Evaluation of Drought Tolerance in Arkansas Cowpea Lines at Seedling Stage

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Abstract. Cowpea [*Vigna unguiculata* (L.) Walp.] is not only a healthy, nutritious, and versatile leguminous crop; it also has a relatively high adaptation to drought. Research has shown that cowpea lines have a high tolerance to drought, and many of them can survive more than 40 days under scorching and dry conditions. The cowpea (Southern pea) breeding program at the University of Arkansas has been active for more than 50 years and has produced more than 1000 advanced breeding lines. The purpose of this study was to evaluate the drought-tolerant ability in Arkansas cowpea lines and use the drought-tolerant lines in cowpea production or as parents in cowpea breeding. A total of 36 University of Arkansas breeding lines were used to screen drought tolerance at the seedling stage in this study. The experiment was conducted in the greenhouse using a randomized complete block design (RCBD) with two replicates, organized in a split-plot manner, where the drought treatment (drought and nondrought stress) as the main plot and the cowpea genotypes as the subplot. Drought stress was applied for 4 weeks, and three drought-tolerant-related traits were collected and analyzed. Results showed that cowpea breeding lines: ‘17-61’, ‘17-86’, ‘Early Scarlet’, and ‘ARBlackeye #1’ were found to be drought tolerant.

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adaptation to drought (Hall and Patel, 1985). However, because most cowpeas are grown in the dry, subtropic area, drought is still one of the significant abiotic constraints for cowpea production. In some drier places in Niger, the average yield is nearly 20 times less than the average yield in the United States, where water is abundant (Agbicodo et al., 2009).

Research has shown that there is a considerable difference among cowpea varieties in drought tolerance (Mai-Kodomi et al., 1999). Many cowpea breeding programs have been created to breed drought-tolerant cowpea varieties, and some cowpea cultivars were proven to be drought tolerant. For example, the cowpea cultivars Machakos 66 and Katumani 80, released by the Kenya National program, are drought-resistant varieties. Early-maturing varieties are also developed to avoid many late-stage biotic and abiotic constraints, including drought (Ehlers and Hall, 1997); however, research showed that these varieties were vulnerable to mid-season drought (Thiaw et al., 1993).

Many studies show that cowpea has a strong ability to tolerate drought in the vegetative stage; however, cowpea drought tolerance at the seedling stage has not gained much attention (Muchero et al., 2008). Because vegetative-stage drought-tolerant cowpea has been extensively studied, it is crucial to explore more seedling-stage drought-tolerant cowpea lines for future researchers to study more about the seedling stage drought-tolerant mechanism and genes in the genome of cowpea. In a water stress test, although most of the cowpea seedlings were stunted, many of them survived for 43 d under very hot and dry conditions and recovered after they were irrigated (Hall, 2012). Various studies also found that the cultivars that showed seedling stage drought tolerance had a big chance to survive the whole life cycle in the drought stress environment with a reasonable level of yield (Hall, 2012; Muchero et al., 2013; Singh et al., 1999). Because of the strong ability of cowpea to tolerate drought, and its relatively small genome size (about 620 Mb) (Arumuganathan and Earle, 1991), cowpea can be an “ideal model to study the molecular mechanisms of drought tolerance in crops” (Agbicodo et al., 2010).

The varieties with strong drought tolerance would maintain a higher photosynthetic rate under drought conditions (Reddy et al., 2004). There are two main aspects of this identification: adaptability in crop morphology and anatomy; and the adaptability of physiological and biochemical reactions, and plants. The commonly used identification indicators include morphological indicators, growth and development indicators, and physiological and biochemical indicators.

The cowpea (Southern pea) breeding program at the University of Arkansas has been active for more than 50 years and has produced more than 1000 advanced breeding lines and released more than 12 varieties (Brar et al., 1999). The varieties have been used by the processing industry and are widely grown by producers for fresh-market. The program has developed a diversity of

With global climate change and related problems intensifying, water shortage is becoming increasingly prominent. The development of agriculture is inseparable from water resources, and its sustainability is facing severe challenges. One-third of the world’s arable land has been in a state of drought or semidrought for a long time. Drought has developed into a global problem and is a major agricultural disaster. The production loss caused by drought exceeds the total loss caused by other environmental factors. A quarter of the world’s population is threatened by this problem, and most of them are from the developing countries of Africa and Asia.

Among all leguminous crops, cowpea [*Vigna unguiculata* (L.) Walp.] has a relatively high

Table 1. The chlorophyll content and chlorophyll decrease of three types of leaves in total 39 cowpea lines during 4 weeks.

