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EVALUATION OF COWPEA MAGIC RECOMBINANT INBRED LINES FOR SEEDLING-STAGE DROUGHT TOLERANCE

ÉVALUATION DE LIGNES RECOMBINANTES CONSANGUINES MAGIC DE NIÉBÉ POUR LA TOLÉRANCE À LA SÉCHERESSE AU STADE PLANTULE

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ABSTRACT

Drought stress causes yield losses of 35-65% in cowpea (*Vigna unguiculata* (L.) Walp.) in sub-Saharan Africa (SSA), under moderate to severe water deficits. Hence, drought tolerance is vital even in crops with inherent tolerance characteristics. This study evaluated F_0 lines of the Multi-parent Advanced Generation Inter-cross (MAGIC) recombinant inbred lines (RILs) and their eight founder parents, for seedling-stage drought tolerance for the Sudan Savanna Zone of Ghana. A screenhouse experiment was conducted using an augmented design, with three replications and a wooden box screening technique. Plants were watered daily with 8.2 L of borehole water for 10 days after planting. Drought stress was then imposed for 21 days, after which watering was resumed to assess the recovery of each genotype. The moisture content within the box decreased from 37.39% at the imposition of drought to 2.45% at the time of stress removal. Significant ($P < 0.05$) genotypic differences were observed for leaf wilting index (0.77 - 1.00), delayed leaf senescence (3.50 - 5), stem greenness (1 to 5), regrowth (0 - 5) and survival rate (0 to 100%), indicating substantial genetic variation. Drought symptoms increased progressively from 7 to 21 days after stress imposition. Strong positive correlations were detected among traits, with survival rate having the highest correlation coefficient with all drought traits, making it the most reliable indicator of drought tolerance at the seedling stage. Based on post-drought survival, 57 genotypes were classified as highly tolerant (>50% survival), 74 as tolerant (20-49%), 37 as susceptible, and 140 as highly susceptible. The high level of susceptible genotypes identified was partly attributed to the stringent conditions of the wooden box techniques that leave little room for escape. The wooden box method proved effective for discriminating seedling-stage drought responses. The phenotypic data obtained would be useful for QTL mapping to identify

markers for marker-assisted selection. This also calls for further field validation of the findings of this study. The identified tolerant genotypes provide valuable genetic resources for improving drought resilience in cowpea breeding programmes.

Key Words: Leaf wilting index, *Vigna unguiculata*

RÉSUMÉ

Le stress hydrique entraîne des pertes de rendement de 35 à 65 % chez le niébé (*Vigna unguiculata* (L.) Walp.) en Afrique subsaharienne (ASS) en cas de déficits hydriques modérés à sévères. Par conséquent, la tolérance à la sécheresse est vitale, même chez les cultures possédant des caractéristiques intrinsèques de tolérance. Cette étude a évalué les lignées F9 issues de croisements inter-populations avancés à parents multiples (MAGIC) et leurs huit parents fondateurs, afin d'analyser la tolérance à la sécheresse au stade plantule pour la zone de savane soudanienne du Ghana. Une expérience en serre a été menée selon un dispositif bloc augmenté, avec trois répétitions et une technique d'évaluation en bacs de bois. Les plantes ont été arrosées quotidiennement avec 8,2 L d'eau de forage pendant 10 jours après le semis. Un stress hydrique a ensuite été imposé pendant 21 jours, après quoi l'arrosage a été repris pour évaluer la capacité de récupération de chaque génotype. La teneur en humidité à l'intérieur du bac a diminué, passant de 37,39 % lors de l'imposition de la sécheresse à 2,45 % au moment de l'arrêt du stress. Des différences génotypiques significatives ($P < 0,05$) ont été observées pour l'indice de flétrissement foliaire (0,77 - 1,00), le retard de sénescence foliaire (3,50 - 5), la verdeur de la tige (1 à 5), la reprise de croissance (0 - 5) et le taux de survie (0 à 100 %), ce qui indique une variabilité génétique substantielle. Les symptômes de la sécheresse ont augmenté progressivement de 7 à 21 jours après l'imposition du stress. De fortes corrélations positives ont été détectées entre les caractères. Le taux de survie présentait le coefficient de corrélation le plus élevé avec l'ensemble des caractères liés à la sécheresse, ce qui en fait l'indicateur le plus fiable de la tolérance à la sécheresse au stade plantule. Sur la base de la survie post-sécheresse, 57 génotypes ont été classés comme hautement tolérants (survie > 50 %), 74 comme tolérants (20-49 %), 37 comme sensibles et 140 comme hautement sensibles. La proportion élevée de génotypes sensibles identifiés a été partiellement attribuée aux conditions strictes de la technique des bacs en bois, qui laissent peu de place à l'évitement du stress. La méthode des bacs en bois s'est avérée efficace pour différencier les réponses à la sécheresse au stade plantule. Les données phénotypiques obtenues seront utiles pour la cartographie des QTL afin d'identifier des marqueurs pour la sélection assistée par marqueurs. Cela appelle également à une validation ultérieure de ces résultats sur le terrain. Les génotypes tolérants identifiés constituent de précieuses ressources génétiques pour améliorer la résilience à la sécheresse dans les programmes de sélection du niébé.

Mots Clés: Indice de flétrissement foliaire, *Vigna unguiculata*

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is a major legume, particularly among smallholder farmer communities in the tropical and sub-tropical regions, where drought is a major production constraint (Sehgal *et al*., 2016). As a major source of plant protein, minerals and vitamins, cowpea serves as food and feed for humans and livestock, respectively (Affrifah, Phillips and Saalia, 2022). Although the crop is considered relatively drought-tolerant, its production is considerably affected by low moisture conditions and high temperatures (Nkomo *et al*., 2021; Omomowo and Babalola, 2021; Kim *et al.*, 2025).

