

Phenotyping cowpea (*Vigna unguiculata* L. Walp) for seedling drought stress tolerance using leaf wilting index, delayed senescence and tolerance indices

Utlwang Batlang

ubatl@buan.ac.bw

National Agricultural Research Development Institute, Botswana University of Agriculture and Natural Resources

Chipo Nkomazana

National Agricultural Research Development Institute, Botswana University of Agriculture and Natural Resources

Goitseone Malambane

National Agricultural Research Development Institute, Botswana University of Agriculture and Natural Resources

Research Article

Keywords: Chlorophyll content, drought tolerance indices, principal component analysis, stem greenness, recovery

Posted Date: September 17th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7518906/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Title: Phenotyping cowpea (*Vigna unguiculata* L. Walp) for seedling drought stress tolerance using leaf wilting index, delayed senescence and tolerance indices

Authors:

Chipo Nkomazana ORCID: <https://orcid.org/0000-0001-5966-4512>

Department of Field Crops and Horticulture, National Agricultural Research Development Institute, Private Bag 00177, Gaborone, Botswana

Goitseone Malambane ORCID: <https://orcid.org/0000-0002-3022-6938>

Department of Crop and Soil Sciences, Botswana University of Agriculture and Natural Resources, Private 0027, Gaborone, Botswana

Utlwang Batlang, ORCID: <https://orcid.org/0000-0002-2381-7529>

Department of Crop and Soil Sciences, Botswana University of Agriculture and Natural Resources, Private 0027, Gaborone, Botswana

ABSTRACT

Botswana's semi-arid climate and variable rainfall patterns make early-season drought a threat to cowpea production. Selecting cowpea for drought tolerance at seedling stage is critical for maintaining survival and maximising yield potential in water-limited areas. This study evaluated 30 cowpea genotypes for drought tolerance at the seedling stage using a wooden box technique. Drought stressed cowpea showed a gradual increase in leaf wilting and a decrease in relative water content and chlorophyll content. Genotypes such as Mahutohuto and Wild cowpea demonstrated resilience to stress, showing regrowth and recovery, while ER7, B500 and B138 did not. Calculations for Stress Susceptibility Index (SSI), Stress Tolerance Index (STI), Tolerance (TOL), Mean Productivity (MP), Biomass Drought Resistance (BDR), Yield Reduction (YR) and Relative Drought Index (RDI) were based on yields under drought (Ys) and irrigated (Yp) conditions. These tolerance indices exhibited genotypic variation and correlated with yield under both water conditions. Principal component analysis indicated significant relationships between yield and both STI and BDR, yielding coefficients of determination of 0.93 and 0.97 as well as 0.69, and 0.44 under drought-stressed and non-stressed conditions, respectively. This suggests that STI and BDR are reliable indicators for selecting drought-tolerant cowpea. Mahutohuto, a landrace from Kalahari sandveld, was recognized as highly tolerant, along with other tolerant types, which include a mutant and a wild cowpea. These genotypes are valuable resources for drought tolerance breeding. Further investigations involving transcriptome profiling in Mahutohuto may reveal the genetic factors contributing to drought tolerance by identifying transcriptional regulators of the relevant genes.

Keywords: Chlorophyll content, drought tolerance indices, principal component analysis, stem greenness, recovery

Acknowledgements

The authors would like to express their gratitude to the Botswana University of Agriculture and Natural Resources (BUAN) and the National Agricultural Research and Development Institute (NARDI) for providing free seed materials and research facilities. Additionally, we extend our thanks to the International Atomic Energy Agency (IAEA) in Vienna, Austria, for providing radiation facilities.

INTRODUCTION

Water scarcity is a fundamental constraint to global agriculture and becoming an increasing problem due to climate change. According to IPCC (2022), climate change is evident through the increasing frequencies and greater intensities of erratic extreme weather events such as dry spells with high temperature, and low precipitation resulting in drought spells. Climate change is therefore aggravating food insecurity and malnutrition, making attaining zero hunger by 2030 almost impossible (FAO 2022).

The Food and Agriculture Organisation (FAO) of the United Nations recognizes pulses as critical crops in the global effort to achieve food security. Cowpea (*Vigna unguiculata* L. walp) is particularly suitable for sustainable development in the three dimensions; social, economic and environmental (UN DESA 2023). It is cultivated on 14.5 million hectares worldwide, with about 84% of this area in Africa and mainly used for food, animal feed and seed (Boukar et al. 2018). Cowpea provides essential protein, minerals and vitamins, making it a cost-effective way to balance the diet and improve overall health in many African countries (Poudel et al. 2025). When compared to many field crops, cowpea performs relatively well in drought stressed conditions. However, drought may still have a detrimental effect on yield especially if occurs at seedling and flowering stages, making it necessary to breed crops for drought tolerance (Ajayi et al. 2020; Boukar et al. 2020). According to Manneh et al. (2024), there is a need in Africa to screen and breed for tolerant and water use efficient cultivars, as cowpea is largely farmed under rainfed conditions, with regular exposure to periodic droughts.

Many researchers have worked on various drought tolerance selection methods using tolerance and/or susceptibility indices (Farshadfar et al. 2013). Stress tolerance index (STI) is a useful marker for determining the potential of tolerance and yield of plants under stress (Fernandez 1992). Other indices useful for identifying tolerant genotypes to water deficit have been proposed, such as stress susceptibility index (SSI), stress tolerance (TOL) and mean productivity (MP), relative drought index (RDI), biomass drought resistance (BDR) and yield reduction percent (YR) (Fernandez 1992; Fischer and Maurer 1978; Rosielle and Hamblin 1981;).

Drought tolerant genotypes have also been identified using plant traits other than tolerance indices, such as root characteristics (Matsui and Singh 2003), leaf wilting scales (Mai-Kodomi et al. 1999), stem greenness, chlorophyll content and drought recovery (Belko et al. 2012). The leaf wilting index is a reliable and quantitative measure for determining wilting intensity and drought-tolerant genotypes (Nkomo et al. 2020). Stem greenness is also an important indicator of drought tolerance as genotypes that maintain greener stems under stress tend to exhibit higher relative water content and proline content (Nkoana et al. 2019). Several studies have applied these scales and tolerance indices on cowpea (Ajayi 2022; Batlang et al. 2018; Basolle et al. 2024; Sanogo et al. 2023) and related crops such as mungbean and common bean to identify drought tolerant genotypes (Sanchez-Reinoso et al. 2020; Tekle et al. 2020).

Botswana has cowpea germplasm collections of more than 1500 maintained by National Plant Genetic Resources Centre (NPGRC) under the Ministry of Land and Agriculture (Molosiwa and Makwala 2020). The accessions include wild and local landraces, as well as improved varieties from the Institute of Tropical Agriculture (IITA). There is a significant pool of cowpea genetic resources which has been explored mostly in phenotypic characterisation and yield stability (Molosiwa et al. 2020). While some research has been conducted on cowpea's drought tolerance (Batlang et al. 2018; Nkomazana and Batlang 2018), it remains insufficient, posing challenges

for the success of drought tolerance breeding programs. The mechanism of drought tolerance occurring at leaf level during the seedling stage is an important criterion in determining drought tolerance type in cowpea (Ravelombola et al. 2018). Mai-Kodomi et al (1999) described 2 types of drought tolerance in cowpea; Type 1 involves growth cessation and uniform tissue desiccation, while Type 2 involves continued slow growth with potential leaf senescence and shedding. These strategies reflect cowpea's diverse adaptation to drought stress and very important in cowpea breeding programs. This study was undertaken to evaluate cowpea genotypes for their resistance to seedling-stage drought stress using drought tolerance indices. The research findings will provide information on developing drought-tolerant varieties to combat the effects of climate change.

Materials and Methods

Plant material

A total of 30 cowpea accessions from three institutions; the National Agricultural Research and Development Institute (NARDI), Botswana University of Agriculture and Natural Resources (BUAN), and the Ministry of Lands and Agriculture-National Plant Genetic Resources Centre were used in the study (Table 1). Among these accessions, 5 are released varieties, four are cultivars, one is a wild cowpea, and the remaining 19 are landraces collected from small-holder farmers across the country. Among the 4 cultivars is an M5 Tswana mutant cowpea (Mutant 33) from NARDI, which was developed through gamma irradiation at the International Atomic Energy Agency (IAEA).