		Chlorophyll content																		Chlorophyll Inflect Index (%)											
		New trifoliolate						First trifoliolate						Unifoliolate						New trifoliolate			First trifoliolate			Unifoliolate					
		Nonstress			Stress			Nonstress			Stress			Nonstress			Stress			Nonstress			Stress			Nonstress & stress					
	1	8	15	22	1	8	15	22	1	8	15	22	1	8	15	22	1	8	15	22	1	8	15	22	15	22	15	22			
Cowpea line	Source	42.23	47.34	46.77	36.90	45.77	47.03	30.77	8.84	43.32	44.61	43.55	28.65	42.92	38.47	13.59	5.80	38.47	33.74	25.58	16.00	40.84	25.53	5.70	5.20	34.2	76.1	68.8	79.8	77.7	67.5
P1255774	Susceptible control																														
09-741	AR breeding line	40.36	44.06	43.25	36.19	41.13	44.04	33.50	20.91	42.66	43.25	43.48	30.12	42.83	41.63	25.18	6.20	39.43	32.88	30.75	17.47	38.53	30.95	12.11	5.80	22.5	42.2	42.1	79.4	60.6	66.8
17-133	AR breeding line	35.60	48.30	52.20	44.20	39.70	48.40	36.50	25.78	42.20	48.20	47.40	35.70	44.68	38.50	28.18	6.00	36.70	22.30	21.60	16.00	39.08	32.70	20.50	5.30	30.1	41.7	40.6	83.2	5.3	66.9
Epic	AR breeding line	35.91	41.62	42.79	37.57	41.19	44.45	35.77	20.22	38.51	41.96	40.92	31.12	39.75	38.98	27.58	6.36	34.37	31.78	30.79	20.60	38.31	31.10	12.55	6.29	16.4	46.2	32.6	79.6	59.2	69.5
17-109	AR breeding line	40.61	47.70	46.48	40.75	42.51	44.21	34.38	15.33	42.10	44.77	44.64	35.62	41.34	40.06	29.81	5.90	38.06	36.96	35.68	26.59	37.87	31.17	10.04	5.50	26.0	62.4	33.2	83.4	71.8	79.3
17-129	AR breeding line	40.23	42.90	45.73	40.00	41.97	45.45	36.11	22.98	41.00	44.14	46.00	37.31	41.77	40.23	34.51	8.33	41.04	34.92	33.71	30.45	41.13	31.94	22.22	8.50	21.0	42.6	25.0	77.7	34.1	72.1
17-131	AR breeding line	27.80	36.30	39.30	31.05	40.59	46.80	38.71	29.58	32.10	40.90	42.50	35.70	39.99	33.80	31.29	12.83	35.65	35.80	31.80	16.00	36.39	29.60	16.96	5.70	1.5	4.7	26.4	64.1	46.7	64.4
17-135	AR breeding line	41.52	45.33	47.18	39.28	43.12	48.10	41.22	26.83	43.97	45.28	48.90	33.08	43.43	44.31	32.57	9.92	40.62	38.81	32.97	18.54	39.13	33.18	5.70	5.60	12.6	31.7	33.4	70.0	82.7	69.8
17-81	AR breeding line	38.08	41.72	44.39	39.48	40.28	45.58	40.31	34.82	41.48	41.01	40.21	33.89	41.14	39.16	26.27	10.29	39.74	34.42	32.73	21.61	37.39	27.96	7.45	5.80	9.2	11.8	34.7	69.6	77.2	73.2
16-166	AR breeding line	36.58	43.64	42.75	40.04	38.21	41.34	35.87	24.20	39.85	44.07	43.59	37.37	38.72	37.59	28.19	10.08	36.90	34.92	34.04	23.72	35.07	29.42	8.99	5.20	16.1	39.6	35.3	73.0	73.6	78.1
EarlyScarlet	AR breeding line	46.84	45.28	48.23	34.48	45.67	50.08	43.70	29.32	42.39	43.55	42.25	33.10	45.82	41.22	30.92	5.80	39.03	40.23	36.40	28.95	36.98	31.68	28.00	5.30	9.4	15.0	26.8	82.5	23.1	81.7
Empire	AR breeding line	37.04	45.23	47.75	44.22	39.70	43.78	39.84	27.71	42.05	45.47	47.96	38.20	41.18	39.35	35.77	17.67	38.07	36.76	34.71	31.65	36.73	31.38	19.19	9.33	16.6	37.3	25.4	53.8	44.7	70.5
09-692	AR breeding line	44.59	48.15	49.39	39.89	39.49	46.83	37.01	24.73	45.19	47.05	47.68	39.30	40.16	38.24	29.53	10.71	39.68	38.47	38.34	32.73	36.53	28.85	25.80	5.30	25.1	38.0	38.1	72.8	32.7	83.8
P1293568	Tolerant control	45.75	49.20	44.31	39.85	46.03	46.44	36.62	34.58	42.81	41.63	41.96	36.44	43.81	41.64	37.98	25.88	37.85	35.77	34.67	25.84	40.82	33.14	19.78	5.90	17.3	13.2	9.5	29.0	43.0	77.2
17-117	AR breeding line	43.01	49.43	48.34	41.16	43.75	46.70	38.98	26.00	39.44	44.39	43.58	34.38	43.60	40.10	29.08	7.21	40.38	41.43	40.60	34.49	42.40	33.80	24.58	7.98	19.4	36.8	33.3	79.0	39.5	76.9
ARblackeye#1	AR breeding line	40.97	46.82	50.26	46.45	39.67	47.30	42.17	33.58	43.86	47.44	50.64	42.13	43.08	42.26	39.54	23.80	41.89	40.46	40.36	32.41	40.77	36.81	31.62	13.63	16.1	27.7	21.9	43.5	21.7	58.0
09-529	AR breeding line	45.91	50.53	49.28	44.29	47.25	46.15	44.38	26.03	47.16	48.78	46.95	45.34	47.04	40.74	39.64	16.45	43.93	42.25	38.44	32.46	41.74	33.53	31.02	15.66	9.9	41.2	15.6	63.7	19.3	51.8
P1349674	Tolerant control	43.73	48.11	47.79	41.55	44.79	47.78	40.46	29.45	42.35	44.75	46.99	35.13	43.73	42.16	38.01	19.94	40.93	37.86	37.54	14.63	39.56	33.78	21.31	7.33	15.3	29.1	19.1	43.2	43.2	49.9
EarlyAcre	AR breeding line	42.55	48.32	52.05	48.08	42.36	43.79	35.33	22.16	47.77	47.66	50.62	41.06	47.39	40.10	31.47	14.86	44.99	40.40	35.98	25.58	41.51	31.93	15.77	5.40	32.1	53.9	37.8	63.8	56.2	78.9
EarlySetSelect	AR breeding line	43.42	46.57	47.02	43.73	46.31	45.50	40.13	27.79	44.12	45.28	47.86	41.05	47.99	44.07	38.46	12.75	38.35	36.32	38.08	25.20	40.11	30.38	16.37	5.50	14.7	36.5	19.6	68.9	57.0	78.2
01-1781	AR breeding line	37.84	40.44	37.82	37.22	40.95	40.14	37.12	25.62	38.00	40.57	40.28	35.32	40.80	35.69	29.17	6.75	33.93	32.44	36.04	28.18	33.71	26.97	22.96	9.84	1.8	31.2	27.6	80.9	36.3	65.1
Ebony	AR breeding line	39.11	40.89	41.40	34.13	43.53	41.16	36.15	26.21	37.37	40.92	41.13	32.78	42.63	36.20	31.24	14.18	36.97	32.47	34.15	23.58	35.28	25.54	16.13	5.40	12.7	23.2	24.0	56.8	52.8	77.1
17-118	AR breeding line	45.00	51.48	50.04	46.60	44.73	43.28	36.30	27.54	43.22	48.80	45.90	40.68	45.67	40.11	33.07	15.55	42.08	40.55	38.22	32.93	40.55	29.48	17.01	5.80	27.5	40.9	28.0	61.8	55.5	82.4
09-211	AR breeding line	39.21	44.66	42.70	41.29	47.35	44.03	39.21	30.58	39.71	42.27	42.51	36.86	45.95	40.39	33.31	10.33	38.55	36.07	38.10	29.94	40.24	32.32	25.64	5.90	8.2	25.9	21.6	72.0	32.7	80.3

(Continued on next page)

Table 1. (Continued) The chlorophyll content and chlorophyll decrease of three types of leaves in total 39 cowpea lines during 4 weeks.

