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Improvement of leaf wilting scoring system in cowpea (*Vigna unguiculata* (L) Walp.): From qualitative scale to quantitative index

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Abstract

Leaf wilting is a fundamental trait used in drought tolerance evaluation. However, its application is limited by a scoring procedure, which is associated with visual assessment and classification of genotypes based on qualitative scales. These limitations present significant challenges to non-experienced researchers. This current research was conducted, in order to improve the scoring system from qualitative scale to quantitative index in cowpeas. Thirty-six cowpea accessions were evaluated in a glasshouse. Plants were stressed for four weeks from the third week after germination, during which the total number of leaves, number of wilting leaves, International Board on Plant Genetic Resources (IBPGR) scale, Mai-Kodomi (MAIK) scale and relative water content (RWC) were scored. Stem greenness and regrowth were scored after re-watering the plants for two weeks. Leaf wilting index (LWI) was calculated as the ratio between the number of wilting leaves and the total number of leaves per plant. Highly significant variation ($P<0.0001$) was observed among accessions for LWI; wilting scales; relative water content; re-growth; and stem greenness. Strong correlations between LWI and drought tolerance traits were also observed. The strong correlations of LWI and drought related traits indicate that LWI could be used in scoring for wilting in cowpeas. An index of less than 0.6 appears to be ideal, in order to identify the drought tolerant genotypes, after four weeks of moisture stress. By counting the number of individual wilting leaves, the challenges associated with other wilting scales could be reduced: thus making it an easier and more reliable method for measuring wilting levels in cowpeas and similar crops.

Keywords: Leaf wilting index, wilting scale, drought tolerance, vegetative stage, Malawi.
Abbreviations: LWI_Leaf Wilting Index; RWC_Relative Water Content; IBPGR_International Board on Plant Genetic Resources, MAIK_Mai-kodomi et al Scale.

Introduction

Cowpea is an important legume crop grown in the dry regions of Africa, Asia and parts of both North and South America, with Africa accounting for 66% of the global production (Ehlers and Hall, 1997). This crop enhances the livelihoods of subsistence farmers, due to its adaptation to dry regions and multiple uses. Cowpea is well known for its drought tolerance, compared to other grain legumes grown within dry regions (Singh et al., 1999a): and its uses can be broadly grouped into culinary, fodder and soil improvement functions (Timko and Singh, 2008). Cowpea grains are rich in proteins and carbohydrates, while the leaves and fresh pods provide a low cost source of vitamins and minerals. In addition to its culinary functions, the crop provides good fodder for livestock and it fixes nitrogen in the soil. Despite its relative drought tolerance, the value of the crop is significantly affected by yield reductions under severe water stress (Kumar et al., 2008). Agbicodo et al. (2009) and Hall (2004, 2012) have provided detailed reviews on drought tolerance research in cowpeas. Both International and National Research Institutions are working on cowpeas germplasm for drought tolerance: and some genotypes have been released for use by farmers and breeders. These drought tolerant genotypes have been identified through the use of several traits including: root characteristics (Matsui and Singh, 2003); stomata conductance (Agbicodo, 2009; Labuschagne et al., 2008); leaf membrane stability (Labuschagne et al., 2008); molecular markers (Agbicodo, 2009; Muchero et al., 2008, 2010); and leaf wilting scales (Mai-Kodomi et al., 1999; Nkouannessi, 2005; Singh et al., 1999b; Watanabe et al., 1997). The use of multiple traits for evaluation affirms the complexity of drought tolerance in cowpeas, in addition to other crops. Despite the availability of several traits for drought tolerance evaluation, leaf wilting still remains a fundamental indicator for drought response: and it reduces the complexities associated with drought evaluation in crops. Three scales for scoring leaf wilting have been used in cowpea. Bioversity International, previously referred to as the International Board on Plant Genetic Resources (IBPGR), developed a 1-9 scale, where 1 represents normal and 9 represents dead and dry plants under moisture stress (IBPGR, 1983). The IBPGR scale has been used to evaluate germplasm for drought tolerance (Nkouannessi, 2005). Similarly, Singh et al., (1999b) developed a 1-5 scale, where 1 represents green turgid leaves and 5 represents completely dead plants. Several studies have applied this scale when screening cowpeas for drought tolerance (Agbicodo, 2009; Belko et al., 2012; Mai-Kodomi et al., 1999; Muchero et al., 2008). In contrast to the Singh et al. scale, Watanabe (1997) developed and used a 1-5 scale, where 1 represents susceptible genotypes and 5 represents highly tolerant genotypes. Similar wilting scales have been used in other crops, including sorghum (Xu et al., 2000); tall fescue (Huang et al., 1998); sugar beet (Ober et al., 2005); *Cicer* spp. (Canci and Toker, 2009); and soybean (Charlson et al., 2009). Use of different wilting scales confirms that leaf wilting is an important indicator for drought evaluation. Although different wilting scales have been successfully used when screening cowpea and other crops for drought tolerance, several limitations have been identified (Xu et al., 2000). The existing leaf wilting scales are associated with biased scoring, due to visual and qualitative assessment. Furthermore, the use of these scales requires experience to systematically and uniformly score for leaf wilting. These limitations have contributed to imprecise results, which point to a need for further research to enhance the ease and reliability of drought tolerance assessment. This current research was conducted, in order to improve the scoring procedure for wilting in cowpea, by identifying a quick and simple scoring method that reduces the challenges associated with the existing wilting scales. The results from this research will be useful to researchers working on drought tolerance in cowpeas and similar crops.