Cowpea is affected by two main drought types: seedling stage drought and terminal drought (Tengey *et al.*, 2023). Seedling stage drought occurs in the early stages of active

vegetative growth, where root establishment and overall plant health are crucial. Hence, seedling stage drought often has severe effects on the cowpea plant. Terminal drought, on the other hand, occurs during the active reproductive phase of cowpea and often affects the yield and quality of the cowpea (Ravelombola *et al*., 2020; Nunes *et al*., 2022; Ritte *et al*., 2025). Both drought types present serious challenges to cowpea production; hence, the need to identify tolerance genotypes and or breed for drought tolerance in cowpea improvement programmes. The current trend of erratic rainfall patterns and unpredictable climatic conditions has further worsened the situation for smallholder cowpea farmers, especially in low-income countries (Tefera *et al.*, 2024).

Mechanisms adapted by crop plants in dealing with drought stress are either through escape, drought avoidance or drought tolerance (Oguz *et al.*, 2022; Pamungkas *et al.*, 2022). The escape and avoidance mechanisms are known not to be reliable because they help plants avoid exposure rather than withstand the stress physiologically, with their effectiveness depending on environmental conditions, timing and seasonal predictability (Haghpanah *et al.*, 2024). Thus, the drought tolerance mechanism has been the best bet in combating drought stress in several crop species.

Plant breeders screen for drought stress tolerance by targeting traits such as stay-green, leaf wilting index, regeneration percentage, survival rate, and the ability of the crop to maintain a yield in the presence of the stress. Other determinants of drought tolerance in cowpea include stomatal conductance (Verbree e*t al*., 2014; Bartlett *et al*., 2016), chlorophyll fluorescence (Corsolini and Carta, 2015), delayed leaf senescence (DLS) and Abscisic acid (ABA) levels (Mitchell *et al*., 1998; Singh e*t al,* 1999; Muchero *et al*., 2013).

The methods used for assessing for cowpea seedling stage drought tolerance in the screen house include the plastic bags method, the plastic pot method and the wooden box or drought stress box method (Nkomo *et al*., 2022). Some studies on cowpea drought tolerance include the identification of three accessions of cowpea (TVu-199, TVu-207, TVu-235, and TVu-241) to be moderately tolerant among 10 cowpea accessions screened for drought tolerance at the seedling stage using plastic bags in the screen house (Ajayi *et al*., 2017). Muchero *et al.* (2008) also reported that stem greenness, survival, and recovery dry weights were useful traits to screen cowpea genotypes for their ability to withstand drought stress at the seedling stage. Fatokun *et al.* (2012) concluded that drought at the seedling stage reduces the number of days to flowering by 12 days and grain yield per plant was also reduced to 67.28%.

Despite these works on drought in cowpea, the lines to be used in the present study have not been screened for drought; also, the scale of screening is large in this study. This study evaluated F9 lines of Multi-parent Advanced Generation Inter-cross (MAGIC) recombinant inbred lines (RILs) and their eight founder parents, for seedling-stage drought tolerance for the Sudan Savanna Zone of Ghana.

MATERIALS AND METHODS

Experimental site. The study was conducted in a screenhouse at the Council for Scientific and Industrial Research (CSIR) - Savannah Agricultural Research Institute (SARI) station in Manga, located in the Upper East Region of Ghana. This study targeted Ghana's Sudan Savanna agro-ecological zone. This zone generally endures a long dry season of 7 to 8 months and receives an annual rainfall of 250 mm (Manga Meteorological Station).

Planting materials. Three hundred RILs ($F_{8:9}$) of Multi-parent Advanced Generation Inter-cross (MAGIC) cowpea population were used, together with their 8 founder parents (Table 1). These parents had several varying important traits (Table 1), resulting in

TABLE 1. Founder parents of the Multi-parent Advanced Generation Inter-cross (MAGIC) population and their traits relevant to sub-Saharan Africa and other production areas

Name	Source	Agronomic trait	Resistance or tolerance trait
Suvita2	INERA	High-yielding under drought in Senegal, Burkina Faso, andMozambique; large dark-brown seed	Drought-tolerant, resistant to Striga, foliarthrips and Macrophomina disease
CB27	UCR	High yielding under drought in Mozambique; large black-eyeseed; photoperiod insensitive; erect growth habit; early maturing	Heat-tolerant, resistant to root-knot nematode,Fusarium wilt and foliar thrips
IT93K-503-1	IITA	High-yielding under drought in Senegal; brown-eyed seedstay-green under drought	Drought-tolerant, resistant to nematodes,Fusarium wilt and Macrophomina
IT89KD-288	IITA	High-yielding under drought in Burkina Faso and Nigeria; brown-eye seed; photoperiod sensitive	Resistant to root-knot nematode
IT84S-2049	IITA	High-yielding under drought in Burkina Faso; brown-eyed seed;erect growth habit	Resistant to aphid, bacterial blight, virusesand root-knot nematode
IT82E-18	IITA	High-yielding under drought in Mozambique; early maturing, light-brown seed coat colour; photoperiod insensitive	Broadly adapted, resistant to root-knotNematode
IT00K-1263	IITA	High-yielding under drought in Mozambique and Nigeria; dark-brown seed; stay-green under drought	Resistant to Striga, aphid, Fusarium wilt androot-knot nematode
IT84S-2246	IITA	High-yielding under drought in Burkina Faso and Mozambique;dark-brown seed	Resistant to aphid, bacterial blight, virusesand root-knot nematode

Source: (Huynh *et al.*, 2018). INERA, Institut de l'Environnement et des Recherches Agricole, Burkina Faso; UCR, University of California–Riverside, United States; IITA, International Institute of Tropical Agriculture, Nigeria

substantial variation for selection among their population. Two parents among the eight (Suvita2 and IT93K-503-1) possessed drought-tolerant traits. The development of the MAGIC population took place at the University of California, Riverside (Huynh *et al.*, 2018), following the scheme depicted by Cavanagh *et al.* (2008).