Cowpea line	Source	Chlorophyll content												Chlorophyll Infect Index (%)											
		New trifoliolate						First trifoliolate						Unifoliolate						First trifoliolate					
		Nonstress			Stress			Nonstress			Stress			Nonstress			Stress			Nonstress & stress			Nonstress & stress		
		1	8	15	22	1	8	15	22	1	8	15	22	1	8	15	22	1	8	15	22	1	8	15	22
16-181	AR breeding line	41.92	45.37	42.99	36.40	38.95	38.93	33.83	23.18	42.39	43.82	41.78	32.56	41.07	33.63	21.65	5.80	39.09	35.43	32.00	20.48	36.57	21.78	12.66	5.70
07-303	AR breeding line	38.48	43.67	42.73	38.83	37.49	43.60	41.42	36.29	37.82	41.88	40.58	35.98	38.06	33.00	23.43	11.83	35.96	33.98	32.24	23.82	35.01	24.76	8.53	5.90
17-127	AR breeding line	37.30	46.50	40.80	33.08	41.62	40.11	33.01	19.72	39.00	43.40	35.63	28.01	40.79	33.97	26.17	7.84	34.20	32.22	33.97	19.80	34.89	20.62	5.70	5.10
17-40	AR breeding line	41.28	45.70	35.05	31.98	50.53	46.96	37.29	23.35	39.42	40.90	38.45	35.98	46.58	39.17	30.44	7.65	37.10	32.90	25.67	26.63	39.64	30.86	15.58	8.50
17-61	AR breeding line	34.81	47.29	49.58	49.41	44.78	45.49	42.76	26.41	42.71	47.33	48.18	41.47	48.34	45.34	42.92	14.03	40.17	40.61	35.25	35.34	41.49	35.71	21.39	18.73
09-393	AR breeding line	44.37	45.28	42.23	37.14	46.84	46.81	34.78	17.91	40.72	43.35	37.63	33.43	42.11	37.09	22.62	7.05	39.72	34.82	36.72	20.55	37.38	28.39	28.45	6.39
09-714	AR breeding line	36.40	48.40	45.60	46.90	40.03	39.73	33.90	27.48	42.00	47.10	43.70	43.60	40.25	36.83	29.89	7.86	39.20	42.80	37.98	23.28	37.63	32.89	11.81	5.70
17-102	AR breeding line	31.58	50.68	47.80	39.88	41.62	47.48	41.55	23.19	42.20	47.50	45.20	31.72	43.67	42.83	34.25	7.27	39.10	38.98	30.65	16.00	39.90	34.27	22.65	5.30
17-107	AR breeding line	42.38	48.06	50.92	48.99	37.68	45.75	37.55	20.81	44.00	50.40	51.86	43.18	43.33	43.70	36.07	11.30	43.91	42.82	32.38	30.28	39.66	33.67	9.75	13.97
17-111	AR breeding line	45.29	48.98	49.21	44.32	42.03	42.28	37.13	17.31	44.30	45.81	44.79	38.30	44.07	37.69	25.15	5.80	39.98	37.49	40.88	30.48	35.23	30.99	23.13	5.50
17-114	AR breeding line	42.40	47.41	45.07	43.13	45.42	43.57	34.04	19.22	40.95	43.10	41.19	37.29	43.96	39.08	27.87	9.00	39.06	38.59	38.09	27.75	39.73	30.41	14.42	5.50
17-124	AR breeding line	42.85	47.78	47.72	53.47	40.41	45.44	43.25	33.21	45.75	51.10	54.19	42.30	45.35	42.92	39.33	19.50	44.58	45.12	41.72	30.35	42.25	37.51	20.58	5.70
17-128	AR breeding line	43.40	44.45	42.64	42.15	43.14	41.47	34.51	22.10	41.05	41.49	40.44	38.16	43.92	38.14	31.83	12.38	37.63	35.70	42.70	24.14	37.19	29.83	20.21	7.73
17-137	AR breeding line	46.27	48.95	46.29	49.09	49.48	45.08	43.62	27.90	43.98	46.79	45.24	43.35	45.68	41.03	35.54	14.35	44.33	41.64	37.00	25.82	45.95	40.02	25.94	5.60
17-86	AR breeding line	36.73	43.69	44.19	42.33	41.14	45.54	42.50	33.62	38.33	42.77	43.12	38.85	42.21	39.14	28.40	12.14	38.19	36.42	33.58	16.58	37.98	29.96	18.75	5.20

AR = Arkansas; 1, 8, 15, and 22 = days after drought stress (week 0, week 1, week 2, and week 3).

cowpea types with a range of mature seed patterns and colors, including blackeye, pink-eye, and red holstein, and various seedcoat colors, including black, brown, green, cream, and yellow. However, the seedling stage drought-tolerance ability of that group of cowpea lines remains unknown. The purpose of this study was to evaluate the drought-tolerant ability in Arkansas cowpea lines to use drought tolerance in cowpea breeding. The specific research objectives of this research are to evaluate drought tolerance in cowpea lines at the seedling stage under greenhouse conditions.

Materials and Methods

Plant material. A total of 36 Arkansas cowpea lines were used in this study for drought-tolerance evaluation. Also, two previously reported drought-tolerant lines ‘PI293568’ and ‘PI349674’, and one drought-susceptible line, ‘PI255774’ (Ravelombola et al., 2018) were used as controls (Table 1).

Greenhouse evaluation for drought tolerance. The indoor evaluation was carried out in the greenhouse at the Arkansas Agricultural Research and Extension Center, Fayetteville, AR. During the experiment, the day/night temperatures in the greenhouse was maintained at 25 °C/20 °C. The screening method was similar to the “Wooden Box” screening methodology established by Singh et al. (1999) and Verbree et al. (2015) with minor modifications (Ravelombola et al., 2018). All cowpea lines were planted in Sterilite polypropylene boxes (Sterilite Corporation, Townsend, MA; dimensions 88.6 cm × 42.2 cm × 15.6 cm) that were filled with Sunshine Mix #1 Natural & Organic (Agawan, MA) up to 10.5 cm high; 6 L of tap water was added into each box 2 d before planting to make sure that the potting mix was at its field capacity stage when seeds were planted.

The experimental design was an RCBD with two replicates per genotype, organized

in a split-plot manner, with the drought treatment as the main plot and the cowpea genotypes as the subplot. For each replicate, the 39 cowpea lines (36 tested lines plus three controls) were planted with a total of eight boxes, where four boxes were used as the control with watering without drought stress, and the other four boxes as the treatment group with drought stress (Fig. 1).

For each box, a total of ten 7.5-cm spaced rows were designed across the box length. Each testing cowpea line was planted within each row. A total of six uniform and vigorous plants were kept at each row when the first trifoliate leaf began to expand. Fertilizer (Miracle-Gro, Detroit, MI) was applied 1 week after the emergence of cowpea seed from the medium. For each row in every box, 150 mL of tap water was irrigated every 3 d until the first trifoliate leaf was fully developed. Drought stress was imposed for the stress treatment by stopping water irrigation when the first trifoliate was utterly expanded, and water stress lasted for 4 weeks (week 0 to week 3) until some cowpea genotypes were utterly dead, indicating susceptibility to drought stress. The control (unstressed of drought) treatment was irrigated with 150 mL of tap water every 3 d continuously.

Measurements. Three drought-related traits, chlorophyll content in trifoliate and unifoliate leaves, overall plant healthiness, and the main stem lodging, were measured from the 39 cowpea lines.

Leaf chlorophyll content was measured using the SPAD-502 Plus Chlorophyll Meter (Spectrum Technologies, Inc., Plainfield, IL). For each plant, three types of leaves: unifoliate (U), first trifoliate (T), and new (second) trifoliate (NT) were measured separately (Fig. 2). Measurements were taken weekly after drought stress was imposed. For each leaflet of the unifoliate or trifoliate, measurements were taken three times to avoid edge effect, and the average of the three measurements was kept as the final chlorophyll

content value. To evaluate the drought tolerance in each cowpea line by chlorophyll content change, the parameters Chlorophyll Inflect Index (González, 1996), was estimated using the following formulas: Chlorophyll absolute decrease (CD) = CC – CS; Chlorophyll Inflect Index (CII) = 100%* CD/(CC), where CC = chlorophyll content for control, and CS = chlorophyll content for drought stress.

The overall plant’s healthiness scores were measured using a 1 to 5 scale (1 = plant completely green, 2 = new trifoliate completely green with chlorotic unifoliate and trifoliate, 3 = new trifoliate is chlorotic, 4 = severe signs of necrosis on all leaves with a green growing tip, and 5 = dead plants) (Fig. 3). Overall plant healthiness was measured twice per plant basis in the third and fourth week after first applying drought stress. To reduce the error in the statistical analysis, we converted the score value into a percentage for calculation. The “Healthiness” value was calculated as follows: 100% * (5-Overall plant healthiness score-1)/4. To

Fig. 2. Three types of leaf for chlorophyll content. U = unifoliate leaf; T = first trifoliate leaf; NT = new trifoliate leaf.

Fig. 1. Greenhouse phenotyping experiments for cowpea drought tolerance: drought stress was applied for (A) 1, (B) 8, (C) 15, and (D) 22 d.

Fig. 3. Overall plant healthiness assessed on a 1 to 5 scale: 1 = green (100% health), 2 = green new trifoliate with chlorotic unifoliate and first trifoliate (75% health), 3 = chlorotic new trifoliate with necrotic unifoliate and first trifoliate (50% health), 4 = severe signs of necrosis on all leaves with a green growing tip (25% health), 5 = dead plants (0% health).

see how healthiness changed over weeks, the parameter of Healthiness Infect Index (HII) was used with the following formulas: Healthiness absolute decrease (HD) = HC – HS; HII = 100%* HD/(HC), where HC =

Healthiness for control, and HS = Healthiness for drought stress.

