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Screening and Evaluation of Cotton Germplasm in Zambia for Tolerance to Water Stress

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ABSTRACT

Forty cotton genotypes of varied origin were subjected to controlled moisture stress in a rainout shelter at Magoye (16°S, 27.6°E) in Zambia with the major objective being identification of moisture tolerant genotypes. Two experiments were used for this purpose; the box and pot experiments. Genotypes with favourable performance under the rain-out shelter were selected for further evaluation under field conditions in six locations during the rainy season. The genotypes in the trials exhibited varying responses to water stress. Significant variation ($p>0.10$) was observed among trial genotypes for seedling wilting, seedling survivability after stress, canopy temperature and plant tissue moisture content after stress. CAM 26 was the most consistent and high performing cotton genotype in the rainout shelter with mean survival after stress of 84%. Multilocation evaluation revealed considerable variation in field performance among trial genotypes. SRG 06 emerged as the most consistent genotype with the highest seed cotton yield in low rainfall (<500mm) test environments and considerably high yields in high rainfall (>500mm) test environments. Subject to further evaluation for performance in fibre quality parameters, SRG 06 is the most suitable genotype for cultivation in low rainfall production environments in Zambia. CAM 26 is recommended for use in cotton breeding programmes as a source of genes for moisture stress tolerance.

KEYWORDS: Stress tolerance, drought, cotton, canopy temperatures, and seedling survivability

INTRODUCTION

Cotton in Zambia is grown on more than 200,000 hectares with more than 160,000 small scale farmers involved in its production annually. The crop has over the years played a very important role in the social economic aspects of Zambia through employment creation by various players in the cotton value chain. Estimates indicate that Zambia earns more than USD 80m from exports of cotton lint (Cotton News 2008).

One of the major challenges in Zambia's cotton sector is low productivity. The average lint yield in Zambia of 260kg/ha is much lower than the world average of 780kg/ha (ICAC 2012). A number of factors have been attributed to the low productivity of Zambian cotton. Adverse weather conditions such as drought occurring during the growing season features prominently among these factors. Globally, areas affected by drought are expanding and the trend is expected to accelerate (Burke et al 2006). Over the last decade, the frequency of droughts and length of dry spells within growing seasons in Zambia have intensified due to climate change. Hence there is need to identify and develop technologies such as cotton varieties that are tolerant to water stress. Identification, development and dissemination of such technologies are likely to reduce the yield losses induced by extreme drought in cotton.

Drought stress can significantly reduce cotton productivity by affecting many agronomic traits such as reduction in size and number of bolls per plant, plant height and above ground dry matter (Malik et al 2006). Several studies have established the sensitivity of cotton to water stress during various developmental phases. Kriegal et al., (1993) identified four basic stages and these are seedling establishment, square formation, early flowering to flowering peak and boll development to ripening. Oosterhuis (1990) established that the most critical window for drought impact on cotton productivity happens between 45 and 65 days after planting which coincides with time from first square to first flower formation. Cotton genotypes that exhibit resilience amidst water stress at critical stages of seedling establishment and reproductive stages of development are desirable especially in situations of unreliable rainfall distribution.

Dilbeck and Quisenberry (1993) suggested two approaches to screening for drought tolerance on cotton plants. The first method involves screening and observing plant size, leaf wilting and leaf drop. The other approach uses observations on leaf size, leaf dry weight and plant height. Canopy temperature is also related to water stress in cotton and has been used to separate cotton germplasm in relation to their water conservation ability (Hatfield et al., 1987). Factors that cause increased canopy temperature have positive effect on dry matter accumulation (Hatfield et al., 1987). Germplasm with warmer canopy temperature will also have decreased evapotranspiration given that all other conditions are equal. Malik et al (2006) used various physiological parameters to select for drought tolerance in cotton. Such parameters included transpiration rate, relative water content, excised leaf water loss and chlorophyll content.