Results

Leaf Wilting Index (LWI)

Highly significant differences ($P<0.0001$) among genotypes were observed for leaf wilting indices (Table 1). Signs of wilting were observed after one week of stress, as shown by the leaf wilting index after the first week of stress (LWI 1). Accessions 471, 1805, 2229, 2232, 2883, 3215 and 3419 showed signs of wilting in the first week. Accession 2232 had the most wilting leaves and a high LWI 1 of 0.72 after the first week of stress. After the first week, no wilting was observed in 17 of the accessions. Increases in LWI were observed in the second to fourth weeks. The highest LWI for the second, third and fourth weeks were 0.77, 0.89 and 1.00, respectively, compared to the lowest values of 0, 0 and 0.23. The highest values were observed in accession 2232 and the lowest values were scored in 601, 645 and 3254 in the second week and 3254, in both the third and final weeks.

Wilting Scales

The IB scale showed highly significant variation ($P<0.0001$) among genotypes (Table 1). After the second week of stress, the highest and lowest values were 6.00 and 1.00, respectively. The highest IB2 value was scored for 2232: and the lowest for 601, 645, 3254 and 3425. After the third week, the highest value of IB 3 increased to 7.00, while the lowest remained at 1.00. The highest and lowest values for IB 3 were scored for 2232 and 3254, respectively. After the fourth week, the IB 4 lowest and highest values increased to 1.25 and 7.50. The lowest and highest values were observed for 3254 and 2232, respectively. Complementary to the IB scale, the MAIK scale also showed highly significant variation ($P<0.0001$) among genotypes. In the second week, a lowest value of 1.00 and a highest value of 3.75 were observed. The lowest was for accessions 3254, 601 and 645, while the highest value was observed for accession 2232. In the third week, the lowest value remained at 1.0 and the highest value increased to 4.00. The lowest value was scored for accessions 601, 645 and 3254, while the highest value was scored for accession 2232. Similarly, in the fourth week, the lowest value of 1.25 was for accessions 601 and 3254 and the highest value of 4.50 was for 2232.

Relative Water Content (RWC)

Highly significant ($P<0.0001$) variation for RWC was observed among the genotypes (Table 1). The RWC, after two weeks of stress (RWC 2), recorded the highest value (0.81) for accession 3254, followed by 479, 320, 2226 and 3422. The lowest value (0.55) was observed for 2232. The highest value of RWC 4 (0.57) was also observed in accession 3254. Other accessions with high RWC 4 values were 479, 601, 645 and 2226. The lowest RWC 4 values were observed in accessions 517, 535 2232, 2234 and 3215.

Re-growth and Stem Greenness (STG)

Both re-growth and stem greenness showed highly significant differences ($P<0.0001$) among genotypes (Table 1). For regrowth, a score of 5.00 was observed for accession 645, followed by accessions 2226, 601, 479 and 3254. Accessions 2231, 2232, 2883 and 3215 did not show any re-growth, as indicated by a score of 1.00. For stem greenness, accession 3254 scored highly (4.88), followed by 479, 2226, 601, 645, 2227 and 3420. Stems were completely yellow and dry for accessions 517, 2231, 2232, 2883 and 3215.

Correlation between LWI and Drought Tolerance Traits

Correlation analysis showed strong association between leaf wilting indices LWI and other variables measured (Table 2). The LWI strongly correlated with characters ($P<0.0001$) measured within the same week. For example, LWI 2 correlated strongly with IB 2, MAIK 2 and RWC 2, while LWI 4 strongly correlated with IB 4, MAIK 4, RWC 4, re-growth and stem greenness. In general, LWI positively correlated with IB and MAIK scales, but negatively correlated with RWC, regrowth and stem greenness.

Discussion

Wilting is the most common sign of drought stress in plants. By definition, wilting is the loss of rigidity, leading to a flaccid state, due to the turgor pressure falling to zero (Taiz and Zeiger, 2010). In this study, some accessions had high leaf wilting indices (LWI 1) after the first week of stress (Table 1), e.g. accession 2232. On the contrary, accessions 479, 601, 645, 2226 and 3254 had low LWI values during the whole stress period and they maintained active canopies, even at a very low soil moisture content of 2.9% (data not shown). These results support previous studies in cowpea, which also identified leaf wilting within the first week of water stress (Fatokun et al., 2009; Mai-Kodomi et al., 1999; Muchero et al., 2008). Therefore, our findings on LWI demonstrate that susceptible genotypes could be identified during the initial stages of stress, as is the case for accession 2232, which showed high wilting index after the first week of stress. The early wilting genotypes suggest the presence of physiological characteristics that enhance water loss from the leaf tissues. For instance, early wilting genotypes keep their stomata open after the initiation of drought, whereas late wilting genotypes close their stomata during the initial phase of stress (Agbicodo et al., 2009). As stress advances, early wilting genotypes dry and drought tolerant genotypes survive through stomatal closure, in addition to osmotic adjustment, which involves accumulation of osmolytes such as prolines (Jaleel et al., 2009; Singh and Reddy, 2011). Stomata closure is regarded as the first defence mechanism under water stress conditions. However, when stress increases, drought tolerant genotypes accumulate osmolytes as a second line of defence.