The main stem lodging scores were measured using a 1 to 3 scale (1 = vigorous, green main stem, 2 = mildly wilting and light green

main stem, and 3 = completely lodged and yellow main stem) (Fig. 4). The main stem lodging score was assessed on a per-plant basis in the fourth week after the drought stress was initiated. The “Lodging” value was calculated as follows: Lodging = 100%* (3-Overall plant lodging score-1)/2. To estimate how the lodging increased over weeks, the parameter “Lodging Infect Index” (LII) was used with the following formulas: Lodging absolute increase (LI) = LS – LC; LII = 100%* LI/ (1 – LC), where LC = Lodging for control, LS = Lodging for drought stress.

Phenotypic data analysis. The cowpea drought-tolerant data were analyzed using analysis of variance (ANOVA) with the general linear models procedure of JMP Genomics 9 (SAS Institute, Cary, NC). The distribution of the data was drawn using “Distribution,” and the scatter plot matrix graphs for chlorophyll content were generated by JMP Genomics 9 (SAS Institute). The broad-sense heritability (H^2) was estimated using the following formula:

$$H^2 = 100 * (\sigma_G^2 / \sigma_P^2)$$

$$= 100 * \left(\frac{\sigma_G^2}{\sigma_G^2 + \sigma_e^2} \right),$$

where σ_P^2 is phenotypic variance, σ_G^2 is genotypic variance, and σ_e^2 is variance associated with the experimental error; σ_G^2 and σ_e^2 were obtained using the following formula:

$$\sigma_G^2 = (\text{MSG} - \text{MSE}) / n0;$$

$$n0 = (\text{observations} / \text{blocks in the study})$$

$$\sigma_e^2 = \text{MSE}.$$

Canonical discriminant analysis and drought tolerance index. To fully assess drought tolerance, we need to standardize

Fig. 4. Plant main stem lodging score assessed on a 1 to 3 scale: 1 = vigorous, green main stem (0% lodging); 2 = mildly wilting, light green main stem (50% lodging); 3 = completely lodged, yellow main stem (100% lodging).

Fig. 5. Chlorophyll content change of unifoliate, trifoliate, new trifoliate under drought stress (green plot) and nonstress (red plot) in 4 weeks.

Table 2. The healthiness, healthiness change, lodging and lodging increase in total 39 cowpea lines during 4 weeks,