Other approaches used by scientists include drought induction at cotton seedling stage to screen and select cotton lines for drought tolerance. The major advantage of this procedure is that it enables one to test a large number of entries at once

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(Longenberger et al 2006). This study was conducted in order to identify cotton genotypes that are tolerant to drought as well as develop selection criteria for drought tolerance for cotton in Zambia.

MATERIALS AND METHODS

Forty cotton genotypes obtained from the germplasm collection at Cotton Development Trust in Zambia were studied for tolerance to water stress at Magoye research station in Zambia. The genotypes studied are shown in the table below;

Table 1- Genotypes tested for water stress tolerance

Code	Name	Origin	Code	Name	Origin
1	Chureza	Zambia	21	BP-52	West Africa
2	F 135	Zambia	22	SRIF 4-7	West Africa
3	CDT II	Zambia	23	PDO 109	West Africa
4	INTSC-01	Zambia	24	BIII-F3 X CZA 87	Zambia
5	INTSC-02	Zambia	25	ZIM II	Zimbabwe
6	INTSC-03	Zambia	26	ZIM III	Zimbabwe
7	INTSC-04	Zambia	27	TURK A	Turkey
8	INTSC-05	Zambia	28	TURK B	Turkey
9	INTSC-06	Zambia	29	YENI	Brazil
10	INTSC-07	Zambia	30	CDT II 02	Zambia
11	INTSC-08	Zambia	31	CDT II 03	Zambia
12	MF-20Kr	Zambia	32	CDTII 06	Zambia
13	CA 223	Zambia	33	CDT II 08	Zambia
14	CA 336	Zambia	34	CDT II 09	Zambia
15	MFKF-20Kr	Zambia	35	STAM 279A	West Africa
16	MCZA-20Kr	Zambia	36	IRMA D 742	West Africa
17	MG-15Kr	Zambia	37	C 2101	Zambia
18	MCF-30Kr	Zambia	38	C 2102	Zambia
19	T 120-78	West Africa	39	C 2104	Zambia
20	Z 146-76	West Africa	40	CAM 26	Cameroon

(i) Identification of seedling drought tolerant cotton genotypes

This part of the experiment consisted of three parts; box screening, box screening and field screening. All three experiments were laid out in a randomised complete block design.

Box screening experiment- Wooden boxes measuring 120cm x 50cm x 7cm depth made of 2.5cm thick planks were constructed and placed in a rain protected shelter (screen house) (Singh, 2000). These boxes were then be filled with river sand (passed through a 2mm screen), to ensure the minimum possible water retention required for appropriate droughty conditions, and watered to saturation (Alabi et al 2003). Spacing inside the boxes was at 5cm x 2.5cm. Forty elite cotton germplasm lines including commercial varieties were planted in the wooden boxes during the first week of September 2011 at Magoye (16°S, 27.6°E) when the heat units were high enough to support cotton growth and development. Each box contained 20 lines of 20 stands (40 plants per row). Each of the entries was replicated four times and each replication was composed of two boxes. Water was applied in the boxes using watering cans every day from planting up to emergence. Water stress was imposed at different times after emergence (7days after planting) for periods of 10 days per stress cycle. Only two stress cycles were used in the experiment. Counts of permanently wilted plants for each variety were carried out along with the collection of two to five plant samples from the thinned plants after each stress treatment. These samples were used to determine the water conservation ability of trial genotypes as determined by plant tissue moisture content after stress. The number of surviving plants in each plot was counted 48 hours after resumption of water application.