Table 1. Variation among 36 cowpea accessions for leaf wilting, relative water content, re-growth and stem greenness (STG)

Accession	IB 2	IB 3	IB 4	MAIK 2	MAIK 3	MAIK 4	Regrowth
169	2.50EFG	3.00CDEF	5.75CD	1.25GHI	1.75CDEF	3.00ABCD	1.50E
320	2.50EFG	2.00EFG	2.5FG	1.25GHI	1.50DEF	1.75 FGH	4.75AB
391	2.75DEF	2.50DEFG	5.50CD	1.38BCDEF	1.75CDEF	2.75BCDE	2.00DE
399	2.50EFG	2.50DEFG	5.00DE	1.13HI	1.75CDEF	2.75BCDE	1.50E
411	2.50EFG	2.50DEFG	4.50DE	1.50EFGHI	1.75CDEF	3.00ABCD	2.00ED
414	2.50EFG	2.00EFG	5.25DE	1.25GHI	1.50DEF	2.75BCDE	1.75ED
421	2.75DEF	2.5DEFG	5.50CD	1.38FGHI	1.75CDEF	3.00ABCD	2.00ED
426	2.75DEF	3.00CDEF	1.38FGHI	1.75CDEF	2.75BCDE	2.25DE	0.06BCD
436	3.00CDE	3.00CDEF	4.75DE	1.50EFGHI	1.75CDEF	3.00ABCD	1.50E
471	3.75B	4.5BC	5.25DE	2.38B	2.50BC	2.5CDEF	1.75ED
479	1.00I	1.75FG	2.00G	1.25GHI	1.50DEF	1.50GH	4.5AB
517	3.50BC	4.50BC	5.75CD	2.13BCD	2.50BC	3.50AB	1.25E
535	2.75DEF	6.00BCD	6.00ABCD	1.50EFGHI	2.50BC	3.50AB	2.25DE
544	2.75DEF	3.00CDEF	5.75CD	1.63DEFGH	1.75CDEF	3.25ABC	2.50CDE
570	3.00CDE	4.00BCD	3.75EF	1.75CDEFG	2.50BC	2.25DEFG	2.50CDE
601	1.00I	1.00G	1.75G	1.00I	1.00F	1.00F	4.5AB
645	1.0I	1.00G	2.00G	1.00I	1.00F	1.00F	5.00A
753	3.25BCD	4.00BCD	5.00DE	2.00BCDE	2.25BCD	3.00ABCD	3.25BCD
823	3.00CDE	3.5BCDE	5.75CD	1.50EFGHI	2.00BCDE	2.75BCDE	2.00DE
1805	3.63BC	4.5BC	4.75DE	2.25BC	2.50BC	3.00ABCD	1.25E
2218	3.5BC	5.00B	4.50DE	1.88BCDEF	2.75B	2.50CDEF	1.25E
2223	2.50EFG	3.00CDEF	5.75CD	1.25GHI	1.75CDEF	3.00ABCD	1.50E
2226	2.50EFG	2.00EFG	2.5FG	1.25GHI	1.50DEF	1.75FGH	4.75AB
2227	2.75DEF	3.5BCDE	2.5FG	1.63DEFGH	2.25BCD	2.00EFGH	4.00ABC
2229	3.00CDE	3.5BCDE	5.50CD	1.88BCDEF	2.50BC	3.00ABCD	1.50E
2231	3.75B	4.5BC	7.00BC	1.88BCDEF	2.25BCD	3.25ABC	1.00E
2232	6.00A	7.00A	7.50A	3.75A	4.0A	4.0A	1.00E
2234	3.00CDE	2.5DEFG	5.50CD	1.75CDEFG	1.75CDEF	3.25ABC	1.50E
2883	3.25BCD	4.00BCD	6.00BCD	2.25BC	2.50BC	3.00ABCD	1.00E
3215	3.50BC	4.00BCD	7.25AB	2.13BCD	2.75B	3.50AB	1.00E
3254	1.00I	1.00G	1.25G	1.00I	1.00F	1.00F	4.5AB
3419	3.50BC	4.0BCD	6.00DE	1.88BCDEF	2.50BC	3.00ABCD	1.75ED
3420	2.25FGH	2.00EFG	4.5DE	1.13HI	1.25EF	2.75BCDE3.25BCD	3.25BCD
3422	2.00GH	2.5DEFG	5.00DE	1.13HI	1.25EF	2.50CDEF	2.50CDE
3425	1.75H	2.25EFG	6.00BCD	1.25GHI	2.00BCDE	3.25ABC	2.25DE
3442	3.25BCD	4.00BCD	4.5DE	1.88BCDEF	2.25BCD	2.75BCDE	1.75DE
Mean	2.82	3.18	4.81	1.65	2.01	2.73	2.25
Minimum	1.00	1.00	1.25	1.00	1.00	1.00	1.00
Maximum	6.00	7.00	7.50	3.75	4.00	4.50	5.00
LSD	0.70	1.59	1.59	0.59	0.81	0.84	1.59
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Table 1. Continued.