Experimental procedure. Treatments included 300 RILs ($F_{8:9}$) MAGIC cowpea population and their 8 founder parents; and drought-induced stress in the order of withdrawal of watering (10 days after planting), period of drought stress (21 days, thus 3 weeks), and resumption of watering (14 days, thus 2 weeks). The experiment was laid out in a randomised complete block design, with 2 replications. It was repeated 2 times. The experiment was conducted in a screen-house, using the "wooden box" screening technique (Mai-Kodomi *et al*., 1999) for drought screening. Twenty-six wooden boxes of dimensions 140 cm in length, 72 cm in width and 16 cm in depth, were used with a wood thickness of 2.5 cm.

To prevent leakages and uneven distribution of water, the boxes were lined with polythene sheets to the full depth. The boxes were filled with well-drained sandy loamy soil to a depth of 13 cm, making a 3 cm allowance for watering. The populations were planted in single rows, with an inter-row distance of 10 cm and intra row distance of 7.2 cm; each box contained 11 rows (populations) with 9 plants per row.

Two seeds each of the 300 MAGIC populations and 8 founder parents, as checks, were sown and thinned to one seedling a week after emergence. Seedlings were watered with 8.2 litres of borehole water per box, at 2-day intervals until all unifoliate leaves were fully open. Watering was terminated (drought imposition) on the emergence of the first trifoliate leaves; this was 10 days after planting. Drought imposition lasted for 21 days after its imposition. At the end of drought imposition, watering continued for two weeks with the same volume of water (8.2 L) to determine the recovery ability of the genotype. Weeds were controlled by hand pulling to keep the setup clean and reduce plant weed competition. Soil moisture content declined from 37.39 % at the imposition of drought to 2.45% at the end of the stress period. There was no minimum soil moisture condition set for the experiment. This environment was rather harsh, but under these conditions, there was no room for genotypes to adopt an escape mechanism. The minimum and maximum temperatures recorded during the experiment were 19.4 and 33.84 °C, respectively, with a relative humidity ranging from 22 to 28%.

Data collection. Soil moisture was determined using the gravimetric method. The Leaf Wilting Index (LWI) was calculated using Mai-Kodomi *et al*., (1999) scale as a ratio of the number of leaves showing wilting per plant (NWL) to the total number of leaves per plant (TNL) at 14 and 21 days of drought imposition. Delayed leaf senescence of unifoliate and trifoliate leaves was determined with a minimum score of 1 and a maximum score of 5, at 14 and 21 days of drought imposition period, where 1 was a normal green and turgid unifoliate and trifoliate leaves and 5 was completely senescence unifoliate and trifoliate leaves. Recovery parameters scored were stem greenness, regrowth and survival rates, after the 2 weeks of re-watering.

Stem greenness was scored using 1-5 scale, where 1 was a normal green and turgid stem, and 5 was completely dried leaves. Regrowth was scored based on a 1,3 and 5 scale; where 1 was no regrowth; 3 was regrowth from axillary buds, and 5 was regrowth from apical meristem. The survival rate of each genotype was also scored as the ratio of the total number of regenerated plants to the total number of plants per row, expressed as a percentage.

Statistical analysis. All data collected were analysed using the PROG GLM function in SAS software, version 9.4. Signifcant means

were separated using the Least Significant Difference (LSD) at P<0.05. Correlational analysis was done using the software XLSTAT, version 19.03.

RESULTS

Physiological drought parameters. Generally, the impact of drought imposition on the crop physiological response parameters (wilting, defoliation, and senescence) was non-evident until after 7 days, as most seedling leaves and stems remained green, with reduced growth rates (Table 2). However, 14 days of the drought imposition resulted in 70% defoliation and wilting of unifoliate and trifoliate leaves in most genotypes; this marked the beginning of the wilting stage of the trifoliate leaves. Twenty-one (21) days after drought imposition, most of the seedlings had unifoliate and trifoliate leaves, and stems were completely wilted, with only a few genotypes showing green patches on their trifoliate leaves and moderate green stems (Fig. 1).

The signs of physiological drought began with wilting, followed by senescence, dropping of the leaves and finally the entire seedlings fell off. The results of visual assessment rating for delayed senescence of unifoliate and trifoliate leaves, wilting of leaves and leaf wilting index (LWI) showed significant differences between the MAGIC cowpea populations (Table 3). There was a significant (P<0.05) treatment (the genotypes) observed for delayed leaf senescence and wilting for unifoliate and trifoliate leaves (Table 2).