Pot screening experiment-The pot experiment was used to screen for drought tolerance from seedling stage to early flowering stage. Three replicates of genotypes were assessed in 120 pots of dimension 20cm diameter by 20cm height filled with loamy sand made of a mixture of sieved river sand and top soil in a ratio of 1:1.NPK fertilizer type (10:20:10) was mixed with soil prior to pot filling at a rate equivalent to 200kg/ha. A space of 5cm was left at the top of the pot for watering. Pots were watered to saturation before sowing 10 seeds per pot. The pots were kept saturated by constant irrigation until emergence. At ten days after planting, irrigation was halted in order to impose the moisture stress for ten days. Data collection included plant tissue moisture content and wilting scores after water stress application at various stages of growth between 10-45 days after sowing. Moisture content was determined by getting plant samples, drying them in an oven and calculating the difference in weight before and after drying. Two plants per pot were left to grow to maximum canopy formation (~56 Days after sowing). At maximum canopy formation, a ten day stress treatment was applied. After this period, canopy temperatures were measured above the canopies of the stressed cotton genotype using a hand held infrared thermometer (OS51, SE OMEGA, Stamford, CT, USA). Five measurements of canopy temperatures were made at 0-45°, 10 cm above a fully grown potted plant at flower initiation stage.

(ii) Determination of the mechanisms for drought tolerance

In order to effectively utilize the drought tolerant cotton germplasm lines, there was need to ascertain the mechanism involved in drought tolerance in the genotypes under study. The plant samples obtained from the pot experiment, box experiment and field screening were subjected to plant tissue moisture content determination at whole plant level after water stress periods. The amount of moisture stored in the shoot system of crop plants after periods of water deficit is an indication of the water conservative ability of the respective genotype and this trait is used for dehydration tolerance under water stress conditions. The samples were weighed fresh and oven dried at 60°C for 48 hours. These samples were re-weighed after oven drying to determine the initial water content of the shoot system of the sampled plants. Data on plant tissue moisture content, wilting percent, canopy temperature and that of plant survivability after moisture stress was subjected to correlation analysis to determine the significance of the relationships among these parameters.

(iii) Field evaluation

This part of the research project was used to evaluate the identified drought tolerant cotton genotypes during the actual growing season for performance in important agronomic and fibre traits. Six locations; Magoye, Magoye 2 Lusitu, Chirundu, Masumba and Nanga) were used for field evaluation. Chirundu, Lusitu and Nanga are located in an agro-ecological region characterised by low rainfall (>800mm) and high temperatures during the growing season. Magoye, Magoye 2 and Masumba are located in an agro-ecological region characterised by medium to high rainfall (800-1000mm). Data was mainly collected on seed cotton yields.

RESULTS

Seedling wilting- A visual count of wilted seedlings was carried out after four days of water stress. Genotypes exhibited a wide range of responses to water stress. The lowest rate of wilting was recorded on CAM 26 with 3.5% while the highest rate of wilting was recorded on STAM 279A with 66.2% wilting. (Table 2)

Seedling survivability-Considerable variation was observed for this trait among trial genotypes. The highest rate of seedling survivability was recorded in CAM 26 with survival rate of 84% while the lowest survival was on CDT II with mean survival of 19.2 %.(Table 2)

Canopy temperature-Canopy temperatures are used to indicate the ability of plant genotypes to conserve water under moisture stress growing conditions. The highest canopy temperature was recorded on C 2102 with 40.5°C while the lowest temperature was recorded on Chureza at 35 degrees Celsius. (Table 2)

Plant tissue moisture content after stress-This parameter measures the ability of plant genotypes to avoid dehydration under water stress conditions. CDT V had the highest plant tissue moisture content of 80.1% while MCZA 20Kr had the lowest plant tissue moisture content at 65 %.(Table 2)

Table 2: Performance of cotton genotypes in the box and pot experiments of the rainout shelter