	Cowpea line	Source	Healthiness (%)										Healthiness Inlect Index (%)										Lodging (%)						Lodging Inlect Index (%)											
			Nonstress					Stress					Nonstress & stress					Nonstress					Stem					Nonstress & stress												
			1					8					15					22					1					8					15					22		
			1	8	15	22	22	1	8	15	22	22	1	8	15	22	22	1	8	15	22	22	1	8	15	22	22	1	8	15	22	22	15	22						
	P1255774	Susceptible control	98.6	96.8	95.3	93.3	93.3	85.5	90.8	33.4	14.5	84.5	65.0	4.0	2.5	5.8	0.0	2.5	4.0	5.8	0.0	10.0	33.3	79.2	25.3	67.6														
	09-741	AR breeding line	91.0	99.4	91.3	99.6	94.1	91.9	62.5	43.8	56.1	31.5	56.1	0.0	0.0	3.4	0.0	0.0	4.0	3.4	0.0	10.0	26.7	64.6	18.7	57.9														
	17-133	AR breeding line	95.1	94.3	92.5	92.6	96.4	94.3	47.9	41.8	54.9	48.2	54.9	0.0	0.0	2.5	3.9	5.0	0.0	2.5	3.9	5.0	26.7	68.7	21.7	60.8														
	Epic	AR breeding line	95.5	92.1	96.1	95.3	93.3	98.5	54.3	45.8	52.0	43.6	52.0	0.0	0.0	3.4	4.3	0.0	0.0	3.4	4.3	0.0	5.0	20.0	60.5	51.8														
	17-109	AR breeding line	96.4	99.3	93.4	96.1	83.4	89.0	41.8	18.8	80.5	55.3	80.5	0.0	0.0	2.5	4.0	2.5	0.0	2.5	4.0	2.5	10.0	31.7	83.4	78.4														
	17-129	AR breeding line	95.3	96.1	91.3	98.1	90.6	95.1	58.3	33.4	36.2	66.0	66.0	0.0	0.0	2.5	2.8	4.5	0.0	2.5	2.8	4.5	0.0	7.5	16.7	58.3	11.1	49.4												
	17-131	AR breeding line	92.4	95.8	95.5	95.1	90.9	91.0	56.3	47.9	41.1	49.7	41.1	2.5	2.5	4.7	4.0	0.0	3.3	15.0	45.8	5.7	37.8	37.8	37.8															
	17-135	AR breeding line	99.1	97.0	93.0	90.8	75.3	96.9	62.5	54.1	32.8	40.4	40.4	0.0	0.0	2.5	5.7	2.5	5.0	6.2	15.0	39.6	3.5	34.6																
	17-81	AR breeding line	96.8	98.1	95.5	95.3	88.6	96.5	68.8	66.8	56.6	31.7	56.6	2.5	2.5	2.8	3.1	0.0	2.5	2.8	3.1	0.0	10.0	26.7	64.6	21.0	58.4													
	16-166	AR breeding line	94.6	93.9	97.5	91.3	86.8	93.1	66.6	39.6	31.7	56.6	31.7	56.6	0.0	0.0	6.1	4.0	0.0	6.1	4.0	0.0	3.3	15.0	35.5	2.8	27.5													
	EarlyScarlet	AR breeding line	94.4	91.1	96.0	96.0	90.3	97.8	77.0	68.8	19.8	28.4	34.4	2.5	0.0	5.2	6.6	0.0	0.0	5.2	6.6	0.0	2.7	20.0	37.5	9.7	24.4													
	Empire	AR breeding line	93.6	99.0	92.8	95.3	94.9	98.9	72.9	62.5	21.4	34.4	2.5	0.0	5.2	6.6	0.0	0.0	5.2	6.6	0.0	2.7	20.0	37.5	9.7	24.4														
	09-692	AR breeding line	95.3	94.9	94.6	97.3	90.6	91.4	45.9	31.3	51.5	67.9	51.5	0.0	0.0	2.9	6.5	5.0	10.0	28.3	70.9	22.5	57.8	57.8																
	P1293568	Tolerant control	97.6	94.8	91.6	92.5	78.6	91.6	83.3	68.8	9.1	25.7	9.1	25.7	2.5	0.0	4.8	5.7	0.0	4.8	5.7	0.0	4.0	20.0	39.6	10.5	28.3													
	17-117	AR breeding line	96.5	96.6	92.4	95.9	91.3	95.5	54.1	43.8	41.4	54.4	41.4	2.5	0.0	4.9	2.5	2.5	0.0	4.9	2.5	2.5	6.0	26.7	62.5	16.8	57.5													
	ARBlackeye#1	AR breeding line	96.8	96.0	97.3	91.3	90.8	97.6	79.3	66.8	18.5	26.8	18.5	0.0	2.5	6.4	4.8	0.0	2.5	6.4	4.8	0.0	2.7	13.3	33.3	0.5	23.7													
	09-529	AR breeding line	96.8	94.0	94.3	97.5	84.5	87.9	56.3	45.9	40.3	52.9	40.3	0.0	0.0	3.3	6.4	0.0	0.0	3.3	6.4	0.0	4.0	23.3	47.9	16.7	35.0													
	P1349674	Tolerant control	96.5	93.8	95.1	96.3	85.4	95.3	89.6	73.0	5.8	24.2	5.8	24.2	0.0	2.5	6.9	3.9	0.0	2.5	6.9	3.9	0.0	5.3	26.7	43.7	12.9	36.0												
	EarlyAcre	AR breeding line	95.8	93.1	98.3	98.5	84.1	94.4	54.1	39.6	44.9	44.9	59.8	2.5	0.0	0.0	4.5	5.0	0.0	0.0	4.5	5.0	5.0	10.0	56.3	10.0	47.4													
	EarlySetSelect	AR breeding line	93.0	95.6	92.0	93.8	90.1	92.5	66.8	58.3	27.4	37.9	27.4	37.9	2.5	0.0	4.6	5.3	0.0	2.5	4.6	5.3	0.0	5.0	15.0	25.0	5.7	14.4												
	01-1781	AR breeding line	91.5	93.6	97.1	95.1	80.6	90.4	62.5	48.0	35.6	49.5	35.6	49.5	0.0	2.5	5.7	3.7	5.0	4.0	20.0	54.1	8.6	46.8																
	Ebony	AR breeding line	93.3	94.4	97.9	95.9	89.0	96.3	66.8	54.1	31.8	43.5	31.8	43.5	0.0	2.5	6.6	3.8	2.5	6.0	28.3	64.6	15.2	57.0																
	17-118	AR breeding line	96.0	91.0	95.3	94.0	86.6	91.6	50.0	37.5	47.5	47.5	60.1	0.0	0.0	5.6	3.1	2.5	10.0	33.3	70.9	22.1	64.6																	
	09-211	AR breeding line	94.6	92.6	97.4	93.8	84.0	88.9	54.1	52.0	44.4	44.5	44.5	0.0	0.0	4.9	5.2	5.0	12.2	40.0	77.1	30.1	66.7																	
	16-181	AR breeding line	94.0	97.1	98.5	97.8	91.5	99.1	58.4	43.8	40.7	55.2	40.7	0.0	2.5	5.6	0.0	0.0	2.5	5.6	0.0	0.0	5.0	26.7	60.4	15.5	60.4													
	07-303	AR breeding line	97.0	91.8	94.9	96.4	83.3	88.3	68.8	62.5	27.5	35.1	27.5	2.5	2.5	3.2	2.5	5.0	8.0	40.0	70.9	33.5	65.9																	
	17-127	AR breeding line	94.0	94.8	95.1	97.4	85.3	93.1	75.0	60.4	21.2	38.0	21.2	38.0	0.0	0.0	2.5	0.0	0.0	2.5	0.0	0.0	8.8	28.3	50.0	23.3	50.0													
	17-40	AR breeding line	94.9	95.0	97.4	93.8	81.0	96.8	58.3	52.1	40.2	44.4	40.2	2.5	2.5	4.1	5.0	8.0	40.0	72.9	35.0	64.8																		
	17-61	AR breeding line	92.3	95.4	99.4	99.3	96.6	91.0	68.8	65.0	30.8	34.5	30.8	2.5	0.0	0.0	3.2	0.0	0.0	3.2	0.0	4.0	16.7	40.0	16.7	29.7														
	09-393	AR breeding line	95.8	95.0	94.9	96.1	84.9	95.3	64.5	43.8	32.0	54.5	32.0	54.5	0.0	0.0	0.0	3.2	0.0	3.2	3.4	0.0	5.3	25.0	64.6	18.6	37.4													
	17-102	AR breeding line	93.3	96.1	95.8	91.6	86.1	96.6	45.9	43.8	52.1	52.3	52.1	52.3	0.0	0.0	3.2	0.0	0.0	3.2	3.4	0.0	5.3	25.0	64.6	18.6	37.4													
	09-714	AR breeding line	94.9	92.9	96.1	98.4	88.8	95.9	73.0	35.4	24.1	64.0	24.1	64.0	0.0	0.0	3.2	2.5	0.0	3.2	2.5	0.0	3.3	18.3	52.1	12.0	47.1													
	17-107	AR breeding line	95.8	95.0	94.9	96.1	84.9	95.3	64.5	43.8	32.0	54.5	32.0	54.5	0.0	0.0	0.0	3.2	0.0	3.2	3.4	0.0	5.3	25.0	64.6	18.6	37.4													
	17-111	AR breeding line	96.1	95.4	93.1	94.8	80.4	97.6	50.0	31.3	46.3	67.0	46.3	67.0	0.0	2.5	4.9	3.2	0.0	2.5	4.9	3.2	0.0	9.5	30.0	73.0	20.2	66.6												
	17-114	AR breeding line	97.6	95.9	95.8	94.5	87.5	90.1	54.1	43.8	43.5	53.7	43.5	53.7	2.5	2.5	5.1	4.6	2.5	5.1	4.6	2.5	6.7	31.7	75.0	21.5	65.9													
	17-124	AR breeding line	93.6	91.9	94.6	95.5	90.9	94.0	68.8	50.0	27.3	47.6	27.3	47.6	0.0	2.5	6.0	2.5	0.0	2.5	6.0	2.5	0.0	5.0	31.7	64.6	19.6	59.6												
	17-128	AR breeding line	93.8	93.0	92.0	96.0	83.1	89.5	60.4	41.6	34.4	56.6	34.4	56.6	0.0	0.0	5.3	6.5	0.0	5.3	6.5	0.0	8.8	26.7	66.7	16.1	53.6													
	17-137	AR breeding line	92.4	95.5	97.1	96.4	95.6	89.0	79.1	56.3	18.5	41.6	18.5	41.6	2.5	0.0	4.7	2.5	0.0	4.7	2.5	0.0	5.0	31.7	58.3	22.2	53.3													
	17-86	AR breeding line	94.8	95.4	91.5	94.3	87.8	88.9	75.0	72.9	18.0	22.7	18.0	22.7	0.0	2.5	2.5	5.7	0.0	2.5	5.7	0.0	7.5	23.3	33.3	18.3	22.0													

three indicators (lodging increase, chlorophyll decrease, and healthiness change) before the final evaluation. The same evaluation weight was used to evaluate the change of chlorophyll content in three types of leaf (unifoliolate, first trifoliolate, and new trifoliolate), and this value was finally evaluated comprehensively. In this study, we used two methods to assess the drought tolerance of each line: 1) canonical discriminant analysis was conducted by JMP 13.0 to draw the scatter distribution for 39 lines based on context parameters; and 2) all breeding lines were scored by their orders in three drought-tolerance parameters one by one, according to the tolerance from small to large (the most tolerance line shall be scored as 1 and most susceptible as 39). Then we added three scores for each line, respectively, to comprehensively evaluate drought tolerance.

Results

Chlorophyll content in trifoliolate and unifoliolate leaves. The drought stress was imposed for 4 weeks (25 Mar. to 22 Apr.), and chlorophyll content difference started to show from the second week (1 Apr. to 8 Apr.). Across the 39 cowpea lines, all three types of leaf (unifoliolate, first trifoliolate, and new trifoliolate) had a significant chlorophyll content drop during the last 2 weeks with drought stress. Overall, unifoliolate and first trifoliolate chlorophyll contents dropped more than new trifoliolate over time; few cowpea lines managed to remain their new trifoliolate chlorophyll at the higher level. During the first 2 weeks, the average chlorophyll increased slightly, after that, the chlorophyll contents decreased sharply with drought stress (Fig. 5). Finally, the average chlorophyll content in unifoliolate, first trifoliolate, and new trifoliolate dropped from 38.6 to 7.0, 43.4 to 11.2, and 40.7 to 25.5, respectively. The chlorophyll contents of unifoliolate, first trifoliolate, and new trifoliolate after 4-week drought stress varied from 5.4 to 22.5, 5.2 to 27.2, and 7.8 to 39.1, respectively. Six cowpea lines: '17-81' (34.8), 'PI293568' (34.6 drought-tolerant control), 'ARBlackeye#1' (33.6), '07-303' (36.3), '17-124' (33.2), and '17-86' (33.6) have the highest new trifoliolate chlorophyll content (Table 1).