Acession	LWI 1	LWI 2	LWI 3	LWI 4	RWC 2	RWC 4	STG
169	0.09BCD	0.13HIJKLM	0.44DEFG	0.85ABCD	0.67CDEFGH	0.36FGHIJ	2.75CDEFG
320	0.13BCD	0.12IJKLM	0.39GHI	0.56GHI	0.76ABCD	0.54AB	4.75A
391	0.00D	0.18FGHIJKLM	0.58BCDEF	0.85ABCD	0.66DEFGH	0.37EFGHIJ	2.38FGH
399	0.03D	0.13HIJKLM	0.55BCDEF	0.87ABCD	0.72ABCDEFG	0.35GHIJ	2.88CDEF
411	0.00D	0.32BCDEFG	0.49CDEFG	0.78BCDEF	0.71ABCDEFGH	0.37EFGHIJ	2.50EFG
414	0.00D	0.10JKLM	0.42DEFG	0.77BCDEF	0.72ABCDEFG	0.49ABCDE	2.63DEFG
421	0.00D	0.15GHIJKLM	0.46DEFG	0.85ABCD	0.65EFGHI	0.43BCDEFGHI	2.63DEFG
426	0.06CD	0.27CDEFGHIJK	0.65ABCD	0.78BCDEF	0.64FGHI	0.36FGHIJ	2.88CDEF
436	0.00D	0.27CDEFGHIJK	0.66ABCD	0.93AB	0.68CDEFGH	0.38DEFGHIJ	2.88CDEF
471	0.22B	0.43BCD	0.75AB	0.87ABCD	0.60HI	0.32HIJKL	2.75CDEFG
479	0.00D	0.14GHIJKLM	0.18HIJ	0.53GHI	0.79AB	0.52ABC	4.75A
517	0.09BCD	0.36BCDEF	0.75AB	0.93ABC	0.61HI	0.29JKL	1.50JK
535	0.06BCD	0.19FGHIJKLM	0.50CDEFG	0.90ABCD	0.70ABCDEFGH	0.31HIJKL	2.38FGH
544	0.03CD	0.18FGHIJKLM	0.56BCDEF	0.78BCDEF	0.67CDEFGH	0.34GHIJK	3.00CDE
570	0.14BCD	0.33BCDEFG	0.58BCDEF	0.81ABCDE	0.64GHI	0.43BCDEFGH	3.00CDE
601	0.00D	0.00M	0.16HIJ	0.43HI	0.75ABCDE	0.56A	4.63A
645	0.00D	0.00M	0.13IJ	0.41IJ	0.74ABCDEF	0.51ABCD	4.63A

753	0.16BCD	0.29BCDEFGHIJ	0.56BCDEF	0.63EFGH	0.69ABCDEFGH	0.41CDEFGHIJ	2.75CDEFG
823	0.00D	0.29BCDEFGHIJ	0.76B	0.92ABC	0.66DEFGH	0.32HIJKL	2.63DEFG
1805	0.25B	0.34BCDEFG	0.55BCDEF	0.83ABCD	0.71ABCDEFGH	0.48ABCDEF	2.38FGH
2218	0.03CD	0.31CDEFGHI	0.53BCDEF	0.85ABCD	0.69BCDEFGH	0.39DEFGHIJ	3.00CDE
2223	0.00D	0.13HIJKLM	0.44DEFG	0.85ABCD	0.67CDEFGH	0.36FGHIJ	2.75CDEFG
2226	0.00D	0.12IJKLM	0.39EFGH	0.56GHI	0.76ABCD	0.54AB	4.75A
2227	0.13BCD	0.32BCDEFGH	0.35FGHI	0.59FGHI	0.67CDEFGH	0.54AB	4.00B
2229	0.25B	0.39BCDE	0.53BCDEF	0.89ABCD	0.68CDEFGH	0.35GHIJ	3.00CDE
2231	0.00D	0.22EFGHIJKL	0.53BCDEF	0.96AB	0.71ABCDEFGH	0.33HIJK	1.75IJ
2232	0.72A	0.77A	0.88A	1.00A	0.55I	0.20L	1.88HIJ
2234	0.00D	0.22EFGHIJKL	0.52BCDEFG	0.82ABCDE	0.71ABCDEFGH	0.31IJKL	2.38FGH
2883	0.22B	0.29BCDEFGHIJK	0.48CDEFG	0.93AB	0.62GHI	0.32HIJKL	1.63J
3215	0.19BCD	0.44BC	0.59BCDEF	0.93AB	0.69BCDEFGH	0.22KL	1.00K
3254	0.00D	0.00M	0.00J	0.23J	0.81A	0.58A	4.88A
3419	0.25B	0.47B	0.63BCDE	0.84ABCD	0.64FGHI	0.39DEFGHIJ	2.75CDEFG
3420	0.00D	0.08KLM	0.28GHI	0.72DEFG	0.77ABC	0.46ABCDEFG	3.25C
3422	0.00D	0.05LM	0.28GHI	0.73CDEFG	0.75ABCD	0.43BCDEFGHI	3.13CD
3425	0.00D	0.26CDEFGHIJKL	0.64BCD	0.88ABCD	0.67CDEFGH	0.32HIJKL	2.25GHI
3442	0.16BCD	0.38BCDEF	0.71ABC	0.89ABCD	0.68CDEFGH	0.38DEFGHIJ	2.88CDEF
Mean	0.09	0.25	0.50	0.78	0.69	0.39	2.87
Minimum	0.00	0.00	0.00	0.23	0.55	0.20	1.00
Maximum	0.72	0.77	0.89	1.00	0.81	0.57	5.00
LSD	0.19	0.20	0.24	0.20	0.11	0.12	0.60
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