All parameters measured for physiological indicators of drought (delayed leaf senescence for unifoliate and trifoliate leaves; and wilting of unifoliate and trifoliate leaves) reached their peak 21 days after the imposition of drought (Table 2). Delayed leaf senescence of unifoliate leaf recorded a maximum score of 5 and a minimum score of 1 for week 2 (14 days of drought imposition) (Fig. 2). A total of 194 genotypes recorded the maximum score, and six genotypes (MAGIC 100, 126, 50, 88, 98 and 99) recorded the minimum score. Delayed leaf senescence of trifoliate leaf score for week 2 ranged from 5 (12 genotypes) to 1 (27 genotypes), the 12 genotypes recorded that maximum score (MAGIC 115, 181, 19, 197, 198, 20, 220, 23, 37, 38, 73 and 75) (Fig. 3).

Wilting of unifoliate leaf at the end of the second week ranged from a score of 2 (234 genotypes) to 0 (69 genotypes); while wilting of trifoliate leaf ranged from a maximum score of 6 to a minimum of 0. MAGIC 173 genotype recorded the minimum wilting score of 0 and MAGIC 191 genotype recorded the maximum

TABLE 2. Analysis of variance for MAGIC cowpea lines evaluated for seedling stage drought tolerance in the screenhouse

Source	DF	Mean square				
		DLSCU	DLSCT	WTU	WTT	LWI
Blocks (REP)	25	0.294**	2.182**	0.142**	9.921**	10.239**
Treatments	307	0.872**	1.269**	0.212**	2.572**	3.106**
Week	2	2476.761**	1464.955**	623.830**	2507.662**	5212.619**
Treatment * Week	614	1.309**	0.976**	0.529**	2.117**	3.374**
Error	897	0.118	0.46	0.041	1.203	1.296
Total	1847					

* Significant at P < 0.05, ** significant at P < 0.01, *** significant at P < 0.0001, ns = non-significant. DLSCU = delayed leaf senescence of unifoliate leaves, DLSCT = delayed leaf senescence of trifoliate leaves, WTU = wilting of unifoliate leaves, WTT = wilting of trifoliate leaves, LWI = leaf wilting index

Figure 1. Physical observation of different stages of the seedling stage, drought screening and expression of drought tolerance and susceptibility under screen house conditions.

A. The emergence of trifoliate leaves. B. Cowpea seedlings 2 weeks after imposition of drought. C. Cowpea seedlings after 3 weeks' drought imposition. D. Regenerated cowpea seedlings 2 weeks after re-watering.

TABLE 3. Analysis of variance for cowpea lines evaluated for seedling stage drought tolerance in the screenhouse after three weeks of drought imposition and two weeks of re-watering

Source	DF	Mean square				
		STG	FSHW	DSHW	Regrowth	Survival rate
Blocks (REP)	25	16.162**	67.418**	15.902**	42.436**	8688.214**
Treatment	307	1.533**	6.369**	1.614**	4.066**	820.657**
Error	281	1.266	4.462	1.091	3.067	589.391
Total	615					

*Significant at $P < 0.05$, ** significant at $P < 0.01$, *** significant at $P < 0.0001$, ns = non-significant
STG = stem greenness, FSHW = fresh shoot weight and DSHW = dried shoot weight