The CII indicates how much the chlorophyll content of that cowpea plant leaf has changed (as percentage) in the drought stress condition compared with the same genotype, healthy cowpea plant in control without drought stress but watering. The higher the CII indicates more chlorophyll content lost compared with the healthy plant (i.e., the higher the CII value, the more susceptible the cowpea line is). The CII value for most cowpea lines started to rise significantly in 2 weeks after the drought stress was imposed. At the end of the fourth week, the CII of unifoliolate, first trifoliolate, and new trifoliolate value for each cowpea line varied from 47.0 to 83.8, 29.0 to 84.9, and 4.7 to 76.1, respectively. Among the 39 cowpea lines, 'PI349674', '17-109', and '17-111' had the

highest CII value, indicating their inability to keep their chlorophyll content stable in the drought stress and showing that their susceptibility to drought stress condition. On the other hand, six cowpea lines, 'PI349674', 'ARBlackeye#1', '09-529', 'PI293568', '17-131', and '17-61' had relatively low CII values, indicating that the six lines were drought tolerant (Table 1).

Overall plant healthiness. Overall plant healthiness scores were recorded in 4 weeks after the drought stress treatment. The plants without stress had an excellent growth con-

dition, whereas the healthiness of stressed plants dropped rapidly in the last 2 weeks (Fig. 6). For week 2, the over plant healthiness varied from 25.0 to 91.8, with an average of 61.8 and an sd of 14.4. For week 3, the plant healthiness score varied from 8.3 to 79.3, with an average of 48.6 and an sd of 16.1 (Table 2). The plant healthiness change was generated using the last 2 weeks of data. The higher value percentage meant more healthiness the plant had kept in the drought stress condition, suggesting the cowpea line was drought tolerant. The healthiness change

Fig. 6. Healthiness change of plant under drought stress (green plot) and nonstress (red plot) in 4 weeks.

Fig. 7. Lodging change of plant under drought stress (green plot) and nonstress (red plot) in 4 weeks.

Table 3. Analysis of variance for chlorophyll content, stem lodging, and plant healthiness.

Trait	Stress	Description	Time	Source	DF	Sum of squares	Mean square	F ratio	Prob > F
Chlorophyll content	Nonstress	New trifoliate	1	Model	38	1,340.87	35.29	1.4279	0.1363
				Error	39	963.79	14.71		
				C. Total	77	2,304.66			
			8	Model	38	758.80	19.97	1.8874	0.0258
				Error	39	412.61	10.58		
				C. Total	77	1,171.41			
			15	Model	38	1,149.13	30.24	2.5423	0.0023
				Error	39	463.91	11.90		
				C. Total	77	1,613.04			
			22	Model	38	2,018.10	53.11	4.6896	0.0000
				Error	39	441.66	11.32		
				C. Total	77	2,459.76			
		First trifoliate	1	Model	38	654.40	17.22	3.2985	0.0002
				Error	39	203.61	5.22		
				C. Total	77	858.01			
			8	Model	38	596.43	15.70	1.8002	0.0357
				Error	39	340.03	8.72		
				C. Total	77	936.46			
			15	Model	38	1,226.52	32.28	3.1197	0.0003
				Error	39	403.50	10.35		
				C. Total	77	1,630.02			
			22	Model	38	1,391.64	36.62	1.2428	0.2511
				Error	39	1,149.23	29.47		
				C. Total	77	2,540.87			
	Stress	Unifoliate	1	Model	38	581.83	15.31	5.4567	0.0000
				Error	39	109.43	2.81		
				C. Total	77	691.27			
			8	Model	38	1,327.38	34.93	4.7569	0.0000
				Error	39	286.39	7.34		
				C. Total	77	1,613.76			
			15	Model	38	1,746.21	45.95	1.5087	0.1028
				Error	39	1,187.91	30.46		
				C. Total	77	2,934.12			
			22	Model	38	2,726.10	71.74	2.5187	0.0025
				Error	39	1,110.84	28.48		
				C. Total	77	3,836.94			
		New trifoliate	1	Model	38	783.87	20.63	2.1257	0.0106
				Error	39	378.47	9.70		
				C. Total	77	1,162.34			
			8	Model	38	527.05	13.87	1.3347	0.1867
				Error	39	405.28	10.39		
				C. Total	77	932.33			
			15	Model	38	932.67	24.54	1.0480	0.4420
				Error	39	913.38	23.42		
				C. Total	77	1,846.05			
			22	Model	38	2,545.54	66.99	2.5124	0.0026
				Error	39	1,039.85	26.66		
				C. Total	77	3,585.39			
		First trifoliate	1	Model	38	497.42	13.09	1.4832	0.1125
				Error	39	344.19	8.83		
				C. Total	77	841.61			
			8	Model	38	686.37	18.06	2.6017	0.0019
				Error	39	270.76	6.94		
				C. Total	77	957.13			
			15	Model	38	2,611.25	68.72	2.9432	0.0006
				Error	39	910.57	23.35		
				C. Total	77	3,521.82			
			22	Model	38	1,979.01	52.08	2.6909	0.0014
				Error	39	754.81	19.35		
				C. Total	77	2,733.82			
		Unifoliate	1	Model	38	511.59	13.46	2.0860	0.0123
				Error	39	251.71	6.45		
				C. Total	77	763.30			
			8	Model	38	1,154.52	30.38	2.4925	0.0028
				Error	39	475.38	12.19		
				C. Total	77	1,629.90			
			15	Model	38	3,924.63	103.28	1.6160	0.0701
				Error	39	2,492.59	63.91		
				C. Total	77	6,417.22			
			22	Model	38	784.27	20.64	2.5472	0.0023
				Error	39	316.00	8.10		
				C. Total	77	1,100.27			

(Continued on next page)

Table 3. (Continued) Analysis of variance for chlorophyll content, stem lodging, and plant healthiness.

