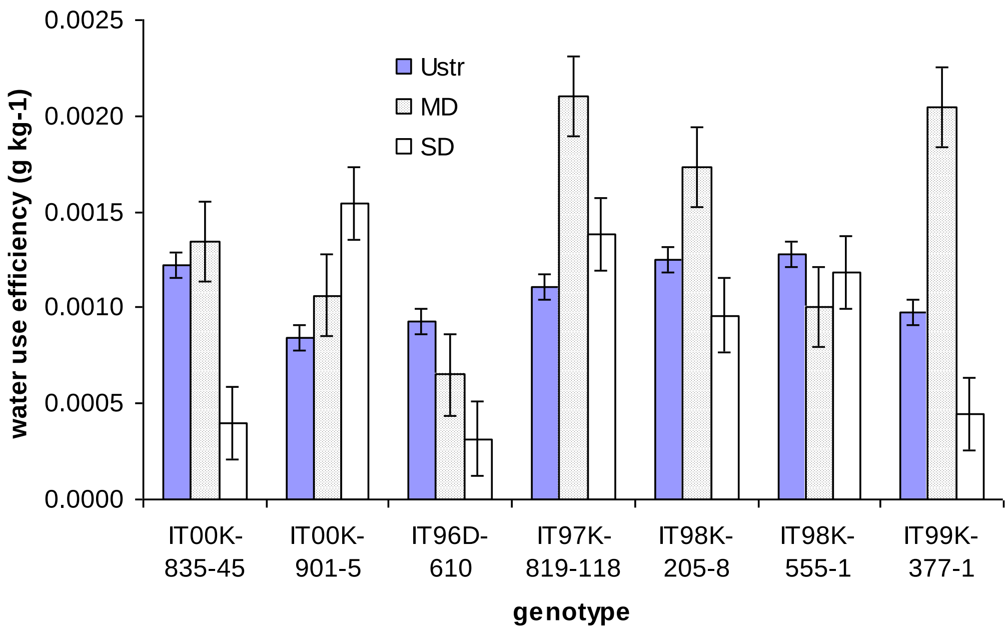

Fig 6: Water use efficiency (WUE) of cowpea under different moisture stress condition

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