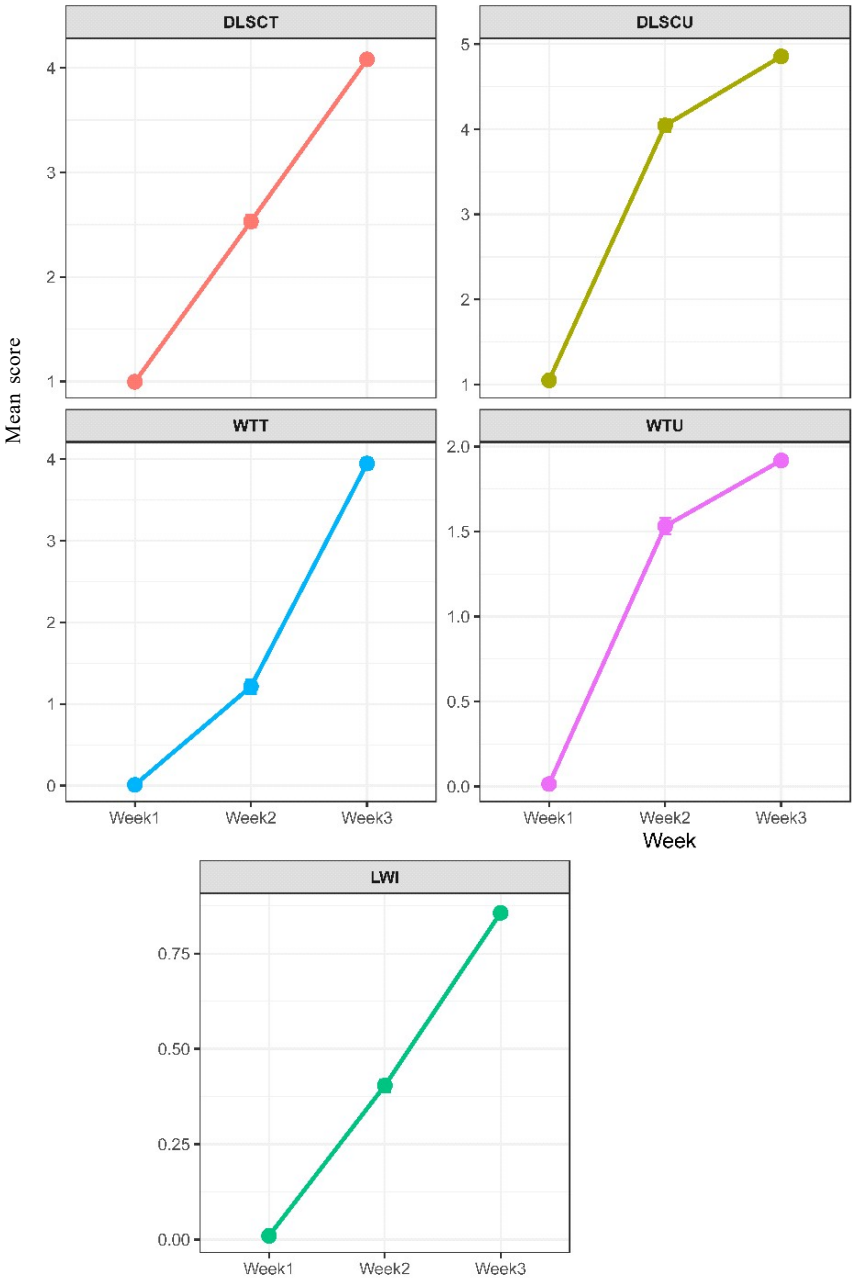

Figure 2. Trend of genotype response traits to seedling stage drought across three weeks. DLSCU =Delayed leaf senescence of unifoliate, DLSCT = Delayed leaf senescence of trifoliate, WTU = Wilting of unifoliate leaves, WTT = Wilting of trifoliate leaves, LWI = Leaf wilting index.

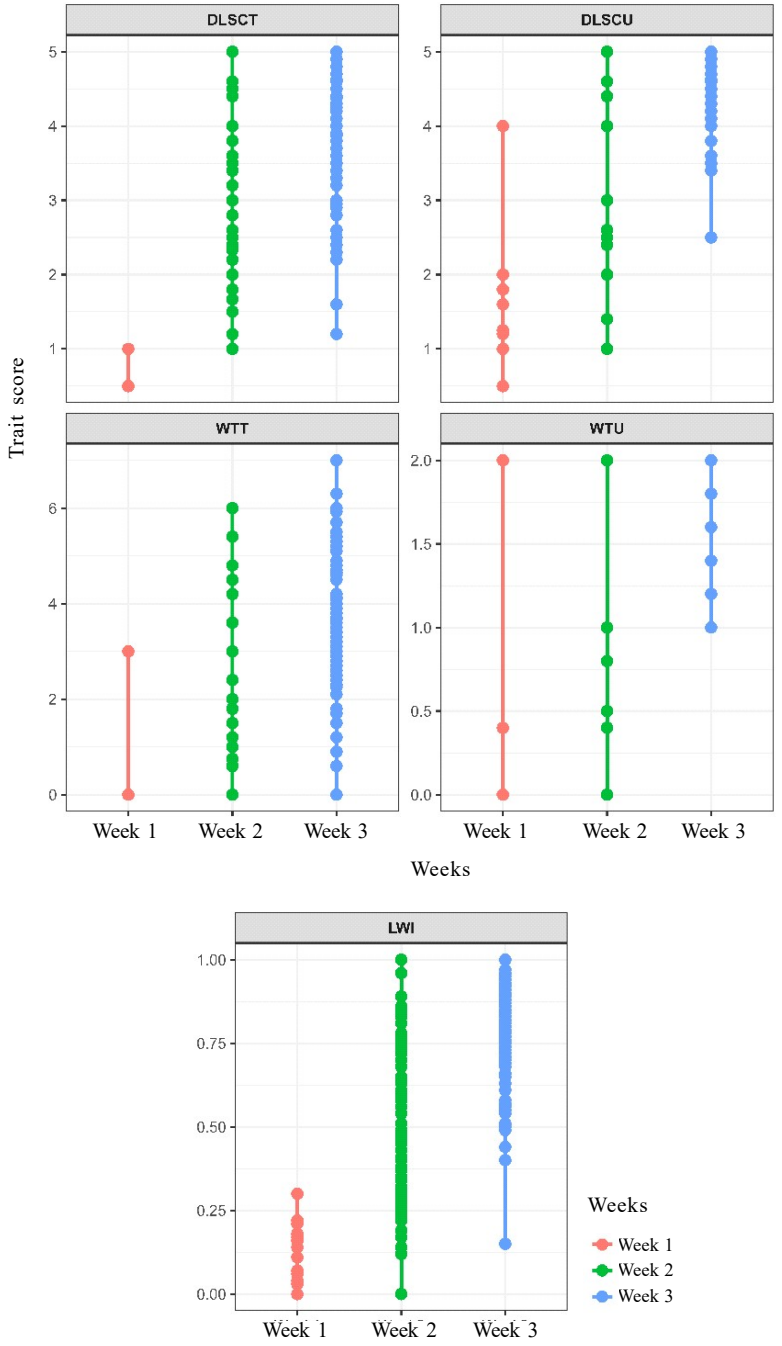

Figure 3. The response of MAGIC cowpea genotypes to seedling stage drought stress. DLSCU = Delayed leaf senescence of unifoliate, DLSCT = Delayed leaf senescence of trifoliate, WTU = Wilting of unifoliate leaves, WTT = Wilting of trifoliate leaves, LWI = Leaf wilting index.

wilting of trifoliate score of 6. Leaf wilting index at the end of the second week ranged from a minimum of 0 which was recorded by 66 genotypes to a maximum of 1 which was recorded by three genotypes (IT00K-1263, IT84S-2049).

At the end of the third week (21 days) of drought imposition, delayed senescence of unifoliate leaf ranged from a maximum score of 5, recorded by 232 genotypes to a minimum score of 2.50, recorded by two genotypes, MAGIC 132 and 221. Delayed senescence of trifoliate leaf ranged from a maximum of 5, recorded by 75 genotypes, to a minimum of 1.2, recorded by MAGIC 221.