Trait	Stress	Description	Time	Source	DF	Sum of squares	Mean square	F ratio	Prob > F
Lodging	Non_Stress	Stem	1	Model	38	42.66	11.23	0.7128	0.0009
				Error	39	61.42	1.57		
				C. Total	77	104.08			
			8	Model	38	46.63	11.23	0.6217	0.0009
				Error	39	76.98	1.97		
				C. Total	77	123.61			
			15	Model	38	240.20	16.32	0.8354	0.0007
				Error	39	295.09	7.57		
				C. Total	77	535.28			
			22	Model	38	190.21	15.01	0.5534	0.0010
				Error	39	352.72	6.04		
				C. Total	77	542.93			
	Stress	Stem	1	Model	38	201.13	15.29	0.8274	0.0007
				Error	39	249.48	4.40		
				C. Total	77	450.61			
			8	Model	38	651.49	7.14	13.9298	0.0000
				Error	39	48.00	1.23		
				C. Total	77	699.49			
			15	Model	39	5,359.86	67.43	5.1270	0.0000
				Error	40	1,072.22	26.81		
				C. Total	79	6,432.08			
			22	Model	39	17,789.99	1,456.12	1.1765	0.0003
				Error	40	15,509.11	387.73		
				C. Total	79	33,299.11			
Healthiness	Non_Stress	Whole plant	1	Model	38	335.18	18.82	0.8395	0.0007
				Error	39	409.78	10.51		
				C. Total	77	744.96			
			8	Model	38	270.53	17.12	1.0086	0.0005
				Error	39	275.28	7.06		
				C. Total	77	545.81			
			15	Model	38	373.71	19.83	1.2770	0.0002
				Error	39	300.34	7.70		
				C. Total	77	674.05			
			22	Model	38	377.16	19.93	1.2350	0.0003
				Error	39	313.44	8.04		
				C. Total	77	690.60			
	Stress	Whole plant	1	Model	38	842.51	22.17	1.8223	0.0000
				Error	39	474.50	12.17		
				C. Total	77	1,317.01			
			8	Model	38	1,994.67	152.49	0.9978	0.0005
				Error	39	2,051.78	52.61		
				C. Total	77	4,046.46			
			15	Model	38	11,554.49	304.07	2.6897	0.0014
				Error	39	4,408.84	113.05		
				C. Total	77	15,963.33			
			22	Model	38	14,919.61	392.62	3.0823	0.0003
				Error	39	4,967.87	127.38		
				C. Total	77	19,887.49			

1, 8, 15, and 22 = days after drought stress (week 0, week 1, week 2, and week 3). C. Total = total count.

Table 4. Broad-sense heritability of chlorophyll content, stem lodging, and plant healthiness,

Trait	Treatment	Description	H ² (%)
Chlorophyll Content	Nonstress	New trifoliolate	61.17
		First trifoliolate	50.40
		Unifoliolate	63.67
	Stress	New trifoliolate	35.70
		First trifoliolate	55.75
		Unifoliolate	52.70
Lodging	Nonstress	Stem	70.44
	Stress		71.91
Healthiness	Nonstress	Whole plant	55.94
	Stress		73.17

percentage varied from 17.4% to 91.6% with an average of 48.9%. Based on the healthiness changes, three cowpea lines, 'PI255774', '17-111', and '17-109' had the lowest healthiness change, indicating their susceptibility to drought stress. The six lines 'PI349674', 'ARBlackeye#1', '09-529', '17-131', 'PI293568', and

'17-61' had the most significant healthiness change, indicating that the six lines were drought tolerant.

Plant main stem lodging scores. Plant main stem lodging scores were recorded on the 4 weeks after drought stress. The stressed plants in the first 2 weeks were all growing

erectly, whereas the lodging of plants was increased rapidly in the last 2 weeks (Fig. 7). The main stem lodging scores varied from 14.5% to 100%, with an average of 58.4% and an SD of 20.5 (Table 2, Supplemental Table 1). Among the 39 cowpea lines, three cowpea lines, '17-118', '16-181', and '17-127' had a stem lodging of 100%, indicating that the three lines were most susceptible to drought stress. On the other hand, six cowpea lines, 'PI349674', '17-61', 'EarlyScarlet', '17-135', '17-86', and 'ARBlackeye#1' had a very low stem lodging score, indicating that the five lines were drought tolerant.

ANOVA, broad-sense heritability, and correlation. We conducted the ANOVA, broad-sense heritability, and correlation analysis for chlorophyll content, stem lodging, and healthiness. According to the ANOVA and broad-sense heritability, the variations of drought-tolerant parameters are most (60% to

drought tolerance indexes and then sorted them in Table 6. Six lines, 'PI349674', 'ARBlackeye#1', '17-86', 'PI293568', 'EarlyScarlet', and '17-61' were evaluated as high tolerance of drought (scored less than 30 in green circle), whereas six lines '17-114', '16-181', '17-118', '17-109', '17-111', and 'PI255774' were assessed as susceptible varieties (scored more than 95 in blue circle). It is worth mentioning that the highest scored line was the susceptible control we used in this experiment. This result was very consistent with the canonical discriminant analysis.

Discussion

Drought tolerance of crops is interacted by a variety of factors and each factor may play a decisive role in tolerance, so the research on drought tolerance is complicated. There are differences in the mechanisms of drought tolerance of crops in different environments, different crop varieties, and different growth stages of the same variety. Therefore, more and more attention has been paid to the comprehensive index method. The comprehensive index method is to use several indicators for comprehensive evaluation

of crop drought tolerance, which can make up for the limitations and shortcomings of single-indicator drought tolerance evaluation, and the evaluation results are closer to the actual situation.

Three drought-tolerance parameters. Plants carry out photosynthesis in the chloroplasts. The first step of photosynthesis is the transformation of light energy into chemical energy by chlorophyll. The content of chlorophyll directly affects the photosynthetic rate (Arjenaki et al., 2012; Nikolaeva et al., 2010). Drought and water shortage can cause chloroplast damage, such as decreased chlorophyll content, ultrastructural damage of chloroplast, decreased enzymatic activity required for the dark reaction, and blocked transport of photosynthetic products, which can accumulate in leaves and cannot be transported to grains, resulting in a traffic jam in leaves (Munné-Bosch et al., 2001). By analyzing the chlorophyll content data in three different types of the leaves over 4 weeks of drought stress, we found that both unifoliate and first trifoliate chlorophyll content dropped to a very low level (chlorophyll content lower than 10) at the end of week 4. The new trifoliate chlorophyll content also

dropped significantly, but the magnitude was not as dramatic as the unifoliate and trifoliate. We also found that some cowpea lines were capable of keeping the new trifoliate chlorophyll content level, which suggested that new trifoliate chlorophyll content and its related parameters (Li et al., 2006), inflect index, can be a good trait to measure for the drought-tolerant assessment. Among the 39 cowpea lines, huge differences were also found in both overall plant healthiness score and plant main stem lodging score, indicating that both traits can contribute to the overall drought-tolerance level for a cowpea line. However, different cowpea lines performed and scored differently based on the trait we measured. Because of the complexity of the drought-tolerant trait, it is hard to use only one parameter to decide the overall drought tolerance of a certain line (Mafakheri et al., 2010).

The relative water content of the leaves can reflect the healthiness of plants, and it can also maintain the greenness and habit in the whole plant (Dedio 1975). Under drought conditions, the cultivars with strong drought tolerance had a higher relative water content of leaves to reduce cell damage caused by water loss and maintain the healthy leaf shape structure and function (Siddique et al., 2000). In the external morphology of the drought-tolerant varieties, the leaf curl degree is low, and the waxy layer is well covered. More studies have shown that the ability of isolated leaves of varieties with strong drought tolerance to retaining water is more durable than that of varieties with weak drought tolerance (Egilla et al., 2005). In this study, we ranked and scored the phenotype of the cowpea leaves by integrating the greenness, structure, curl, and growth of the leaves to evaluate the healthiness of the whole plant in drought stress. Compared with the traditional identification method, which is completely dependent on specific physiological or growth indicators, this method can save time, money, and labor.

Lodging is another parameter of water content in the plant, which can reflect the damage of the drought to plant stem (Kebede et al., 2001). Especially in cowpea breeding, if plants lodged due to water shortage at the seedling stage, the yield of cowpea will still be seriously affected, even if they regain vitality after later irrigation (Rosenow and Clark, 1995). This is why we use lodging as a criterion for drought resistance in this study. However, one issue we need to consider is the weight of this trait, because cowpea has a very high creeping attribute, and many cultivated cowpeas are lodging badly no matter whether it is under drought stress or not. Therefore, the weight of this parameter should be reduced when considering its drought-tolerance characteristics comprehensively.