	Seedling wilting (%) After 4 days of Stress		Canopy temperature(°C)		Plant tissue moisture content (%) After 3 days stress		Seedling survivability 1st Cycle (%)		Seedling survivability 2nd Cycle (%)		mean survivability
1	CAM 26	3.5	C 2102	40.5	CDT V	80.1	INTSC 07	90.1	CAM 26	80.7	84
2	BP-52	5.1	YENI	40.0	C 2104	77.2	CAM 26	87.3	YENI	76	72.5
3	INTSC 05	9.4	BP 52	39.5	CAM 26	77.1	INTSC 05	87.1	BP-52	71.9	77.2
4	CA 223	10.1	C 2101	39.5	SRG 09	77.1	CA 223	85.4	INSTC 07	66.1	78.1
5	YENI	12.3	C 2104	39.5	C 2101	75.4	ZIM II	84.5	INTSC 08	64.5	62.2
6	INSTC 07	17.7	SRG 06	39.5	C 2102	75.2	BP 52	82.5	Chureza	62.7	66.4
7	B III-F3 x	19.8	CDTII 08	39.5	MCF 30Kr	75.2	INTSC 04	82.0	MFKF 20Kr	59.5	68.9
8	MFKF 20Kr	20.6	INTSC 07	39.5	INTSC 01	75.3	CDT V	81.0	PDO 109	58.3	43.8
9	Z 146-76	21.6	INTSC 04	39.5	F 135	74.6	MFKF 20Kr	78.2	INTSC 04	58.2	70
10	INTSC 08	21.9	IRMA D742	39.5	YENI	74.6	TURK B	77.3	INTSC 05	57.7	72.4
11	INTSC 02	22.4	STAM 279 A	39.5	MFKF 20Kr	74.4	CDTII 08	75.5	MCZA 20Kr	54.8	64.0
12	CDT V	22.4	CAM 26	39.0	SRG 06	74.1	MF 20Kr	73.9	INSTC 06	54.5	58.4
13	INSTC 06	22.6	MF 20Kr	39.0	CHUREZA	73.9	MCZA 20Kr	73.1	SRG 09	53.7	63.1
14	SRG 09	23.4	SRIF 4-7	39.0	CDT II 08	73.8	SRG 09	72.8	SRIF 4-7	52	57.0
15	Chureza	23.4	ZIM III	39.0	T 120-78	73.5	SRG 06	72.4	MG 15Kr	49.6	49.8
16	MCF 30Kr	26.6	CDTII 02	38.5	BP 52	73.4	CHUREZA	70.0	INTSC 02	49.1	54.7
17	MF 20Kr	27.4	CA 223	38.5	ZIM II	73.3	TURK A	69.4	MCF 30Kr	48.1	56.4
18	MCZA 20Kr	27.8	INTSC 05	38.5	CDT II	73.0	YENI	69.0	CA 223	46.8	66.1
19	TURK B	28.1	CDT V	38.0	INSTC 07	72.8	MCF 30Kr	64.7	MF 20Kr	46.7	60.3
20	CDT II 03	28.4	CDT II	38.0	IRMA D742	72.8	CDT II 03	64.4	C 2104	46.6	54.8
21	SRG 06	28.9	INSTC 06	38.0	PDO 109	72.7	C 2104	62.9	SRG 06	46.5	59.5
22	CDT II 08	30.0	TURK B	38.0	MG 15Kr	72.6	INTSC 06	62.3	CDT V	44.9	63.0
24	SRIF 4-7	31.5	INTSC 02	37.5	CDT II 03	72.2	T 120-78	60.6	T 120-78	42.5	51.6
25	PDO 109	31.6	TURK A	37.5	INTSC 02	72.2	INTSC 02	60.2	B III-F3 x	38.6	44.2
26	T 120-78	32.0	ZIM II	37.5	INTSC 04	72.2	INTSC 08	59.8	TURK B	36.9	57.1
27	TURK A	33.1	CDTII 03	37.0	TURK B	72.2	CDT II 02	59.5	INSTC 03	36.2	38.8
28	C 2104	34.7	F 135	37.0	INTSC 03	72.1	STAM 279A	56.5	INTSC-01	36	42.7
29	MG 15Kr	35.6	MCZA 20Kr	37.0	BIII-F3 X CZA	71.8	F 135	51.4	CDT II 03	35.2	49.8
30	CDT II	37.8	PDO 109	37.0	SRIF 4-7	71.6	MG 15Kr	49.9	TURK A	33.7	51.6
31	INSTC 03	39.5	BIII-F3 X CZA	36.5	INTSC 08	71.0	BIII-F3 X CZA	49.7	CDT II 08	32.9	54.2
32	F 135	40.8	INTSC 08	36.5	STAM 279A	70.8	INTSC 01	49.4	C 2101	32.8	39.2
33	IRMA D 742	44.7	INTSC 01	36.5	ZIM II	70.4	C 2101	45.4	STAM 279 A	28.5	42.5
34	C 2101	45.3	MFKF 20Kr	36.5	MF 20Kr	70.2	INTSC 03	41.3	C 2102	26.4	35.9
35	C 2102	48.3	MG 15Kr	36.5	Z 146-76	70.2	IRMA D 742	37.2	Z 146-76	24.3	23.4
36	ZIM II	48.4	INTSC 03	36.0	TURK A	69.9	C 2102	32.8	ZIM III	16.5	22.8
37	INTSC 04	49.0	MCF 30Kr	35.5	CDT II 02	69.4	PDO 109	29.2	IRMA D 742	16.2	26.7
38	ZIM III	52.8	T 120-78	35.5	INSTC 05	69.1	ZIM III	29.0	ZIM II	16.1	22.6
39	CDT II 02	54.9	Z 146-76	35.5	CA 223	68.8	CDT II	26.2	F 135	15.6	33.5
40	STAM 279 A	66.2	CHUREZA	35.0	MCZA 20Kr	65.0	Z 146-76	22.5	CDT II	12.1	19.2
	Mean	30.3	Mean	37.9	Mean	72.8	Mean	62.2	Mean	44.6	
	LSD (10%)	29.3**	LSD (10%)	3.4*	LSD (10%)	7.5ns	LSD (10%)	38.8**	LSD (10%)	39.9*	