Wilting of unifoliate leaf score ranged from 2, recorded by one genotype (MAGIC 259), to a minimum of 1 recorded by 13 genotypes (MAGIC 191, 226, 178, 180, 177, 162, 50, 163, 281 and 221). Wilting of trifoliate leaf ranged from a maximum of 7 recorded by one genotype (MAGIC 191) to a minimum of 0 recorded by one genotype (MAGIC 281). Leaf wilting index ranged from a maximum of 1, recorded by 100 genotypes, to a minimum of 0.15, recorded by the genotype MAGIC 281.

Regrowth parameters. Significant differences ($P < 0.01$) were observed among the treatments (genotypes) for fresh shoot weight, dry shoot weight, regrowth and survival rate, except for stem greenness (Table 3).

Stem greenness varied from completely green stem, with a score of 0 (MAGIC 230) to a fully dried or dead stem with a score of 5 (107 genotypes). Fresh shoot weight ranged from a score of 0 (100 genotypes) for dead genotypes to 7.8 g (1 genotype: MAGIC 264). Dry shoot weight also varied from 0.00 g (110 genotypes) to a maximum of 4.40 g (MAGIC 239). Survival rate ranges from 0% (107 genotypes) to 100% (2 genotypes, MAGIC205, MAGIC239). The regrowth score extends from 0 (107 genotypes) to 5 (26 genotypes) (Fig. 4).

Based on the survival rate, the worst genotypes presented a survival rate of 0% at the end of the experiment, with the promising drought-tolerant lines showing greater than 50% survival rate. Table 4 shows the best 20 drought-tolerant lines and the worst 10

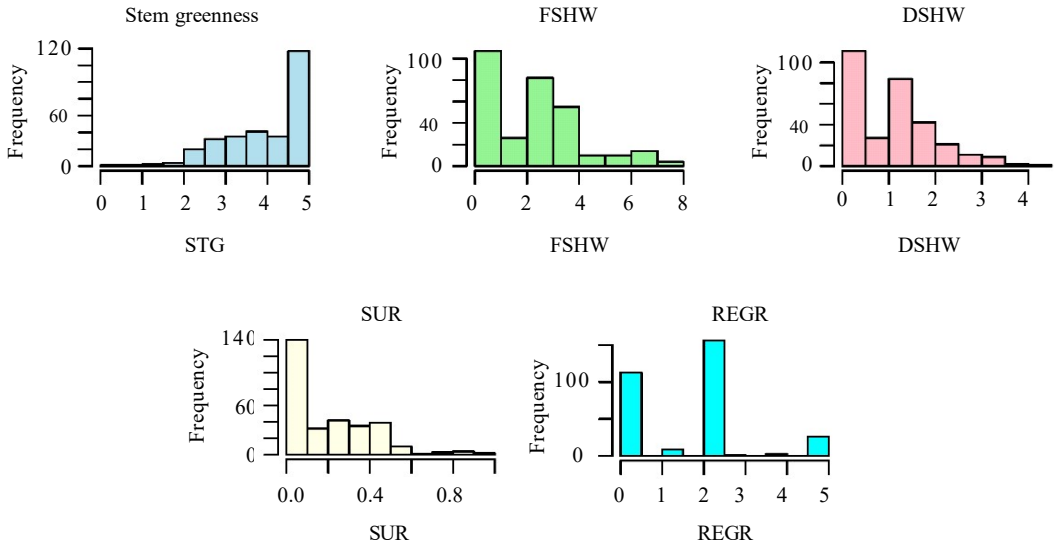

Figure 4. Distribution of the regrowth parameters measured after removal of drought stress. STG = Stem greenness, FSHW = Fresh shoot weight, DSHW = Dried shoot weight, SUR = Survival rate, REGR = Regeneration.

TABLE 4. Genotype response showing the best 20 and worse 10 genotypes tolerant to seedling stage drought stress

Treatments	Stem greenness	Fresh shoot weight	Dried shoot weight	Survival rate	Regrowth
Top 20					
MAGIC 205	1.4	6.3	2.8	100	5
MAGIC 239	2	7.2	3.5	100	5
MAGIC 81	2.2	5.85	2.6	90	5
MAGIC 100	0.8	7	3.3	90	5
MAGIC 236	1.8	6.4	3	90	5
MAGIC 48	2.1	6.5	3.6	85.71	5
MAGIC 235	2.9	5.7	2.3	80	5
MAGIC 79	2.8	4.8	2.2	77.5	5
MAGIC 199	2	6.8	3.2	75	5
MAGIC 98	3.6	5.9	2.4	70	5
MAGIC 244	2.3	6.2	2.85	60	5
MAGIC 264	2.3	7.8	4.4	60	5
MAGIC 161	2.7	6.8	3.1	58.33	5
MAGIC 230	1.3	7.6	3.4	58.33	5
MAGIC 108	3.25	6.1	2.5	57.5	5
MAGIC 88	1.5	6.05	2.75	55	5
SUVITA 2(PC)	3.4	6.8	3.2	55	5
MAGIC 87	2.6	6.05	2.75	51.43	5
MAGIC 281	3.7	5.9	2.7	51.43	4
MAGIC 11	3.1	3.1	1.5	51.25	5
Bottom 10					
MAGIC 91	5	0	0	0	0
MAGIC 92	5	0	0	0	0
MAGIC 93	5	0	0	0	0
MAGIC 133	5	0	0	0	0
MAGIC 134	5	0	0	0	0
MAGIC 96	5	0	0	0	0
MAGIC 97	5	0	0	0	0
MAGIC 137	5	0	0	0	0
MAGIC 99	5	0	0	0	0
MAGIC 139	5	0	0	0	0
Mean	4.08	2.2	1.05	22.49	1.79
t0.05	1.97	1.97	1.97	1.97	1.97
LSD	2.21	4.16	2.06	47.79	3.44

PC = Parent control, LSD = Least significant difference

drought-tolerant lines, along with their responses to the other regrowth parameters.