Table 1 List of 30 Cowpea genotypes evaluated for drought stress tolerance

Code	Genotype	Place of Collection	Growth Habit	Accession type (released/landrace/wild/cultivar)
CW1	ER7	NPGRC	Erect	Released
CW2	B500	NPGRC	Semi prostrate	Landrace
CW3	B138	NPGRC	Semi prostrate	Landrace
CW4	B111	NPGRC	Semi prostrate	Landrace
CW5	B212	NPGRC	Semi prostrate	Landrace
CW6	B281	NPGRC	Semi prostrate	Landrace
CW7	B272-B	NPGRC	Semi prostrate	Landrace
CW8	B154-D	NPGRC	Prostrate	Landrace
CW9	BO75	NPGRC	Semi prostrate	Landrace
CW10	Speckled grey-Hukuntsi	BUAN	Prostrate	Landrace
CW11	B269	NPGRC	Prostrate	Landrace
CW12	Wild cowpea	Lecheng	Prostrate	Wild
CW13	Speckled grey-Lecheng	BUAN	Erect	Landrace
CW14	B478	NPGRC	Semi prostrate	Landrace
CW15	INIA 37	NPGRC	Semi prostrate	Released
CW16	B378	NPGRC	Semi prostrate	Landrace
CW17	INIA 71	NPGRC	Prostrate	Released
CW18	EO60	NPGRC	Prostrate	Landrace
CW19	B154	NPGRC	Prostrate	Landrace
CW20	B342	NPGRC	Semi prostrate	Landrace
CW21	B261-B	NPGRC	Semi prostrate	Landrace
CW22	Mahutohuto	BUAN	Prostrate	Landrace
CW23	Blackeye	NPGRC	Semi prostrate	Released
CW24	E129	BUAN	Prostrate	Landrace
CW25	B163	NPGRC	Semi prostrate	Landrace
CW26	BWC635	NPGRC	Semi prostrate	Released
CW27	Tswana cream	NPGRC	Prostrate	Released
CW28	Mutant 33	NARDI	Prostrate	Cultivar
CW29	B359	NPGRC	Semi prostrate	Landrace
CW30	INIA 30	NPGRC	Semi prostrate	Released

NPGRC- National Plant Genetic Resources Centre; *BUAN*- Botswana University of Agriculture and Natural Resources; *NARDI*-National Agricultural Research and Development Institute

Experimental Design

Cowpea genotypes were screened in a screen house in summer between February 2024 and April 2024 at the National Agricultural Research and Development Institute (NARDI). The institute is located Sebele, Gaborone, Botswana (24° 33' S, 25° 54' E) at an elevation of 994 meters above sea level. The average duration of daylight during the experiment from February to April was 12 hours. Six wooden boxes designed according to Singh et al. (1999) were used (Figure 1). The wooden boxes were 117cm long, 100cm wide, and 12cm high, with a thickness of 2.5cm. The bottom sides of the boxes were lined with polyethylene to ensure even distribution of water. Topsoil collected from NARDI experimental field was mixed with sandy soil at a ratio of 3:1 and each wooden box filled with soil and irrigated to field capacity. A night after watering, 2 seeds per genotype were sown in holes of 2cm depth with a spacing of 10cm between plants and 8cm between rows and thinned to 1 plant per stand after germination. Each box contained 1 row each of the 30 cowpea genotypes with 10 plants constituting a replication. The experimental design was a split plot replicated 3 times with 2 watering regimes.

Drought Treatment

After germination plants were watered to field capacity 3 times a week for seedling establishment. After 21 days of planting, the first trifoliate leaves were completely formed, and watering was stopped for half of the experimental plants for 28 days to induce moisture stress. On the final day of the stress treatment, 3 plants from each row were collected from both watering treatments to evaluate biomass yield and determine the indices of drought tolerance. Following 28 days of moisture stress, the plants were rewatered to field capacity for 14 days to observe which genotypes exhibited high tolerance and the capacity to recover from drought conditions. Soil moisture levels were assessed weekly at a depth of 20cm during the water stress period using a digital soil moisture probe meter (MPM-160-B, ICT International, Armidale, Australia), calibrated following the manufacturer's guidelines.

Leaf wilting and delayed senescence

Leaf wilting index was calculated from the first week of stress to the final week using the Mai-Kodomi (MAIK) scale by Mai-Kodomi et al. (1999) as the ratio between leaves showing wilting signs and the total number of leaves per plant. The Mai-Kodomi scale uses a score of 0-4 where 0 denotes no signs of wilting; 1- denotes 25% of leaves wilting; 2- 50% wilting; 3- yellow and brown leaves with 75% wilting and 4 - leaves completely wilted. A SPAD-502 Plus (Spectrum Technologies, Inc., Plainfield, IL) was used to take chlorophyll measurements from the plants on weekly basis during the period of stress. Stem greenness and regrowth were scored per plant as recovery parameters after 2 weeks of rewatering. Stem greenness was scored using a scale of 0 to 4; 0= leaves and stems completely yellow; 1 =75% of leaves yellow, stem not turgid; 2=50% of leaves yellow, stems not turgid; 3 = 25% leaves yellow, stems less turgid; 4= leaves completely green, stem turgid

The recovery rate (RR) was assessed by counting the number of plants that recovered from stress expressed as a % according to Muchero et al., (2008):

$$RR = \frac{\text{Number of dead plants}}{\text{Number of emerged plants}} * 100$$

Calculations of indices

Seven indices were calculated using the formula in Table 2.

Table 2 Description of drought tolerance indices used in the study and their definitions

No	Index code	Index Name	Formula	Description	References
1	BMP	Biomass Mean Productivity	$BMP = \frac{(Y_{ns} - Y_s)}{2}$	Genotypes with higher BMP will be more desirable	Rosielle and Hamblin (1981)
2	TOL	Tolerance	$TOL = Y_p - Y_s$	Genotypes with low values of TOL are more stable in two different conditions	
3	SSI	Stress Susceptibility Index	$SSI = \frac{(1 - (\frac{Y_s}{Y_{ns}}))}{1 - \frac{Y_s}{Y_{ns}}}$	The greater the index, the greater the susceptibility of the genotype to stress	Fischer and Maurer (1978)
4	RDI	Relative Drought Index	$RDI = \frac{Y_s - Y_{ns}}{\bar{Y}_s / \bar{Y}_{ns}}$	The genotype is relatively drought tolerant if $RDI > 1$ and susceptible if $RDI < 1$	Naghari et al, (2013)
5	STI	Stress Tolerance Index	$STI = \frac{Y_s * Y_{ns}}{\bar{Y}_s^2}$	Genotypes with high STI are more tolerant to drought stress	Naghari et al, 2013
6	BDR	Biomass Drought Resistance	$BDR = (Y_s * (\frac{Y_s}{Y_{ns}})) \bar{Y}_s$	Genotypes with high BDR are more tolerant to drought stress	Fernandez (1992)
7	YR	Yield Reduction	$YR = \frac{Y_{ns} - Y_s}{Y_{ns}} * 100$	Genotypes with low YR are tolerant	Farshadfar et al, (2012)

Data Analysis

The general linear model (GLM) procedure in the SAS 9.4 statistical package (SAS Inc., USA) was used to analyse data. Means of variables that showed significant differences were separated using Least Significant Difference (LSD) with a significance level of 5% ($p < 0.05$). Correlation analysis was conducted between leaf wilting, stem greenness, and recovery rate, as well as between various indices and biomass yield under both well-watered and drought-stressed conditions. A cluster analysis employing the Unweighted Pair Group Method with Arithmetic means (UPGMA) was performed on all calculated indices to create a dendrogram of the genotypes with SAS 9.4 software. The Euclidean method was applied to determine the similarity among data points. Principal Component Analysis (PCA) was carried out using R Studio software version 4.5.0.

Results

Climate conditions in the screenhouse and soil moisture content

The screenhouse recorded daytime temperatures ranging between 19°C and 36.7°C while the relative humidity ranged between 39% and 73% (Fig. 2a). The average soil moisture level decreased from 52% to 36% after 7 days of not watering (Fig. 2b). By the 14th day, the average moisture content in the soil had dropped to 20%, and nearly all plants showed signs of wilting at that point. After 28 days, most plants had reached the permanent wilting point as the soil moisture level dropped further to 2%, leading a decision of rewatering.

Leaf wilting, chlorophyll content and recovery

Most of the cowpea accessions showed significant differences ($P < 0.0001$) in their response to drought stress, particularly in leaf wilting, chlorophyll content, stem greenness and recovery rate (Table 3 and 4). After one week of stress, there were signs of wilting, change of leaf colour and loss of stem turgidity with ER7 and B500 having the most wilted leaves, with a LWI of 1 "indicating 25% wilting," while the other accessions showed no signs. LWI levels increased from the second to the fourth week. At week four B138, B500, and ER7 leaves were completely wilted with an index of 4, followed by B272-B, B154-D, B281 and Speckled grey-Hukuntsi, which showed 75% leaf wilting. The remaining genotypes scored 2 (50% leaf wilting), while Mahutohuto leaves showed 25% wilting with a score of 1. Chlorophyll content in cowpea seedlings decreased with an average of 42.7% as drought intensity increased (Table 4). A total of eight genotypes experienced a chlorophyll content decrease of over 50%, including B500, B163, and B154-D. Five genotypes, namely Inia 37, Wild cowpea, Mahutohuto, Tswana cream, and Speckled grey-Hukuntsi, showed a reduction of 30% or less. The remaining 17 genotypes had a decrease in chlorophyll content that ranged between 30% and 40%. After exposure to 4 weeks of drought stress and 14 days of rewatering, some genotypes exhibited signs of recovery and regrowth. Mahutohuto retained green and turgid stems, while ER7, B500, and B163 showed stem turgidity loss and 75% of the leaves. At 14 days post rewatering, Mahutohuto fully recovered to 100%, followed by Tswana cream, Wild cowpea and Speckled grey-Hukuntsi (85.2, 84, and 83.3%, respectively). Meanwhile ER7, B138, and B163 exhibited the lowest recovery rates, with 26.1, 26.3, and 24.3%, respectively (Table 4). A significant ($P < 0.01$) and positive relationship between stem greenness and chlorophyll content ($r^2 = 0.92$) and between stem greenness and recovery rate ($r^2 = 0.89$) was recorded (Fig. 3) while LWI had a negative relationship with chlorophyll content ($r^2 = 0.24$).