Correlation Analysis

Significant positive relationships were established between seedling survivability and Plant tissue moisture content after stress and wilting percent after stress (Table 3)

Table 3-Correlations between water stress related parameters

	Canopy Temperature	Plant tissue moisture content	Seedling survivability
Canopy Temperature	1		
Plant tissue moisture content	0.17*	1	
Seedling survivability	0.02	0.15*	1
Wilting Percent	0.14*	-0.11*	-0.71**

*,** significant at p=0.1 and p=0.05 respectively

Seed cotton yield

The test environments were grouped into two broad categories depending on the amount of rainfall received. CDT V was the best performing genotype in environments that received more than 500mm or rainfall with seed cotton yield of 1,713kg/ha while SRG 06 had the highest seed cotton yield in environments with less than 500mm of rainfall with 1,315kg/ha. Chureza had the best yielding check variety in low rainfall environments. (Table 4)

Table 4: Genotype performance for seed cotton yields (kg/ha) under field conditions in six environments

Genotype performance in environments with more than 500mm Rainfall						Genotype performance in environments with less than 500mm rainfall					
Genotype	Magoye2	Magoye	Masumba	Mean	Rank	Genotype	Chirundu	Lusitu	Nanga	Mean	Rank
CA 223	916	1206	1578	1233	16	CA 223	415	403	1484	767	15
INTSC 07	1472	1611	1612	1565	5	INTSC 07	280	630	1919	943	9
CAM 26	1546	1285	1718	1516	7	CAM 26	361	796	1951	1036	6
INTSC 05	1267	1773	1478	1506	9	INTSC 05	293	602	1482	792	13
BP 52	1250	1464	1656	1457	11	BP 52	548	805	1542	965	7
INTSC 04	953	1314	1252	1173	17	INTSC 04	492	667	1154	771	14
CDT V (C)	1536	1786	1818	1713	1	CDT V (C)	600	671	1529	933	10
MFKF 20KR	1096	1450	1318	1288	15	MFKF 20KR	525	658	1413	865	12
TURK B	981	1344	1688	1338	13	TURK B	479	602	1014	698	16
CDT II 08	1723	1203	1422	1449	12	CDT II 08	398	1018	2067	1161	2
MF 20KR	1157	2071	1486	1571	4	MF 20KR	253	338	971	521	17
MCZA 20KR	1460	1604	1566	1543	6	MCZA 20KR	581	866	1709	1052	4
SRG 09	1233	2362	1418	1671	2	SRG 09	688	986	1759	1144	3
SRG 06	1612	1792	1562	1655	3	SRG 06	529	1028	2388	1315	1
CHUREZA(C)	1284	1510	1700	1498	10	CHUREZA(C)	644	843	1634	1040	5
TURK A	980	1592	1334	1302	14	TURK A	522	509	1816	949	8
CDT II(C)	1672	1507	1358	1512	8	CDT II(C)	467	679	1485	877	11
Mean	1302	1579	1528	1470		MEAN	475	712	1607		
LSD	641	556	460			LSD	333	353**	1303		