Grouping the 308 MAGIC population on the basis of the ability of the genotypes to rejuvenate and survive resulted in four drought groupings; (i) genotypes with survival rate > 50% (57 genotypes) were considered as highly tolerant to drought, (ii) genotypes with survival rate from 49% to 20% (74 genotypes) were tolerant to drought, (iii) genotypes with survival rate ranging from 19% to 10% (37 genotypes) were susceptible to drought, and (iv) genotypes with survival rate <10% (140 genotypes) were highly susceptible (Fig. 5).

Correlation among parameters. Correlation data for the eight drought traits considered in this study are presented in Table 5. Leaf wilting

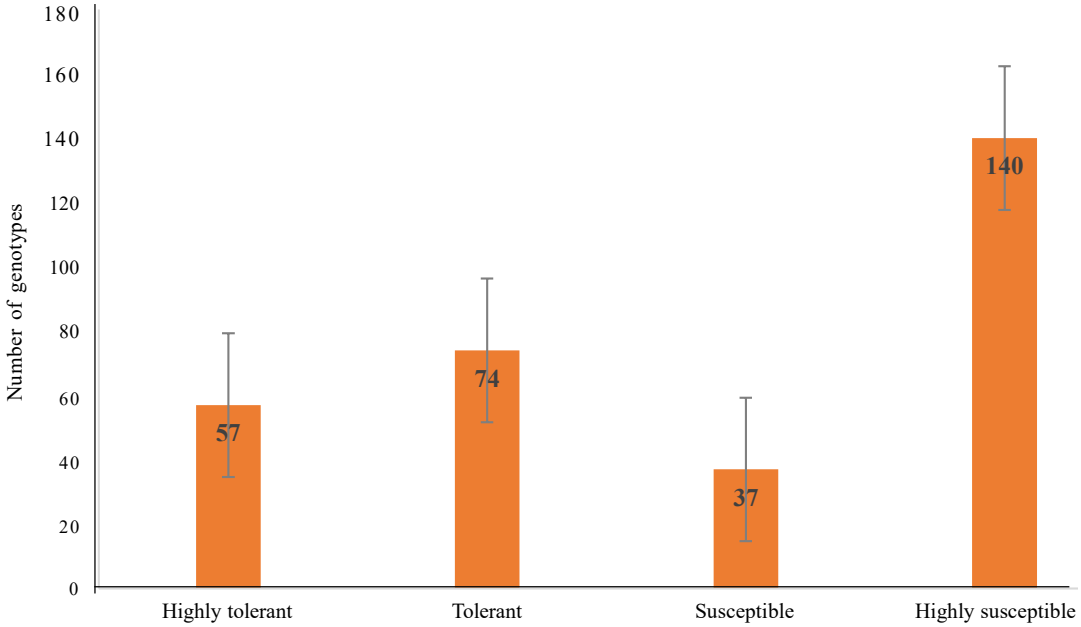

Figure 5. Grouping of cowpea genotypes into levels of seedling stage drought tolerance based on the survival rate.

TABLE 5. Correlations among drought-indicative parameters for cowpea seedling stage screening

	DLSCU	DLSCT	LWI	STG	FSHW	DSHW	RGSC	SUR
DLSCU								
DLSCT	0.438**							
LWI	0.485**	0.831**						
STG	0.446**	0.670**	0.636**					
FSHW	-0.284**	-0.628**	-0.601**	-0.780**				
DSHW	-0.275**	-0.614**	-0.584**	-0.777**	0.986**			
RGSC	-0.266**	-0.629**	-0.589**	-0.790**	0.917**	0.898**		-
SUR	-0.305**	-0.670**	-0.643**	-0.900**	0.844**	0.831**	0.871**	

* Correlation significant at 0.05 and ** significant at 0.01 levels. DLSCU = Delayed leaf senescence of unifoliate, DLSCT = Delayed leaf senescence of trifoliate, LWI = Leaf wilting index, STG = Stem greenness, FSHW = Fresh shoot weight, DSHW = Dried shoot weight, RGSC = Regrowth score and SUR = Survival rate

index (LWI) showed a negative correlation with regrowth score and survival rate, but was positively correlated (r = 0.636) with stem greenness. Significant positive correlation (P<0.01) was also observed between regrowth score and survival rate (r = 0.871).

DISCUSSION

Physiological drought parameters. At the early stages of drought imposition, the plants maintained relatively slight wilting (1%) at periods of high evaporative demand. This response could be attributed to the ability of cowpea to initially maintain tissue water status through drought-avoidance mechanisms such as stomatal closure, osmotic adjustment and reduced transpiration; thereby delaying the onset of severe wilting symptoms (Muchero *et al*., 2008; Nunes *et al*., 2022). Hence, the observed lack of severe drought stress in week one was due to the plants adjusting to the drought imposed to support the plant during the initial stages of drought imposition. However, as drought imposition extended to the second and third week, soil moisture drastically reduced, evaporative demands increased, and genotypes differentiation for drought was more pronounced.