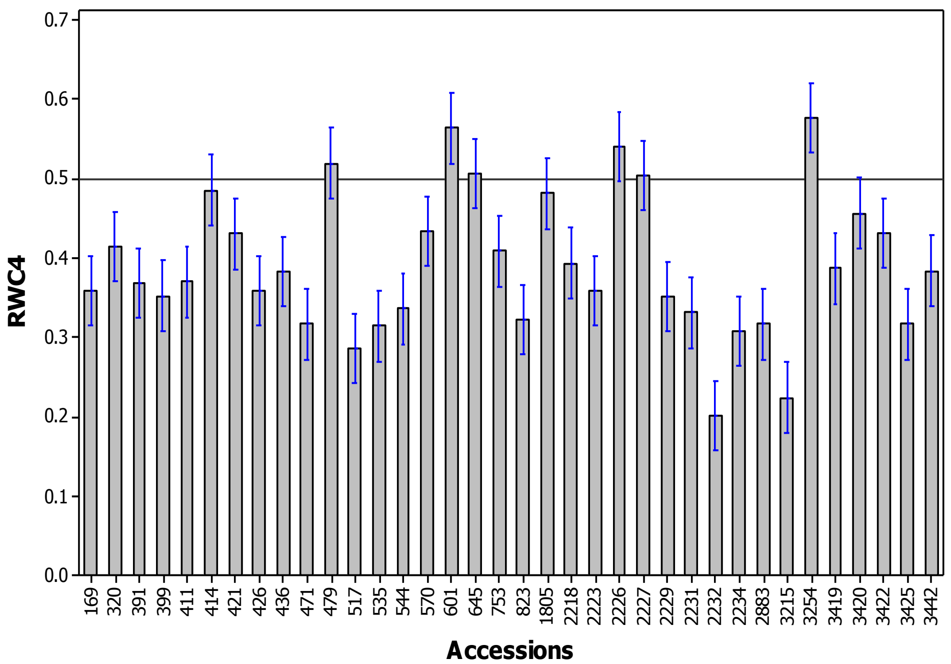

Fig 1. Classification of 36 accessions based on relative water content (RWC) after four weeks of moistures stress.

In cowpea, Sharma and Kumar (2008) reported stomata conductance, leaf water potential and osmotic adjustment as key mechanisms in maintaining water in leaf tissues. Therefore, genotypes that maintained high RWC in this study may exhibit desirable physiological characteristics, such as stomata closure, high leaf water potential and the accumulation of osmolytes, leading to survival at low moisture content of 2.9%. A lack of such desirable physiological traits in the susceptible genotypes may have contributed to significant wilting in the early stages of stress.

In this study, strong correlations between leaf wilting indices (LWI) and RWC, stem greenness, regrowth and wilting scales suggest that LWI can be used as a proxy trait for assessing drought tolerance in cowpea. Strong correlations existed between LWI and other drought tolerance traits (Table 2). In particular, the LWI negatively correlated with RWC, stem greenness and regrowth, which are reliable traits that have been extensively used in screening genotypes for drought tolerance (Agbicodo, 2009; Kumar et al., 2008; Mai-Kodomi et al., 1999; Muchero et al., 2008; Singh et al., 1999b). The strong correlations between LWI and other

Table 2. Pearson correlation coefficients between leaf wilting indices (LWI) and wilting scales (IB and MAIK), relative water content (RWC), stem greenness (STG) and regrowth.