Table 3 Variability among 30 cowpea genotypes for leaf wilting, stem greenness and recovery in response to drought

Accession	LW1	LW2	LW3	LW4	SG	Recovery rate (%)
ER7	1	2	3	4	1	26.1
B500	1	2	3	4	1	65.1
B138	0	1	3	4	1	26.3
B111	0	1	1	2	1	43.9
B075	0	1	2	2	2	65.5
B154	0	2	3	2	3	80.6
B478	0	1	2	2	3	55.6
B163	0	1	3	2	2	24.3
B342	0	1	2	2	2	62.2
B212	0	2	3	3	2	31.6
E129	0	1	1	2	3	61.5
B272-B	0	2	3	3	2	69.0
B154-D	0	2	3	3	3	53.9
Tswana Cream	0	1	2	2	2	85.2
B269-1	0	2	1	2	1	52.2
B359	0	1	2	2	2	43.5
Speckled grey-Lecheng	0	1	2	2	2	69.5
Blackeye	0	1	2	2	3	77.8
Inia 30	0	1	2	2	2	54.6
B281	0	1	2	3	3	24.6
Inia 71	0	1	2	2	2	52.2
E060	0	1	2	2	3	58.3
Speckled grey-Hukuntsi	0	1	2	3	3	83.3
B378	0	1	2	2	2	36.1
Wild -Lecheng	0	1	1	2	4	84.0
Inia 37	0	1	2	2	3	47.1
BWC635	0	1	1	2	3	82.6
Mutant 33	0	1	1	2	3	58.3
B261-B	0	1	1	2	2	73.9
Mahutohuto	0	0	1	1	4	100
LSD	0	0.69	0.84	0.94	1.04	0.97
P<0.05	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Stress greenness scores: 0 -leaves and stems completely yellow; 1 -75% of leaves yellow, stem not turgid; 2- 50% of leaves yellow, stems not turgid; 3 – 25% leaves yellow, stems less turgid; 4- leaves completely green, stem turgid. Leaf wilting scores: 0- no signs of wilting; 1- 25% of the leaves wilting; 2- moderately (50%) wilting; 3- yellow and brown leaves with 75% wilting; 4- leaves completely wilted

Table 4 Variability in chlorophyll content (SPAD) among 30 cowpea genotypes in response to four weeks of drought stress.

Accession	Wk1	Wk2	Wk3	wk4	% Reduction
ER7	36.9 ^b	32.6 ^b	28.01 ^b	22.4 ^b	39.3
B500	54.7 ^a	43.8 ^a	29.4 ^b	22.3 ^b	59.2
B138	44.6 ^{ab}	37.3 ^{ab}	28.9 ^b	21.5 ^b	51.8
B111	59.8 ^a	48.5 ^a	38.9 ^{ab}	36.1 ^a	39.6
B075	48.1 ^{ab}	43.5 ^a	36.8 ^{ab}	30.1 ^{ab}	37.4
B154	44.5 ^{ab}	43.1 ^a	30.7 ^b	27.8 ^{ab}	37.5
B478	55.6 ^a	45.7 ^a	30.7 ^b	25.8 ^{ab}	53.6
B163	46.3 ^{ab}	36.4 ^{ab}	30.1 ^b	19.5 ^b	57.9
B342	42.8 ^{ab}	44.3 ^a	33.8 ^{ab}	24.6 ^{ab}	42.5
B212	50.3 ^a	44.8 ^a	37.4 ^{ab}	26.6 ^{ab}	47.1
E129	50.3 ^a	40.7 ^a	32.2 ^{ab}	28.0 ^{ab}	44.3
B272-B	52.9 ^a	46.3 ^a	38.6 ^a	29.8 ^{ab}	43.7
B154-D	52.5 ^a	41.3 ^a	34.2 ^{ab}	22.8 ^b	56.6
Tswana cream	47.3 ^{ab}	41.5 ^a	36.5 ^{ab}	34.4 ^a	27.3
B269-1	46.2 ^{ab}	42.9 ^a	35.2 ^{ab}	21.6 ^b	53.2
B359	49.9 ^a	42.6 ^a	33.6 ^{ab}	28.1 ^{ab}	43.7
Speckled grey-Lecheng	49.2 ^a	39.9 ^a	30.8 ^b	28.4 ^{ab}	42.3
Blackeye	52.2 ^a	40.9 ^a	31.2 ^b	24.6 ^{ab}	52.9
Inia 30	52.3 ^a	44.2 ^a	31.6 ^b	28.4 ^{ab}	45.7
B281	51.7 ^a	40.1 ^a	35.5 ^{ab}	24.7 ^{ab}	52.2
Inia 71	56.5 ^a	44.5 ^a	38.3 ^{ab}	34.8 ^a	38.4
E060	51.4 ^a	43.10 ^a	32.6 ^{ab}	28.9 ^{ab}	43.8
Speckled grey-Hukuntsi	49.3 ^{ab}	45.4 ^a	42.2 ^a	34.9 ^a	29.2
B378	53.2 ^a	43.7 ^a	34.1 ^{ab}	25.2 ^b	52.6
Wild -Lecheng	57.4 ^a	48.3 ^a	43.3 ^a	40.3 ^a	29.8
Inia 37	54.4 ^a	45.7 ^a	42.7 ^a	40.6 ^a	25.4
BWC635	43.8 ^{ab}	38.7 ^a	32.9 ^{ab}	26.7 ^{ab}	39.0
Mutant 33	48 ^a	43.2 ^a	38.4 ^{ab}	35.0 ^a	27.1
B261-B	52.3 ^a	44.2 ^a	37.2 ^{ab}	29.6 ^{ab}	43.4
Mahutohuto	59.6 ^a	50.4 ^a	49.2 ^a	44.3 ^a	25.7
Mean	50.4	42.9	32.1	28.9	42.7
LSD	18.45	14.06	22.59	19.06	
P<0.05			0.001		

Stress greenness scores: 0 -leaves and stems completely yellow; 1 -75% of leaves yellow, stem not turgid; 2- 50% of leaves yellow, stems not turgid; 3 – 25% leaves yellow, stems less turgid; 4- leaves completely green, stem turgid. Leaf wilting scores: 0- no signs of wilting; 1- 25% of the leaves wilting; 2- moderately (50%) wilting; 3- yellow and brown leaves with 75% wilting; 4- leaves completely wilted

Assessment of drought tolerance in cowpea using stress tolerance indices

Different drought indices were estimated based on the biomass yield of genotypes under non stress (Y_p) and stressed (Y_s) environment (Table 5). The results indicate that TOL, SSI, BMP, and YR were highest in B500, B111, ER7, and B138, while the lowest values were recorded in Mahutohuto, Tswana cream, Inia 30, B359 and E129. For STI, RDI, and BDR, the highest values were observed in Mahutohuto, BWC635, Speckled grey-Hukuntsi, B212, B359, and Inia 30 across the indices, whereas ER7, B500, B138, B478, and B475 exhibited lower indices.

Table 5: Tolerance indices for cowpea genotypes under stress and non stressed conditions

CODE	Genotype	Yp	Ys	TOL	STI	SSI	BMP	RDI	BDR	YR
CW1	ER7	21.4	1.2	13.43	0.61	1.54	10.12	0.13	0.45	94.72
CW2	B500	26.3	1.8	24.47	0.92	1.47	12.23	0.22	1.46	90.75
CW3	B138	15.4	1.3	14.13	0.47	1.49	7.07	0.2	0.77	91.71
CW4	B111	20.6	5	15.63	2.38	1.24	7.82	0.57	9.13	76.08
CW9	B075	9.4	2.6	6.87	0.61	1.18	3.43	0.65	6.11	72.84
CW14	B154	13.5	3.4	10.1	1.18	1.15	5.05	0.69	6.78	70.75
CW19	B478	8.5	1.8	6.67	0.32	1.23	3.33	0.58	3.47	75.49
CW20	B163	13.9	2.7	11.1	0.87	1.27	5.55	0.52	3.97	78.29
CW25	B342	16.3	5.5	10.83	2.60	1.15	5.42	0.7	13.83	70.55
CW5	B212	15.5	10.3	4.67	3.94	0.47	2.33	1.43	52.57	29.15
CW7	E129	17.2	10.7	6.53	4.19	0.61	3.27	1.48	44.34	37.64
CW8	B272-B	11.8	6.6	5.23	2.10	0.71	2.62	1.34	24.61	43.57
CW11	B154-D	7.5	2.9	4.6	0.64	0.98	2.3	0.94	7.75	60.39
CW13	Tswana cream	8.1	4.6	3.53	0.83	0.7	1.77	1.35	18.04	43.08
CW23	B269-1	9.7	6.1	3.62	1.35	0.61	1.81	1.48	26.08	37.78
CW24	B359	16.8	11	5.87	5.93	0.47	2.93	1.68	48.9	29.14
CW27	Speckled grey-Lecheng	13.8	6.8	7.03	2.21	0.82	3.52	1.18	22.84	50.23
CW29	Blackeye	8.8	4.1	4.73	1.01	0.88	2.37	1.09	12.77	54.05
CW30	Inia 30	8.8	5.9	2.93	1.22	0.55	1.47	1.57	26.07	33.71
CW6	B281	23.6	10.3	13.4	5.38	0.9	6.7	1.06	32.32	55.43
CW10	Inia 71	16.4	7	9.43	2.97	0.99	4.72	0.92	22.5	61.12
CW12	E060	18.6	10.3	8.33	4.60	0.73	4.17	1.3	40.63	45.1
CW15	Speckled grey-Hukuntsi	20.6	11.4	9.13	5.70	0.74	4.57	1.29	43.41	45.88
CW16	B378	18.2	7.2	11	3.04	0.98	5.5	0.94	18.98	60.45
CW17	Wild -Lecheng	20.5	8.3	12.27	4.39	0.98	6.13	1.34	22.93	60.27
CW18	Inia 37	20.6	9.3	11.27	5.45	0.94	5.63	1.03	31.38	57.72
CW21	BWC635	23.1	11.8	11.3	7.22	0.76	5.65	1.27	41.42	46.67
CW26	Mutant 33	19.8	9.1	10.7	4.67	0.79	5.35	1.21	29	48.95
CW28	B261-B	18.4	9.1	9.3	3.88	0.82	4.65	1.18	30.33	50.51
CW22	Mahutohuto	29.8	18.9	10.9	15.35	0.64	5.45	1.68	81.64	39.56