The present study thus confirmed that, although there was inherent comparative tolerance to drought in the population, the cowpea genotypes demonstrated significant variations in response to drought stress. These responses were manifested in several discrete physiological and morphological characteristics that were clearly related to the ability of cowpea seedlings to tolerate and recover from seedling drought stress (Fig. 3). It was also observed that, within genotypes, individual cowpea seedlings expressed different levels of survival and ultimate recovery in response to drought stress. This observation is similar to the result reported by Fatokun *et al*. (2012), that genotypic differences play key roles in the variation in response of cowpea to drought stress.

MAGIC 236, 48, 98 and parental check, Suvita2 wilted gradually, but maintained green stems until the seedlings were re-watered (Fig. 1). These genotypes appear to have adopted a mechanism to reduce their transpiration rate to avoid the depletion of moisture conserved; therefore, recording a lower wilting index when compared to other surviving genotypes. This was in line with findings reported by Muchero *et al*. (2008) and Pungulani *et al.* (2012); that cowpea genotypes adapted mechanisms such as stem greenness, delayed senescence, and reduced wilting to reduce the effect of drought stress. The wilting of leaves and, hence, the decrease in growth of seedlings observed was attributed to the loss of water *via* transpiration, thus making leave wilting index an indicator of drought tolerance in cowpea.

The slow-wilting observed in this experiment was not a result of deep-water extraction by roots of the cowpea genotypes because the seedlings were planted in shallow boxes of soil, meaning that this trait is associated with water saving mechanism in the shoot (Kunert and Vorster, 2020; Franco-Navarro *et al*., 2025). In leguminous grains like cowpea, soybean (*Glycine max*), peanut (*Arachis hypogaea*) and chickpea (*Cicer arietinum*), traits including lower transpiration rate under vapour pressure deficits (VPD) have been identified as a soil water-saving strategy in legume lines tolerant to drought (Sadok *et al.*, 2012; Sinclair *et al*., 2015). However, MAGIC 91, 92, 133, 137 and 139 cowpea lines wilted faster, which resulted in low survival rates (Table 5). This finding conforms to the observations made elsewhere in soybeans (Ries *et al*., 2012) and common beans (*Phaseolus vulgaris*) (Mukeshimana *et al.*, 2014) who also observed that higher wilting is associated with lower survival.

Regrowth parameters. The ultimate measure of drought tolerance is the ability of the plant to survive and regrow after removal of drought stress. In the present study, the regrowth

parameters, including stem greenness, fresh shoot weight, regrowth score, survival rate and dried shoot weight, were significant across gynotypes and provided a strong basis for classifying genotypes based on the drought response. All parameters were observed to have significant differences among the genotypes, except for stem greenness, which showed that there was enough stress response variability for stress tolerance selection. A plant's ability to tolerate drought stress conditions at any given growth stage is determined not only by its adaptability to survive under stress, but also by its unique potential to resume growth (Yang *et al.*, 2021). Hence, in the present study, the survival and regrowth score was the major determinant of drought tolerance.

This study has revealed a drastic reduction in seedling survival rate from the onset to the end of drought stress (Fig. 1), which represents an essential distinguishing parameter among drought-tolerant and susceptible cultivars (Nkomo *et al.*, 2021). Several genotypes recorded higher survival rates than their parental checks (Table 5), implying the presence of transgressive segregants (Huang *et al*., 2016; Mvula *et al*., 2018). Though intermittent drought during the growing season usually leads to a decrease in total plant yield, a major factor might be attributed to a fall in the number of plant stands due to seedling death (Engelbrecht *et al.*, 2007).

This phenomenon makes it more important for researchers who target yield improvement to have an interest in selecting for a high survival rate, especially at the vegetative stage. Hence, genotypes with a high survival rate displayed a quick morphological recovery when they were re-watered (Table 5). Various characteristics have been used to assess seedling stage drought tolerance, including the study of Sadok *et al*. (2012), who reported that slow wilting in soybeans was associated with drought tolerance.

Parameter correlations. Stem greenness showed a highly significant positive correlation to survival. Cowpea lines, MAGIC 236, 48, 100, 205, 230 and 88 exhibited high stem greenness scores during the period of drought stress with relatively high survival rates. This corroborates observation was also made in sorghum (*Sorghum bicolour* (L.) Moench), where maintenance of stem greenness highly correlated with chlorophyll content of the leaf and drought tolerance (Xu *et al*., 2000).

Survival rate correlated negatively with leaf senescence, leaf wilting index, and stay greenness, but positively with fresh shoot weight, dry shoot weight and regeneration score (Table 5). This is consistent with the physiological response process in plants, as drought stress increases, leaf senescence, leaf wilting and stem greenness decrease. Genotypes with a high survival rate will exhibit lower senescence and wilting, along with high shoot weight and a high regeneration score. The regeneration scores also showed a similar trend to the survival rate of other studies in cowpea.

CONCLUSION

Fifty-seven MAGIC cowpea genotypes out of a total of 300 were identified as highly tolerant to the seedling stage drought for the Sudan Savanna Zone of Ghana. These genotypes will serve as resources for Ghana's cowpea improvement programmes. The wooden box technique of drought screening proved effective in this study, following the gradual reduction of soil moisture during the period of drought stress and the effective differentiation of drought tolerance lines. Survival rate from the study stands out as the most informative measure of drought using the wooden box technique. Further studies could leverage the diverse set of the MAGIC population for terminal drought tolerance for the identification of lines that combine seedling and terminal stage tolerance.

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