	LWI 1	LWI 2	LWI 3	LWI 4	IB 2	IB 3	IB 4	MAI K2	MAI K3	MAIK 4	REGROW TH	RWC 2	RWC 4
WI2	0.83												
LWI3	0.48	0.77											
LWI4	0.35	0.63	0.85										
IB2	0.81	0.87	0.74	0.66									
IB3	0.75	0.85	0.71	0.65	0.91								
IB4	0.36	0.54	0.76	0.89	0.61	0.58							
MAIK2	0.91	0.89	0.63	0.54	0.94	0.89	0.54						
MAIK3	0.81	0.92	0.74	0.67	0.89	0.93	0.61	0.90					
MAIK4	0.36	0.56	0.76	0.85	0.59	0.55	0.93	0.53	0.61				
REGROWTH	-0.36*	-0.58	-0.74	-0.91	-0.63	-0.66	-0.84	-0.54	-0.64	-0.79			
RWC2	-0.62	-0.76	-0.80	-0.68	-0.71	-0.70	-0.62	-0.70	-0.75	-0.61	0.60		
RWC4	-0.42	-0.64	-0.80	-0.84	-0.64	-0.59	-0.89	-0.60	-0.67	-0.86	0.77	0.68	
STG	-0.35	-0.58	-0.75	-0.87	-0.61	-0.61	-0.92	-0.55	-0.64	-0.89	0.90	0.62	0.84

* Significant at $P<0.05$ the rest significant at $P<0.0001$.

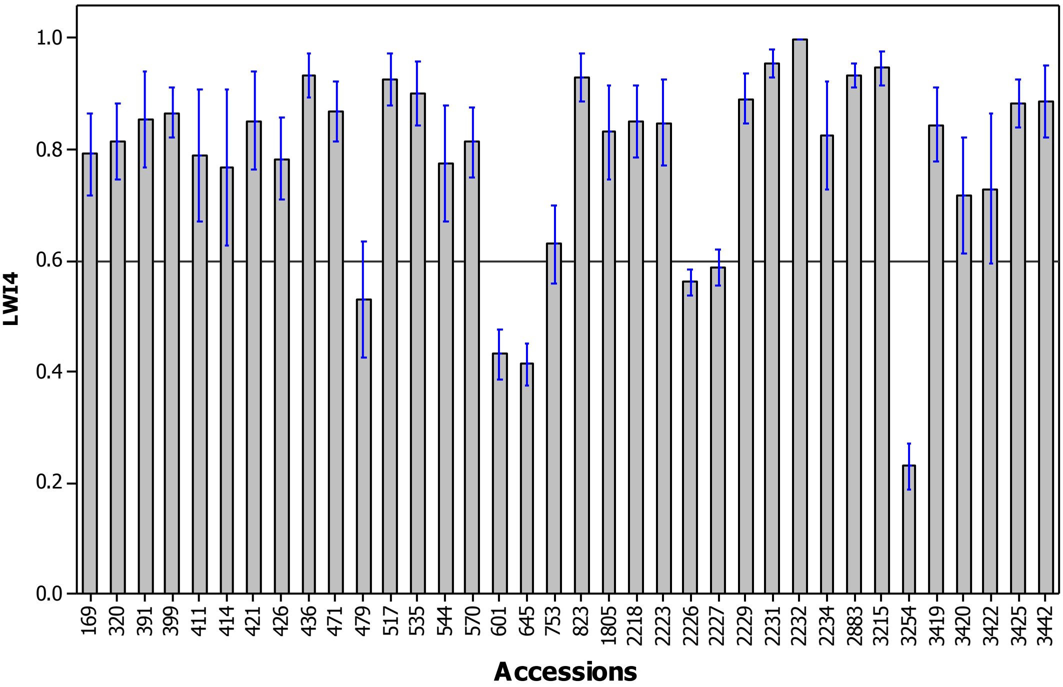

Fig 2. Classification of 36 accessions based on the leaf-wilting index (LWI) after four weeks of moisture stress.