Yp, yield under non stressed conditions; *Ys* yield under stress; *BDR*, biomass drought resistance, *STI*, stress tolerance index; *SSI*, stress susceptibility index; *YR*, yield reduction percent; *TOL*, tolerance; *BMP*, biomass mean productivity; *RDI*, relative drought index

Correlation analysis

To determine the most desirable drought tolerance index, the coefficients between *Yp*, *Ys* and other quantitative indices of drought tolerance were calculated (Table 6). Biomass yield in non stressed conditions (*Yp*) was significantly and positively correlated with *BMP*, *TOL*, *STI* and *BDR* and had a negative and insignificant relationship with *BR*. Yield under stressed conditions exhibited a significant positive correlation with *RDI*, *STI*, and *BDR*, while showing a negative correlation with *SSI* and *YR*. However, the relationship with *TOL* was found to be non-significant.

Table 6 Correlation coefficients between cowpea drought tolerance indices

	Yp	Ys	BMP	TOL	SSI	RDI	STI	BDR	YR
Yp	1								
Ys	0.56 ^{ns}	1							
BMP	0.72***	0.17 ^{ns}	1						
TOL	0.73***	-0.17 ^{ns}	1.00***	1					
SSI	0.09 ^{ns}	-0.72***	0.71***	0.71***	1				
RDI	-0.10 ^{ns}	0.71***	-0.72***	-0.72***	-0.99***	1			
STI	0.69***	0.93***	0.04 ^{ns}	0.04 ^{ns}	-0.47**	0.47*	1		
BDR	0.44*	0.97***	-0.28 ^{ns}	-0.28 ^{ns}	-0.79***	0.79***	0.88**	1	
YR	0.09 ^{ns}	-0.72***	0.71***	0.71***	0.99***	-0.1***	-0.47***	-0.79***	1

***, **, * Significant at P<0.0001, 0.001, 0.01 and ^{ns} denotes non significance

Yp, yield under non stressed conditions; Ys, yield under stress; BMP, biomass mean productivity; TOL, tolerance; BDR, biomass drought resistance; STI, stress tolerance index; SSI, stress susceptibility index; YR, yield reduction percent; RDI, relative drought index

Principal component and cluster analysis

A principal component analysis (PCA) and biplot analysis were used to determine the interrelationship between indices. In Table 7, the first four principal components (PC) had eigenvalues greater than one and accounted 99.77% of the total accumulated variation. PC1 contributed the most variability of 62.35% followed by PC2 34.3% while PC5 contributed the least of 0.2%. PC1 and PC2 were interpreted and used to create a biplot in (Fig 5 and 6) because they contributed most of the variation in the dataset. The biplot classified the drought indices into five groups; group 1 and 2 had accessions with high values of SSI, TOL and low STI whereas group 5 had accessions with high STI and BDR. The cosine of the angle between the vectors of 2 indices approximates the correlation between them, for example G5 indices (STI, Ys, BDR) were positively correlated and the same conclusion can be made for G3 (BR, SSI). G1 (Yp) and G2 (BMP, TOL) were negatively correlated with G4 (RDI). The biplot assigned genotypes to each of the indices groups; Group 1 had 2 genotypes, group 2 had 7 genotypes, group3 and 4 each had 10 genotypes and group 5 consisted of 1 genotype. To categorise genotypes according to their tolerance levels, a dendrogram divided the accessions into 5 groups ranging from highly sensitive to highly tolerant. ER7 and B500 showed high sensitivity, Blackeye and Tswana cream showed moderate tolerance, whereas Mahutohuto demonstrated high tolerance (Fig. 7)

Table 7 Principal components of drought tolerance indices and their eigen values, variability and cumulative variability of cowpea genotypes subjected to water stress

	PC1	PC2	PC3	PC4	PC5
Yp	0.000	0.566	0.183	0.134	-0.291
Ys	0.344	0.321	-0.159	0.345	-0.611
BMP	-0.288	0.403	0.345	-0.056	0.188
TOL	-0.288	0.403	0.345	-0.056	0.188
SSI	-0.412	0.066	-0.358	-0.089	-0.155
RDI	0.412	-0.066	0.360	0.885	0.143
STI	0.261	0.415	-0.550	0.247	0.629
BDR	0.370	0.257	-0.125	-0.879	-0.085
YR	-0.412	0.066	-0.358	-0.089	-0.155
Eigenvalue	5.612 ^{e+00}	3.078 ^{e+00}	2.517 ^{e-01}	2.790 ^{e-02}	0.546 ^{e-02}
Variance %	62.35	34.3	2.8	0.3	0.2
Cumulative variance	62.35	96.66	99.45	99.77	99.99

Yp, yield under non stressed conditions; Ys, yield under stress; BMP, biomass mean productivity; TOL, tolerance; BDR, biomass drought resistance; STI, stress tolerance index; SSI, stress susceptibility index; YR, yield reduction percent; RDI, relative drought index

DISCUSSION

Drought stress is a threat to cowpea productivity and understanding the genotypic variation in tolerance traits is critical in breeding programs for the crop (Carvalho et al. 2020; Liu et al. 2025). A wooden box technique as a method for screening cowpea accessions under drought conditions at seedling stage was used in this study. This method has been utilized in several studies to identify cowpea varieties with improved drought tolerance (Alidu et al. 2019; Singh et al. 1999; Singini et al. 2024). The application of wooden boxes has been found to be the most appropriate, fast and rapid screening approach for seedling drought tolerance for shoot related traits such as the relative water content, leaf wilting, chlorophyll contents and the estimation of proportion of survived seedlings after recovery (Manneh et al. 2024; Muchero et al. 2008). This has been the most successful approach for evaluating large populations and subsequent selection for field drought assessment of genotypes (Singh et al. 1999).

This study revealed that moisture is a very important component in plant growth and development. Watanabe et al. (1997) recommended 3% as the optimum moisture content for discriminating cowpea germplasm into tolerant and susceptible groups. Similarly, Batlang et al. (2018), Nkoana et al. (2020) and Pungulani et al. (2012) identified drought tolerant cowpea genotypes that survived at 2.2 and 2.6% moisture content after 28 days of moisture stress. The 2 % moisture content level for screening germplasm for drought tolerance in this study agrees with their findings. Soil moisture is an essential resource for crop growth and its insufficiency coupled with high temperatures can lead to significant yield reductions or even crop failure (Liu et al. 2025). According to Alidu et al. (2019) and Boukar et al. (2020), the ideal temperature for the growth and development of cowpeas is approximately 30°C, with temperatures exceeding 30°C potentially increasing stress levels. The severity of drought observed in this study may have been exacerbated by the high temperatures recorded during the experimental period.

Drought affects crop growth and development by decreasing soil water availability and suppressing photosynthesis (Chaves et al. 2002). Adaptive responses to drought stress usually arise from physiological and morphological adjustments, and wilting is the first visible adaptive phenotypic change in plants (Pathan et al. 2014). Most of the cowpea genotypes exhibited variations in their reactions to drought conditions in chlorophyll levels, leaf wilting, stem greenness, recovery rates and relative water content. As drought stress intensified, leaves became wilted, stems lost turgidity, and chlorophyll levels decreased, leading to reduced recovery and lower final biomass yield. However, some genotypes displayed lower wilting scores, managed to withstand vegetative drought stress by preserving stem greenness, and were able to recover after being rewatered. Leaves as the primary photosynthetic organs, serve as an indicator of a plant's adaptability to drought stress (Ritte et al. 2025). Drought-tolerant cowpea varieties tend to exhibit a lower reduction in chlorophyll content compared to drought-sensitive varieties when exposed to drought stress (Tengey et al. 2023). This means that drought-tolerant cowpeas are better at maintaining their chlorophyll levels under water-deficit conditions, which is a key indicator of photosynthetic efficiency and overall plant health (Fang and Xiong 2015).