DISCUSSION

Differences were established among cotton genotypes with regard to seedling wilting under drought. There was a significant negative correlation between seedling wilting and survivability. This was expected as the level of wilting indicates the amount of moisture in the plant tissues and that ultimately determines plant survival under moisture stress conditions. The correlations indicate that seedling wilting can be used as drought tolerance selection criteria in cotton at seedling stage. Tavakol and Pakniyat (2007) also established significant correlations between seedling wilting and plant survival in wheat.

Seedling survivability is the ultimate indicator of drought tolerance as it would dictate the final plant population and seed cotton yield after stress. Hameed *et al* (2010) and Logenberger *et al* (2006) relied on seedling survivability in their efforts to identify drought tolerant wheat and cotton genotypes respectively. Cotton genotypes had different performances in seedling survivability after moisture stress. Survivability ranged from 84% to 19.2% signifying considerable variability for this trait among trial genotypes. Genotype CAM 26 had the highest rate of seedling survivability after stress while CDT II had the least survivability rates. However, CDT II performed well under dry field conditions. The difference in performance between protected screening conditions and natural field screening conditions for some genotypes indicates the need to confirm results obtained under protected conditions with field based experimentation. Another suggestion could be that the entire compliment of genotypes tested under protected conditions should also be subjected to field screening.

Canopy temperature is used to measure the ability of plant genotypes to maintain transpiration and gas exchange especially under drought stress. In this study, canopy temperature was used to ascertain the dehydration avoidance of cotton genotypes. This approach is based upon the close, inverse relationship between leaf temperature and transpirational cooling. There was significant variation among trial genotypes with respect to canopy temperature. Correlation analysis revealed an insignificant relationship between canopy temperature and seedling survivability. This is in contrast with Royo *et al* (2002) who found a significant relationship between canopy temperature, plant survival and wheat yield. Our results could emanate from possible interference and errors in recording canopy temperatures.

The variation for plant tissue moisture content among trial genotypes was not statistically significant. However, correlation analysis revealed significant positive relationship between plant tissue moisture content and seedling survivability after stress. Sarobol *et al* (2011) also established significant positive correlation between plant tissue moisture content and survivability. This result is expected because the hydration levels of plants will ultimately determine their chances of survival.

Seed cotton yield data was collected from those cotton genotypes that were subjected to multilocation field evaluation. Considerable variation was observed for mean seed cotton yields among genotypes. Generally, cotton genotypes that had high seedling survivability under protected drought stress conditions also performed well in those locations that had occasional dry spells during the growing season. This entails that screening for drought stress tolerance under protected conditions can be used to identify cotton genotypes with higher productivity under moisture stress field conditions. Britol *et al* (2011) also found differences in seed cotton yield between drought tolerant and drought susceptible cotton genotypes after exposure to moisture stress during the growing season.

Conclusion

A combination of rainout shelter screening and field based evaluation identified CAM 26 as a very moisture tolerant genotype for seedling drought and SRG 06 as suitable genotypes for cultivation in drought prone field environments. CAM 26 can be used in hybridization programmes that are aimed at inducing increased tolerance to drought at seedling and full growth stages. Further analysis for fibre is suggested to determine the commercial merit of growing SRG 06.Chureza was the most productive commercial variety under dryland field conditions. It should therefore, be recommended as a variety suitable for cultivation in drought prone cotton production environments in Zambia.

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