drought tolerance traits suggest the possibility of using this as an easy measure indicator for the more difficult or slower to measure traits associated with drought tolerance in cowpea and other related crops. The values of LWI ranged between 0.00 and 1.00, where 0.00 indicates the non-existence of wilting leaves and 1.00 means that all leaves show wilting signs. The index in the fourth week quantitatively classified all the accessions between 0.23 and 1.00 for the most tolerant and susceptible genotypes, respectively. Determining the cut-off point for the drought tolerant and susceptible genotypes may provide a substantial challenge, in the absence of other evidence. In this study, we propose an index≤0.6 as the cut-off point for determining tolerant genotypes. This cut-off point is supported by results from RWC and recovery assessments. Comparison of classification of the 36 accessions, based on RWC and LWI in the final week of stress (Figures 1 and 2), show that accessions 3254, 601, 645, 479, 2226 and 2227 exhibited both RWC>0.5 and LWI <0.6. In addition to the similarity between RWC and LWI,after four weeks of stress, these accessions fully recovered from water stress (Table 1). Conclusions on an association between RWC>0.5 and recovery ability are supported by Taiz and Zeiger (1998), as reported in Abraham et al. (2004), who identified that genotypes with RWC>0.5 had high chances of recovery. They further reported that most plants undergo disruption of physiological processes when the RWC falls below 50%: and such low RWC contributes to low recovery or complete death of plants. Therefore, we conclude that an index<0.6 is a good threshold for identifying drought tolerant cowpea genotypes, after complete withdrawal of water for a period of four weeks. The LWI positively correlated with the leaf wilting scales (IB and MAIK), which have been previously used in identifying drought tolerant cowpeas (Nkouannessi, 2005; Singh et al., 1999b). LWI compared very well with wilting scales (IB and MAIK), in terms of association with regrowth, relative water content and stem greenness in cowpea (Table 2). LWI 4 strongly correlated with regrowth (r = -0.91), stem greenness (r = -0.87) and RWC 4 (r = -0.84) at $P<0.0001$. At the same level of significance, IB 4 strongly correlated with regrowth (r = -0.84), stem greenness (r = -0.92) and RWC 4 (r = -0.89), whereas MAIK 4 also strongly correlated with regrowth (r = -0.79), stem greenness (r = -0.89) and RWC 4 (r = -0.86). The similarity between LWI and wilting scales, in terms of a strong association with regrowth, stem greenness and RWC, signifies the importance of LWI in drought tolerance

Table 3. List of accessions from Malawi evaluated for drought tolerance.

Accession Number	District of origin	Longitude	Latitude	Accession Number	District of origin	Longitude	Latitude
169	Nkhatabay	34.05E	11.63S	823	Mangochi	35.45E	14.35 S
320	Rumphi	33.68E	10.95S	1805	Salima	34.47 E	13.72 S
391	Nsanje	35.28E	16.73S	2218	Chikwawa	34.78 E	16.02 S
399	Nsanje	35.18E	16.43S	2223	Chikwawa	34.87 E	16.25 S
411	Chikwawa	34.68E	16.37S	2226	Zomba	35.23 E	15.48 S
414	Chikwawa	34.92E	16.40S	2227	Zomba	35.23 E	15.48 S
421	Chikwawa	34.88E	16.08S	2229	Zomba	35.23 E	15.48 S
426	Chikwawa	34.88E	16.08S	2231	Mchinji	33.02 E	13.67 S
436	Chikwawa	34.53E	16.03S	2232	Mchinji	33.07 E	13.62 S
471	Mulanje	35.43E	16.10S	2234	Lilongwe	33.77 E	14.23 S
479	Mulanje	35.33E	16.15S	2883	Likoma	34.73 E	12.09 S
517	Mwanza	34.65E	15.48S	3215	Mzimba	33.43 E	12.10 S
535	Mulanje	35.78E	16.00S	3254	Lilongwe	33.67 E	14.23 S
544	Mulanje	35.60E	15.73S	3419	Balaka	35.13 E	14.80 S
570	Chiradzulu	35.30E	15.95S	3420	Balaka	35.14 E	14.90 S
601	Thyolo	35.25E	16.13S	3422	Chikwawa	34.48 E	15.99 S
645	Thyolo	35.15E	16.25S	3425	Chikwawa	34.77 E	15.96 S
753	Machinga	34.92E	15.05S	3442	Phalombe	35.67 E	15.61 S

evaluation. Application of the LWI would be advantageous to breeders, since it quickly and easily separates genotypes from each other through a quantitative index, in contrast to the qualitative scales, which group genotypes based on visual assessment and predefined classes. The existing wilting scales of IB and MAIK in cowpea are associated with the limitations of qualitative scoring and they require specialised expertise when scoring. In contrast, the LWI, which involves counting the total number of leaves per individual plant and the total number of leaves showing wilting signs, is easy for non-experts, if drought-wilting signs are well understood.

Although the LWI is a better measure for leaf wilting, it should only be applied to crops in which wilting is a good indicator of drought response. In cases where genotypes maintain active growth of apical meristems, by deriving water from lower leaves, the index may classify such genotypes as being susceptible. This could apply to genotypes of cowpea with a Type 2 drought tolerance mechanism identified by Mai-Kodomi et al. (1999). These genotypes derive water from their lower canopies, in order to support apical meristematic growth during water stress. Such genotypes may show relatively high values of LWI ─ and yet fully recover after re-watering. Therefore, the use of LWI in genotypes with Type 2 mechanism could be complemented by other traits, such as regrowth and stem greenness, in order to properly group genotypes into either tolerant or susceptible classes. However, genotypes with a Type 2 mechanism were not encountered in this study. Symptoms of diseases and pests may also limit application of the LWI. For this reason, prior understanding of some diseases and pest symptoms, which could be mistaken for drought wilting, should be given due consideration. This scale is not applicable to aged plants, since old leaves will naturally wilt and drop with age. Therefore, we are proposing that the application of this scale should be confined to the early vegetative stage of drought evaluation.