Sharma and Kumar (2008) identified stomatal conductance, leaf water potential and osmotic adjustment as key mechanisms preventing early wilting in cowpea. Plants that do not close their stomata wilt within a short period of stress as they transpire at a faster rate creating water imbalance in plant tissues (Ritte et al. 2022). This may have been the case with ER7 and B500 as they showed signs of wilting during the initial stages of drought stress and subsequently became dry. Comparable findings were reported in other cowpea studies by Muchero et al. (2008); Pungulani et al. (2012), and in common bean Mukeshimana et al. (2014), where wilting was noted after the first week of stress in genotypes vulnerable to drought. Stem greenness in drought stressed plants can be an indicator of chlorophyll content and overall ability to tolerate drought, with a greener stem typically indicating enhanced photosynthetic activity and better resilience to water deficit (Poudel et al. 2025). Maintenance of stem greenness is often correlated with survival and recovery after water stress (Hasanuzzaman et al. 2022). In the study, it was established that the chlorophyll content in cowpea leaves closely correlates with the greenness of the plant. A greenness score of 0 indicates that the leaves and stems of the plant are completely yellow and dead, while a score of 4 signifies fully green leaves and turgid stems (Muchero et al. 2008). This suggests that decreased chlorophyll levels correlate with lower greenness, aligning with research findings that a strong connection between chlorophyll fluorescence parameters and overall plant mortality when facing environmental stressors (Valladares et al. 2025). Although drought stress typically leads to a decrease in leaf chlorophyll content, certain genotypes can preserve higher levels of chlorophyll or experiencing only a minor decline under stressful conditions, which is often associated with a decrease in chlorophyll degradation and delayed senescence (Guo et al. 2023). This characteristic might contribute to a rapid recovery from drought once water is reintroduced by resuming photosynthesis (Alidu et al. 2019). Both Mahutohuto and the wild cowpea exhibited these traits, and as noted by Manneh et al. (2024), these genotypes are expected to demonstrate greater tolerance to drought stress and can be considered as Type 1 drought tolerant. While some cowpea genotypes exhibited a greater ability to recover from drought stress, other genotypes (ER7, B138, B163, B212) had an irreversible damage with only a small proportion of plants recovering. According to Ritte et al. (2025) this indicates that recovery may serve as a reliable criterion for selecting drought-tolerant cowpea genotypes following extended drought stress and rewatering.

The findings from the correlation analysis suggest that stem greenness could be a reliable measure of chlorophyll content and recovery, demonstrating a significant relationship between these variables. Furthermore, a lower leaf wilting index can also serve as a valuable criterion for selecting drought-tolerant plants (Poudel et al. 2025). As highlighted by Pungulani et al. (2013), this demonstrates a trait of drought resilience that is closely related to the stay-green or delayed senescence characteristic in conditions of drought. The results showed an inverse relationship between LWI and chlorophyll content, a correlation that is supported by the work of Muchero et al. (2008) and Fatokun et al. (2012). According to Ajayi (2022), cowpea genotypes that exhibit low LWI and high recovery rates have developed a mechanism to reduce moisture loss in their tissues by regulating the transpiration rate. In contrast, the genotypes that are highly susceptible undergo significant moisture loss, reduced turgidity, and substantial chlorophyll degradation, which can ultimately result in their death and an inability to recover, depending on the severity of drought (Tengey et al. 2023). Several studies have identified a notable connection between increased relative chlorophyll content and the stay green phenotype in cowpea (Nkoana et al. 2018; Nunes et al. 2022). The stay green trait, which is defined by extended leaf greenness during drought periods, indicates diminished chlorophyll degradation. This trait enables drought-resistant cultivars to maintain their photosynthetic functions for extended periods, consequently enhancing dry matter production. (Kamal et al. 2019). The stay green characteristic is frequently observed in sorghum and has also been noted in certain barley varieties (Hasanuzzaman et al. 2022; Mwamahonje et al. 2021).

While a crop can survive seedling drought stress, it is crucial to assess its ability to maintain productivity under water limited conditions. Drought tolerance indices provide a quantitative measure to assess a plant's ability to withstand drought stress and maintain yield, aiding in the selection of superior genotypes for breeding programs (Islam et al. 2022). The effectiveness of these indices has been confirmed when selecting high yielding and drought tolerant wheat and cowpea genotypes (Sahar et al. 2017; Sanchez-Reinoso et al. 2020; Singini et al. 2024). Most drought tolerance indices evaluated in the study showed high significant differences among the cowpea genotypes at each stress level showing a high degree of discrimination. In performance testing, the genotype with the highest yield generated three times the biomass compared to the one with the lowest yield under normal conditions. When subjected to water stress, high-yielding genotypes, such as Mahutohuto and BWC635, as well as low-yielding ones like B359, B212 and Inia30, maintained consistent performance across the environments, as shown by their percent yield reduction. In contrast, other high-yielding genotypes, including B500 and ER7, lost nearly all their biomass when faced with drought stress. Ravelombola et al. (2020) noted that high-yielding cowpea varieties may encounter decreased yields under stress conditions, especially during drought. Although cowpeas are recognized for their relative resilience to stress, some high-yielding varieties exhibit sensitivity to drought during crucial growth stages, such as early vegetative growth. This vulnerability may result in stunted growth, reduced flowering and yield, and a lower harvest index (Poudel et al. 2025). This could have applied to ER7, B500, and B138, which experienced significant losses under stress conditions. Various indices identified different genotypes as drought tolerant, while others recognized nearly the same genotype for drought tolerance. For instance, Mahutohuto was identified by STI, RDI, and BDR as the most tolerant genotype. In studies concerning drought tolerance, STI is often considered the most suitable index for assessing drought tolerance (Farshadfar et al. 2013). Selection centred on high STI and BMP enables the identification of genotypes that are both high yielding and drought tolerant (Ladoui et al. 2021). Higher TOL values indicate increased sensitivity to stress; therefore, lower values are preferred when selecting tolerant genotypes. A lower SSI and TOL indicate

greater tolerance but does not differentiate between genotypes that excel in both environments and those that only perform well under stress (Basolle et al. 2024; Ravelombola et al. 2020). According to Mitra (2001) and Farshadfar et al., (2012), an index becomes ideal for selecting drought tolerant genotypes if it shows a relatively high correlation with grain yield in both stressed and non stressed conditions. Our results showed that STI and BDR were positively associated with yield in both conditions, with the strongest correlations observed under stress, which aligns with the observations made by Farshadfar et al. (2012).

The PCA results classified the cowpea genotypes into five distinct categories: very sensitive, sensitive, moderately tolerant, tolerant, and highly tolerant. The highly sensitive genotypes had the highest values for SSI, TOL, BMP, Yp, and YR, showing their vulnerability to drought and suitability for irrigated environments only. The tolerant and highly tolerant groups exhibited the greatest values for STI, BDR, and Ys, making them the best indices for both growth circumstances. This suggests that STI and BDR are appropriate indices for screening cowpea seedlings for drought tolerance.

The findings of this study provided a combination tools for screening and determining drought tolerant and susceptible cowpea genotypes at the seedling stage. The study suggests that severe drought at the seedling stage might have a significant effect on yield through reduced biomass accumulation. The combination of a low leaf wilting index, delayed senescence, high leaf water status, and high BDR (biomass drought resistance) and STI (stress tolerance index) are all important traits for selecting drought-tolerant cowpea. These traits contribute to a cowpea's ability to withstand and recover from drought stress by maintaining leaf function, minimizing water loss, and efficiently utilizing water resources. The Mahutohuto landrace, originating from Botswana's Kalahari sandveld, is suggested for use in drought tolerance breeding, along with the genotypes that belong to the tolerant group such as the mutant Tswana cowpea cultivar (Mutant 33) which is in the process of variety release. Although this study was conducted in a screenhouse, it is essential to assess these accessions under field conditions and at various growth stages to evaluate yield, stability, and drought tolerance.

REFERENCES

- Ajayi AT, Gbadamosi AE, Olumekun VO, Nwosu PO (2020) GT biplot analysis of shoot traits indicating drought tolerance in cowpea (*Vigna unguiculata* L. Walp) accessions at vegetative stage. *Int J BioSciences Technol* 13:18–33. <https://doi.org/10.5281/zenodo.4019163>
- Alidu SM, Asante IK, Tongoona P, Ofori K, Danquah A, Padi FK (2019) Development and screening of cowpea recombinant inbred lines for seedling drought tolerance. *J Plant Breed Crop Sci* 11(1):1–10. <https://doi.org/10.5897/JPBCS2018.0768>
- Batlang U, King Jr. C, Ngwako S, Nkomazana C, Malambane G, Mpuisang T, Moroke TS, Ng'uni D, Munkombwe G (2018) Germplasm evaluation for climate adaptation and drought tolerance: The cases of local varieties of maize in Zambia and cowpea in Botswana. In: *Climate change and adaptive land management in southern Africa – assessments, changes, challenges, and solutions* (ed. by Revermann R, Krewenka KM, Schmiedel U et al) *Biodiversity & Ecology* 6, Klaus Hess Publishers, Göttingen & Windhoek, pp 272-281. <https://doi.org/doi:10.7809/b-e.00334>
- Belko N, Zaman-Allah M, Cisse N, Diop NN, Zombre G, Ehlers JD, Vadez V (2012) Lower soil moisture threshold for transpiration decline under water deficit correlates with lower canopy conductance and higher transpiration efficiency in drought-tolerant cowpea. *Funct Plant Biol* 39:306-322
- Boukar O, Abberton M, Oyatomi O, Togola A, Tripathi L, Fatokun CA (2020) Introgression breeding in cowpea (*Vigna unguiculata* L. Walp.). *Front Plant Sci* 11:567425. <https://doi.org/10.3389/fpls.2020.567425>
- Boukar O, Belko N, Chamarthi S, Togola A, Batieno J, Owusu E, et al (2018) Cowpea (*Vigna unguiculata*): genetics, genomics and breeding. *Plant Breed* 138:415–424. <https://doi.org/10.1111/pbr.12589>
- Chaves MM, Pereira JS, Maroco J, Rodrigues ML, Ricardo CP, Osório ML, Carvalho I, Faria T, Pinheiro C (2002) How plants cope with water stress in the field. Photosynthesis and growth. *Ann Bot* (7):907-16. <https://doi.org/10.1093/aob/mcf105>
- Fang Y, Xiong L (2015) General mechanisms of drought response and their application in drought resistance improvement in plants. *Cell Mol Life Sci* 72:673–689. <https://doi.org/10.1007/s00018-014-1767-0>
- FAO (2022) *FAO Strategy on Climate Change 2022–2031*. Rome. <https://www.fao.org>
- Farshadfar E, Elyasi P (2012) Screening quantitative indicators of drought tolerance in bread wheat (*T. aestivum*) landraces. *Eur J Exp Biol* 2(3):577-584
- Farshadfar E, Poursiahbidi MM, Safavi SM (2013). Assessment of drought tolerance in land races of bread wheat based on resistance/ tolerance indices. *Int. J. Adv. Biol. Biomed. Res* 1(2):143-158
- Fatokun CA, Boukar O, Muranaka S (2012) Evaluation of cowpea [*Vigna unguiculata* (L.) Walp.] germplasm lines for tolerance to drought. *Plant Genet Res* 10:171–176. <https://doi.org/10.1017/S1479262112000214>

Fernandez GCJ (1992) Effective Selection Criteria for Assessing Stress Tolerance. In: Proceedings of the International Symposium on Adaptation of Vegetables and Other Food Crops in Temperature and Water Stress (ed. Kuo CG) AVRDC Publication, Tainan, 257-270

Fischer R, Maurer R (1978) Drought Resistance in Spring Wheat Cultivars Grain Yield Responses. Aust J Agric Res 29:897-912. <http://dx.doi.org/10.1071/AR9780897>

Guo J, Hu F, Zhang Y (2023) Effects of drought stress on photosynthetic physiological characteristics, leaf microstructure, and related gene expression of yellow horn. Plant Signal Behav 18(1):2215025. <https://doi.org/10.1080/15592324.2023.2215025>

Hasanuzzaman M, Shabala L, Brodribb TJ, Zhou M, Shabala S (2022) Understanding the Role of Physiological and Agronomical Traits during Drought Recovery as a Determinant of Differential Drought Stress Tolerance in Barley. Agron 12(9):2136. <https://doi.org/10.3390/agronomy12092136>

Hossain MS, Khan MAR, Mahmud A, Ghosh UK, Anik TR, Mayer D, Das A K, Mostofa MG (2024). Differential Drought Responses of Soybean Genotypes in Relation to Photosynthesis and Growth-Yield Attributes. Plants 13(19):2765. <https://doi.org/10.3390/plants13192765>

IPCC (2022) Climate Change 2022: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change (Pörtner HO, Roberts DC, Tignor M, Poloczanska ES et al (eds.) Cambridge University Press. Cambridge University Press, Cambridge, UK and New York, NY, USA, pp 3056. <https://doi.org/10.1017/9781009325844>

Islam MR, Kamal MM, Hossain MF, Hossain J, Mohammad GA, et al (2022) Drought Tolerance in Mung Bean is Associated with the Genotypic Divergence, Regulation of Proline, Photosynthetic Pigment and Water Relation. Phyton-Int J Exp Bot 92(3):955-981. <https://doi.org/10.32604/phyton.2023.025138>

Kamal NM, Gorafi YSA, Abdelrahman M, Abdellatef E, Tsujimoto H (2019) Stay-Green Trait: A Prospective Approach for Yield Potential, and Drought and Heat Stress Adaptation in Globally Important Cereals. Int J Mol Sci 20:5837. <https://doi.org/10.3390/ijms20235837>

Ladoui KK, Mefti M, Benkherbache N (2020) Selection of water stress tolerant barley (*Hordeum vulgare* L.) genotypes by drought tolerance indices. Agribiol 10(1):1805-1812

Liu Z, Lv A, Li T (2025). Intensified Drought Threatens Future Food Security in Major Food-Producing Countries. Atmos 16(1):34. <https://doi.org/10.3390/atmos16010034>

Mai-Kodomi Y, Singh BB, Myers O (1999). Two mechanisms of drought tolerance in cowpea. Indian J Genet Plant Breed 59:309-316

Manneh N, Adetimirin VO, Dieng I, Ntukidem SO, Fatokun CA, Boukar O (2024) Response of Cowpea (*Vigna unguiculata* L. Walp) Accessions to Moisture Stress. Int J Plant Biol 15(4):1201-1214 <https://doi.org/10.3390/ijpb15040083>

Matsui T, Singh BB (2003). Root characteristics in cowpea related to drought tolerance at the seedling stage. Exp Agric 39(1):29-38

- Mitra J (2001). Genetics and genetic improvement of drought resistance in crop plants, *Curr Sci* 80(6):758–763
- Molosiwa OO, Makwala BC (2020). Field evaluation of introduced and local cowpea genotypes performance in Botswana. *Afr J Agric Res* 16:1317-1324
- Nkomazana C, Batlang U (2018) Grain yield water use efficiency of cowpea (*Vigna unguiculata* L. Walp.) in response to planting dates in Botswana. In: Climate change and adaptive land management in southern Africa – assessments, changes, challenges, and solutions (ed. by Revermann, R., Krewenka, K.M., Schmiedel, U., Olwoch, J.M., Helmschrot, J. & Jürgens, N.), pp. 282-287, Biodiversity & Ecology, 6, Klaus Hess Publishers, Göttingen & Windhoek. <https://doi.org/10.7809/b-e.00335>
- Muchero W, Ehlers JD, Roberts PA (2008) Seedling Stage Drought-Induced Phenotypes and Drought-Responsive Genes in Diverse Cowpea Genotypes. *Crop Sci* 48:541
- Mukeshimana G, Amy L, Wayne L, James K (2014) Identification of Shoot Traits Related to Drought Tolerance in Common Bean Seedlings. *J Am Soc Hortic Sci* 139:299-309. <https://doi.org/10.21273/JASHS.139.3.299>
- Mwamahonje A, Eleblu JSY, Ofori K, Deshpande S, Feyissa T, Tongoona P (2021) Drought Tolerance and Application of Marker-Assisted Selection in Sorghum. *Biol* 10(12):1249. <https://doi.org/10.3390/biology10121249>
- Naghavi MR, Aboughadareh AP Khalili M (2013) Evaluation of drought tolerance indices for screening some of corn (*Zea mays* L.) cultivars under environmental conditions. *Not Sci Biol* 5:388–393. <https://doi.org/10.15835/nsb.5.3.9049>
- Nkoana K, Gerrano A, Gwata T (2019) Evaluation of diverse cowpea [*Vigna unguiculata* (L.) Walp.] germplasm accessions for drought tolerance. *Legume Res* 42(2):168-172. <https://doi.org/10.18805/LR-428>
- Nkomo GV, Sedibe M, Mofokeng M (2022) Phenotyping cowpea accessions at the seedling stage for drought tolerance using the pot method. *Open Agric* 7:433-444. <https://doi.org/10.1101/2020.07.10.196915>
- Nunes C, Moreira R, Pais I, Semedo J, Simões F, Veloso MM, Scotti-Campos P (2022) Cowpea Physiological Responses to Terminal Drought—Comparison between Four Landraces and a Commercial Variety. *Plants* 11:593. <https://doi.org/10.3390/plants11050593>
- Olorunwa OJ, Shi A, Barickman TC (2021) Varying drought stress induces morpho-physiological changes in cowpea (*Vigna unguiculata* L. Walp) genotypes inoculated with *Bradyrhizobium japonicum*. *Plant Stress* 20(2):100033
- Pathan SM, Lee JD, Sleper DA, Fritschi FB, Sharp RE, Carter TE, Nelson RL, King CA et al (2014) Two soybean plant introductions display slow leaf wilting and reduced yield loss under drought. *J Agron Crop Sci* 200:231–236
- Poudel S, Valsala L, Sivarathri BS, Hosahalli V, Harkess RL, Bheemanahalli R (2025) Characterization of Cowpea Genotypes for Traits Related to Early-Season Drought Tolerance. *Agric* 15(10):1075. <https://doi.org/10.3390/agriculture15101075>