Materials and methods

Plant Material and Experimental Design

Thirty-six accessions of cowpea (Table 3) collected from various sites in Malawi were planted in 10 litre pots in a glasshouse during the summer period (November 2011 – January 2012) at the Plant Growth Unit, Massey University, Palmerston North, New Zealand. The experiment was laid out in a Randomised Complete Block Design (RCBD) with genotypes (accessions) as treatments, being replicated four times. Four healthy seeds were planted in each pot and after eight days from germination, the seedlings were thinned into two uniform healthy seedlings per pot.

Drought Treatment

Moisture stress was applied by watering plants until the full expansion of the first trifoliate leaves (three weeks after planting), after which water was withdrawn for four weeks, in order to take drought response measurements Muchero et al., (2008). The plants were then re-watered twice a week for a period of two weeks, before taking recovery measurements. The soil moisture content during the water stress period was monitored at 20cm depth, using a Time Domain Reflectrometer (TDR) twice weekly.

Scoring Procedure

The following parameters were recorded after stressing the plants: wilting, using both the IBPGR (IB) (IBPGR, 1983) and Mai-Kodomi (MAIK) Mai-Kodomi et al., (1999) scales: total number of leaves per plant; number of leaves showing wilting signs per plant; and relative water content (RWC). The Leaf Wilting Index (LWI) was calculated weekly, from the first week to the final week of stress, as the ratio between leaves showing wilting signs and the total number of leaves per plant. Both the IB and MAIK scales were scored on a weekly basis from the second week until the end of the stress period. Stem greenness and regrowth were scored as recovery parameters, after the two weeks of re-watering. Stem greenness was scored using a scale of 1-5, where 1 was yellow and 5 was completely green. Regrowth was scored using three categories: i.e. 1 with no re-growth; 3 with re-growth from auxiliary buds; and 5 with re-growth from the apical meristems. Relative water content (RWC) was calculated on new fully expanded leaflets after the second and fourth weeks of stress, as outlined in Bogale et al., (2011). The leaves for RWC were detached from the plant between 10am and 2pm during bright days, in order to avoid the effects of weather conditions on water loss from the detached leaves. Immediately after cutting at the base of the lamina, the leaves were weighed to obtain the fresh weight (FW).

After weighing, the leaves were soaked in deionised water for 24 hours at room temperature for rehydration: and then re-weighed for turgid weight (TW). The leaves were then dried in an oven at 70^0C for 72 hours before dry weight (DW) measurements were taken. The RWC was calculated as follows:

$$RWC = \frac{FW - DW}{TW - DW}$$

In total, 14 variables were used to assess the drought tolerance of the 36 accessions, as outlined below:

- **LWI 1**: Leaf wilting index after the first week of stress
- **LWI 2**: Leaf wilting index after the second week of stress
- **LWI 3**: Leaf wilting index after the third week of stress
- **LWI 4**: Leaf wilting index after the fourth of stress
- **IB 2**: International Board on Plant Genetic Resources scale after the second week
- **IB 3**: International Board on Plant Genetic Resources scale after the third week
- **IB 4:** International Board on Plant Genetic Resources scale after the fourth week
- **MAIK 2**: Mai-Kodomi et al. scale after the second week
- **MAIK 3**: Mai-Kodomi et al scale after the third week
- **MAIK 4**: Mai-Kodomi et al scale after the fourth week
- **RWC 2:** Relative water content after the second week
- **RWC 4:** Relative water content after the fourth week
- **STG:** Stem greenness after two weeks of re-watering
- **Regrowth:** Resumption of growth after two weeks re-watering

Statistical analysis

Data was subjected to Analysis of Variance (ANOVA) using the general linear model (GLM) procedure in the SAS 9.2 statistical package (SAS Inc., USA). Means of significantly different variables were separated using Least Significant Difference at 5% alpha level ($LSD_{0.05}$). Pearson correlation coefficients were calculated, in order to determine the associations between leaf wilting indices (LWI1, LWI2, LWI3 and LWI4) and the already known drought tolerance characteristics (wilting scales, relative water content, re-growth and stem greenness).

Conclusion

The existing wilting scales for drought tolerance in cowpea provide challenges that need to be overcome by an improvement in the scoring system. LWI has been identified as a reliable and easy method that will overcome some of the challenges associated with previous methods of scoring for wilting in cowpea and related crops. By counting individual leaves with wilting signs, the challenges associated with visual assessment would be reduced and it would require non-specific expertise in scoring for wilting. Such objective scoring, which generates a quantitative wilting index, would reduce bias when assigning genotypes into different wilting groups, as is the case with the qualitative scale. Its strong correlation with the key traits associated with drought tolerance, such as stem greenness, high relative water content, regrowth and wilting scales indicates the potential value of LWI in drought tolerance evaluation. In the present case, a cut-off point of LWI4<0.6 identified the same drought tolerant genotypes, as the much more laborious relative water content method. Despite the anticipated limitations of the Type 2 drought mechanism, together with symptoms of diseases, pests and leaf senescence due to age, LWI should be further explored in the field with other crops, in order to ascertain its application as a reliable method of scoring leaf wilting.

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