Pungulani L, Davie K, Lucius N, Modester K (2012) Selection of High Yielding and Preferred Genotypes of Bambara Nut (*Vigna subterranea* L. Verdc) in Malawi. *Am. J. Plant Sci* 3:1802-1808 <https://doi.org/10.4236/ajps.2012.312A221>

Pungulani LLM, Milner JP, Warren MW, Banda M (2013) Improvement of Leaf Wilting Scoring System in Cowpea: From Qualitative Scale to Quantitative Index. *Aust. J. Crop Sci.* 7:1262-1269

Ravelombola W, Shi A, Chen S, Xiong H, Yang Y, Cui Q, Olaoye D, Mou B (2020) Evaluation of Cowpea for Drought Tolerance at Seedling Stage. *Euphytica* 216:123 <https://doi.org/10.1007/s10681-020-02660-4>

Ravelombola W, Shi A, Qin J, Weng Y, Bhattarai G, Zia B, Zhou W, Mou B (2018) Investigation on various aboveground traits to identify drought tolerance in cowpea seedlings. *HortScience* 53:1757–1765

Ritte IP, Egnin M, Idehen O, Mortley D, Bernard GC, Binagwa PH, Brown AP, Bonsi CK (2022) Evaluation of Cowpea Morpho-physiological and Yield Responses to Vegetative and Pre-Anthesis Water-Deficit Stress Tolerance under Greenhouse Conditions. *Eur J Appl Sci* 10:391–41. <https://doi.org/10.14738/aivp.102.11786>

Ritte IP, Egnin M, Idehen O, Mortley D, Bernard GC, Bonsi CK (2025) Morpho-Physiological and Molecular Responses to Seedling-Stage Drought Stress in Different Cowpea Cultivars. *Int. J. Plant Biol.* 16(1):25. <https://doi.org/10.3390/ijpb16010025>

Rosielle AA, Hamblin J (1981) Theoretical aspects of selection for yield in stress and nonstress environments. *Crop Sci* 21:943-946. <http://dx.doi.org/10.2135/cropsci1981.0011183X002100060033x>

Sahar B, Nsarellah N, Birouk A (2022). Roots and Shoots Traits contributing to drought tolerance for bread wheat. *Acta fytotech zootech* 3:247-258. <https://doi.org/10.15414/afz.2022.25.03.247-258>

Sánchez-Reinoso AF, Ligarreto-Moreno GA, Restrepo-Díaz H (2020) Evaluation of drought indices to identify tolerant genotypes in common bean bush (*Phaseolus vulgaris* L.). *J Integr Agric* 19 (1):99-107. [https://doi.org/10.1016/S2095-3119\(19\)62620-1](https://doi.org/10.1016/S2095-3119(19)62620-1)

Sanogo SA, Diallo S, Nyadanu D, Batieno TBJ, Sawadogo N (2023). Selection of Cowpea (*Vigna unguiculata* (L.) walp) Genotypes for Drought Tolerance Using Selection Indices. *Agric Sci* 14(3):384-397 <https://doi.org/10.4236/as.2023.143025>

Sharma KD, Kumar A (2008) Genetic diversity for plant water relations, gaseous exchange, leaf anatomical characteristics and seed yield in cowpea under receding soil moisture. *Indian J. Genet. Plant Breed.* 68:435-440

Tengey TK, Gyamfi RA, Sallah EK, Issahaku M, Ndela DN, Seidu M, Senyabor AF, Affram EI, Amoako OA, Naapoal C (2023) Seedling Stage Drought Screening of Candidate Cowpea (*Vigna unguiculata* L. Walp.) Genotypes. *Cogent Food Agric* 9:2212463. <https://doi.org/10.1080/23311932.2023.2212463>

UN DESA. 2023. The Sustainable Development Goals Report 2023: Special Edition - July 2023. New York, USA.

Valladares F, Dobarro I, Sa'nchez-Go'mez D, Pearcy RW (2005) Photoinhibition and drought in Mediterranean woody saplings: Scaling effects and interactions in sun and shade phenotypes. *J Exp Bot* 56(411):483–494. <https://doi.org/10.1093/jxb/eri037>

Watanabe F, Abe K, Takenaka S, Tamura Y, Maruyama I, Nakano Y (1997). Occurrence of cobalamin coenzymes in the photosynthetic green alga, *Chlorella vulgaris*. *Biosci Biotechnol Biochem* 61:896–897. <https://doi.org/10.1271/bbb.61.896>

Yoseph T, Mekbib F, Fenta BA, Tadele Z, Alemaw G (2020) Screening Mung bean (*Vigna radiata* L. Wilczek) Genotypes for Drought Tolerance. *Ethiop J Agric Sci* 30(4):223-236

List of Figures

Fig.1 Cowpea evaluation for drought tolerance using a wooden box technique.

Fig.2 Climatic conditions of the screenhouse during the study period: February 2025 to April 2024 (a) and soil moisture content over time during drought stress evaluation in cowpea (b).

Fig. 3 Relationship between of recovery rate (a), chlorophyll content (b) and stem greenness (SG) in water stressed cowpea genotypes.

Fig. 4 Relationship between leaf wilting index (LWI) and chlorophyll content in water stressed cowpea

Fig 5 Biplot graph of PCA demonstrating the relationship among the drought tolerance indices in cowpea. *STI*, stress tolerance index; *Yp*, yield under non stressed conditions; *Ys*, yield under stress; *BDR*, biomass drought resistance, *SSI*, stress susceptibility index; *YR*, yield reduction percent; *TOL*, tolerance; *BMP*, biomass mean productivity; *RDI*, relative drought index.

Fig. 6 Biplot graph of principal component analysis showing classification of cowpea genotypes according to the stress tolerance levels. Numbers in red inside the graphs represent cowpea genotypes 1-30; *STI* represents stress tolerance index; *Yp*, yield under non stressed conditions; *Ys* yield under stress; *BDR*, biomass drought resistance; *SSI*, stress susceptibility index; *YR*, yield reduction percent; *TOL*, tolerance; *BMP*, biomass mean productivity; *RDI*, relative drought index

Fig. 7 Dendrogram between groups showing classification of 30 cowpea genotypes based on drought tolerance indices

Statements and declaration**Funding**

The authors declare that no funds and grants were received during the preparation of this manuscript.

Competing interests: The authors declare that they have no relevant financial or non-financial interest to disclose.

Authors contribution

C. Nkomazana: Conceptualization, Investigation, Data curation, Data analysis, Methodology, Visualization, Original draft writing. G. Malambane: Conceptualization, Methodology, Supervision, Investigation, Review and editing. U. Batlang: Conceptualization, Methodology, Project administration and supervision, Resources, Review and editing. All authors read and approved the final manuscript.

Data availability

The data supporting this study is included within this manuscript. Further inquiries concerning materials in the manuscript can be directed to the corresponding author.



Fig. 1 Cowpea evaluation for drought tolerance using a wooden box technique

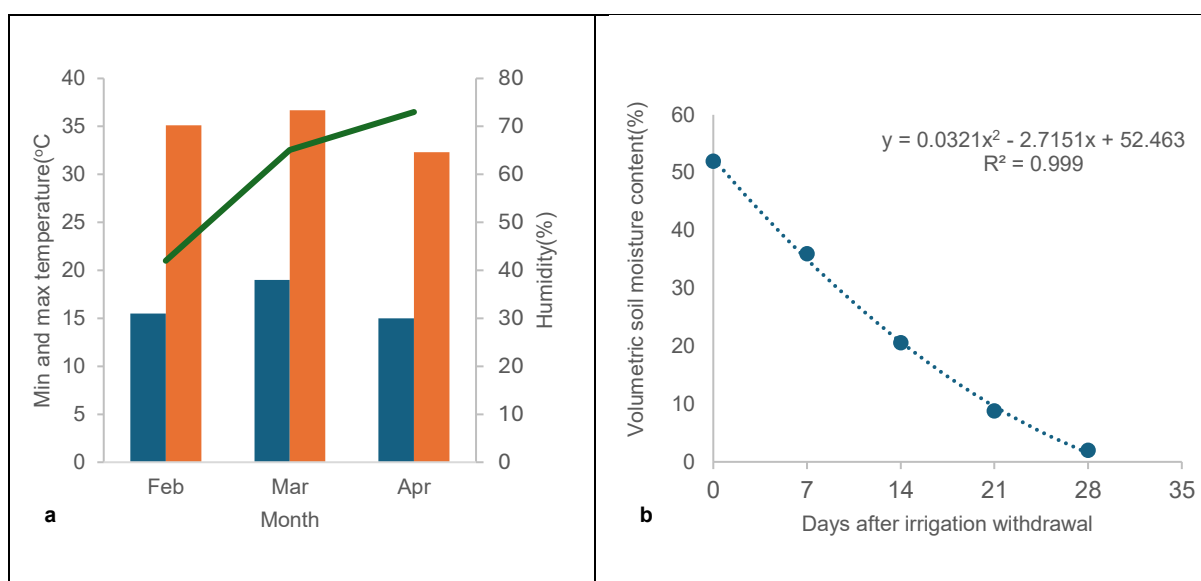


Fig. 2 Climatic conditions of the screenhouse during the study period: February 2025 to April 2024 (a) and soil moisture content over time during drought stress evaluation in cowpea (b)

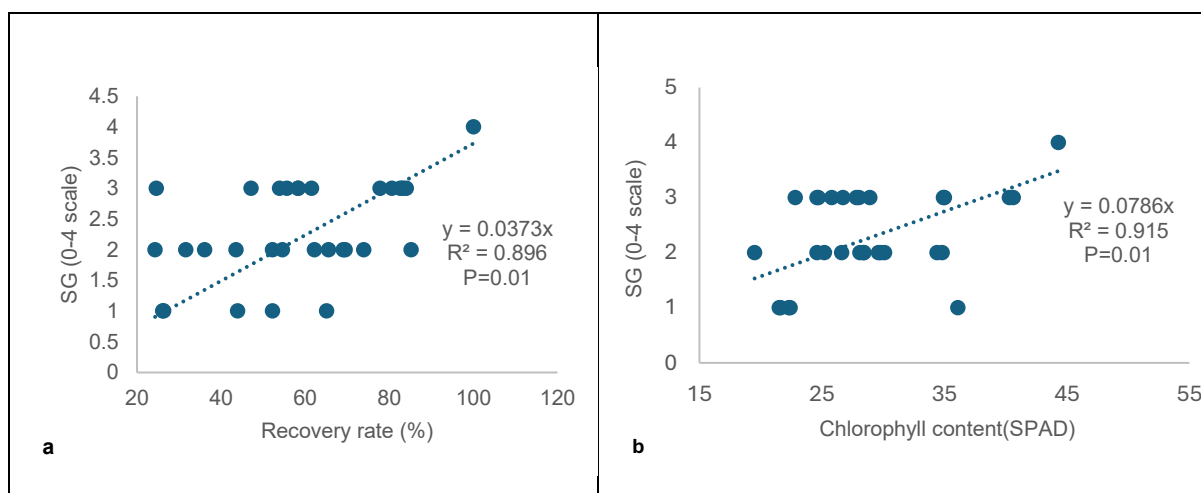


Fig. 3 Relationship between of recovery rate (a), chlorophyll content (b) and stem greenness(SG) in water stressed cowpea genotypes

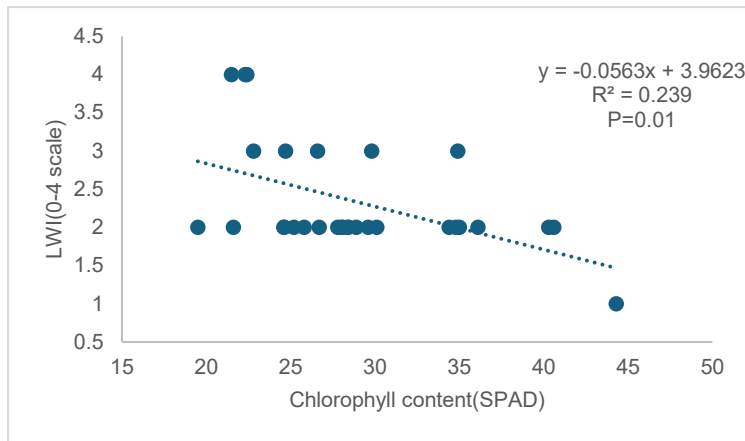


Fig. 4 Relationship between leaf wilting index(LWI) and chlorophyll content in water stressed cowpea

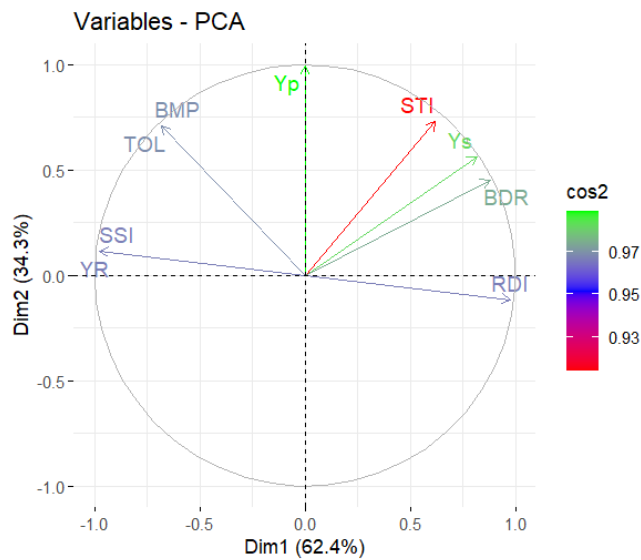


Fig. 5 Biplot graph of PCA demonstrating the relationship among the drought tolerance indices in cowpea. *STI*, stress tolerance index; *Yp*, yield under non stressed conditions; *Ys*, yield under stress; *BDR*, biomass drought resistance, *SSI*, stress susceptibility index; *YR*, yield reduction percent; *TOL*, tolerance; *BMP*, biomass mean productivity; *RDI*, relative drought index

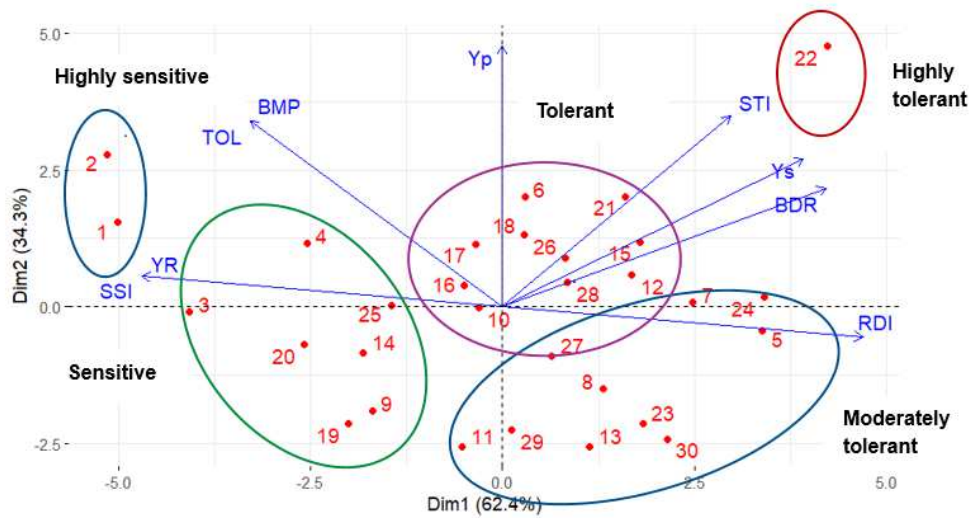


Fig. 6 Biplot graph of principal component analysis showing classification of cowpea genotypes according to the stress tolerance levels. Numbers in red inside the graphs represent cowpea genotypes 1-30; *STI* represents stress tolerance index; *Y_p*, yield under non stressed conditions; *Y_s* yield under stress; *BDR*, biomass drought resistance; *SSI*, stress susceptibility index; *YR*, yield reduction percent; *TOL*, tolerance; *BMP*, biomass mean productivity; *RDI*, relative drought index

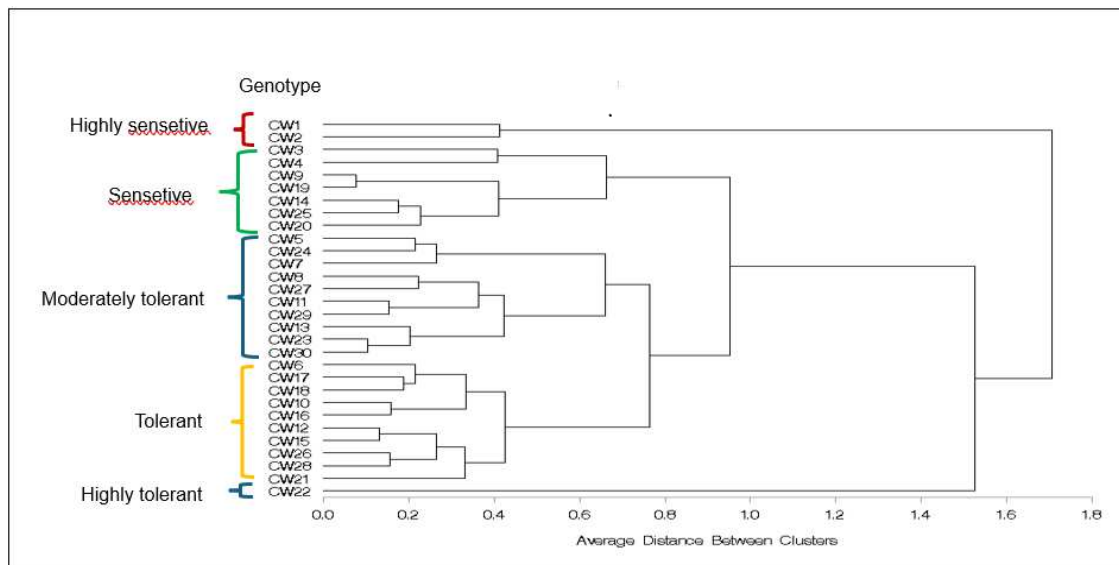


Fig. 7 Dendrogram between groups showing classification of 30 cowpea genotypes based on drought tolerance indices