In this experiment, three parameters, chlorophyll decrease (CII), healthiness change (HII), and stem lodging increase (LII), were used as the main parameters for evaluating drought tolerance. The value of these parameters can be well repeated in our experiment

Table 6. Overall ranking of drought tolerance for 39 cowpea breeding lines.

Cowpea lines	Chlorophyll score	Healthiness score	Lodging score	Tolerant index	Rank
PI349674	1	1	1	3	1
ARBlackeye#1	2	4	6	12	2
17-86	7	3	5	15	3
PI293568	5	2	12	19	4
17-61	6	12	2	20	5
EarlyScarlet	13	5	3	21	6
17-131	4	24	7	35	7
17-81	19	7	9	35	8
17-40	8	16	11	35	9
Empire	10	6	20	36	10
17-137	11	9	19	39	11
17-135	24	13	4	41	12
EarlySetSelect	20	11	13	44	13
09-529	3	25	22	50	14
09-393	22	18	15	55	15
07-303	17	10	29	56	16
01-1781	9	17	30	56	17
17-124	16	14	27	57	18
17-129	21	30	8	59	19
Ebony	12	15	35	62	20
09-211	15	22	26	63	21
17-117	25	26	17	68	22
Epic	26	27	16	69	23
16-166	30	21	18	69	24
17-128	18	23	33	74	25
09-741	32	19	24	75	26
17-127	28	8	39	75	27
EarlyAcre	34	32	10	76	28
09-714	35	20	21	76	29
17-107	27	36	14	77	30
17-102	14	33	31	78	31
17-133	23	31	25	79	32
09-692	29	37	23	89	33
17-114	36	29	32	97	34
16-181	33	28	38	99	35
17-118	31	34	37	102	36
17-109	38	38	28	104	37
17-111	37	35	34	106	38
PI255774	39	39	36	114	39

The score is the ordinal of drought-tolerance parameter among all breeding line. The drought-tolerant index is the sum of three parameters.

(Table 6). We assumed all three traits had the same importance value for the overall drought tolerance and used them to rank each cowpea line for its drought tolerance summed the values of the three traits. In the end, an overall ranking score was given to each cowpea line and the overall ranking score from the sum of the three ranking number drought tolerance. The cowpea lines with the low overall ranking score were considered to be overall drought tolerant, and the cowpea lines with higher overall ranking scores were considered to be drought susceptible.

Data collection and analysis. In the process of data acquisition, to ensure that each breeding line has enough seedlings (six plants) in a treatment, we often keep more than six seedlings. While in the process of data collection we recorded all survived seedlings, which may cause a slight difference in the resolution of three parameters among varieties, but did not affect the estimate of the final results. It is important to note that we used the date of stopping water as the starting time of drought treatment, but at that point, especially during the first week of ceasing water (week 0), there is no drought stress to the plants. It can also be seen from the results that the significant differences between the stress and the nonstress group occurred only after 2 weeks (week 0 and week 1) of drought stress. In particular, the content of chlorophyll increased in both of the treatments during first 2 weeks of drought stress. Therefore, when calculating and evaluating the chlorophyll decrease (CII), healthiness change (HII), and stem lodging increase (LII), we only used and showed those data from the last 2 weeks, thus reducing the bias and making the conclusion more reliable. We collected all the values of the three parameters during 4 weeks, respectively. Therefore, the following results of calculation and analysis were described carefully in all tables for the purpose of providing readers and researchers with sufficient information for reference. However, in the calculation of heritability, we averaged all values from 4 weeks to one final result, because the discussion of heritability difference in each week is not the point of this article.

Screening drought-tolerant lines. The two previous reported drought-tolerant lines 'PI293568' and 'PI349674', and one drought-susceptible line, 'PI255774' were also ranked (Table 5). Cowpea line 'PI293568' was ranked as number 4, and 'PI349674' as number 1 in the overall drought-tolerant ranking, indicating their drought tolerance, and 'PI255774' ranked number 39 as the most susceptible to drought tolerance in the study, which showed and confirmed our previous results (Ravelombola et al., 2018). The three cowpea genotypes showed stable tolerance or susceptibility across the three parameters and were used as controls in this study.

Worldwide, drought is increasingly affecting crop production and yields (Singh

et al., 1997). The effective way to solve this problem is to cultivate drought-tolerant varieties. It is a wise choice to explore new genetic resources with high water utilization characteristics to improve drought resistance. There are some reports on the drought tolerance of cowpea, but most of them were using limited indexes. In this study, the drought resistance of 39 breeding resources was evaluated, and several cowpea materials with different drought tolerance were screened out through water control treatment at the seedling stage for drought stress. The purpose of this study was to identify the drought-tolerance indexes at the seedling stage, screen the drought tolerance breeding resources, and lay a foundation for drought-tolerance gene mapping and identification.

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Supplemental Table 1. Statistics for chlorophyll content, chlorophyll decrease, lodging, lodging increase, healthiness, and healthiness change.

Trait	Treatment	Decription	Date	Mean	SD	Min	Max	Range	Variance	SE	CV	Median	
Chlorophyll content	Nonstress	Unifoliate	1	39.23	3.00	31.55	47.70	16.15	8.98	0.34	7.64	39.20	
			8	36.86	4.58	22.30	48.48	26.18	20.96	0.52	12.42	36.74	
			15	34.82	6.17	16.00	46.95	30.95	38.11	0.70	17.73	35.78	
		First trifoliate	22	25.05	7.06	13.25	37.96	24.71	49.83	0.80	28.18	25.63	
			1	41.72	3.34	32.10	47.80	15.70	11.14	0.38	8.00	41.95	
			8	44.70	3.49	38.83	54.00	15.17	12.16	0.39	7.80	43.90	
		New trifoliate	15	44.35	4.60	33.00	56.72	23.72	21.17	0.52	10.38	43.77	
			22	36.78	5.74	21.00	50.05	29.05	33.00	0.65	15.62	36.09	
			1	40.50	5.47	27.80	52.97	25.17	29.93	0.62	13.51	41.28	
		8	46.06	3.90	36.30	53.53	17.23	15.21	0.44	8.47	45.70		
		15	45.64	4.58	31.70	54.52	22.82	20.95	0.52	10.03	45.60		
		22	41.17	5.65	27.97	53.65	25.68	31.94	0.64	13.73	41.55		
	Stress	Unifoliate	1	38.75	3.15	32.15	47.17	15.02	9.91	0.36	8.13	38.68	
			8	30.89	4.60	15.60	41.63	26.03	21.17	0.52	14.89	31.18	
			15	17.90	9.13	1.80	37.50	35.70	83.34	1.03	51.01	17.77	
		First trifoliate	22	7.03	3.78	5.40	22.53	17.13	14.29	0.43	53.75	5.40	
			1	43.20	3.31	33.70	50.00	16.30	10.93	0.37	7.65	43.40	
			8	39.45	3.53	31.08	47.67	16.58	12.43	0.40	8.94	40.06	
		New trifoliate	15	31.02	6.76	12.92	44.30	31.38	45.74	0.77	21.80	30.77	
			22	11.23	5.96	5.20	27.70	22.50	35.50	0.67	53.05	9.14	
			1	42.75	3.89	33.48	51.62	18.14	15.10	0.44	9.09	42.52	
8		44.78	3.48	34.50	51.33	16.83	12.11	0.39	7.77	44.89			
15		37.99	4.90	26.20	48.20	22.00	23.97	0.55	12.89	38.33			
22		25.45	6.82	7.80	39.08	31.28	46.56	0.77	26.82	26.00			
Chlorophyll Infect Index (%)	Stress	Unifoliate	15	48.33	26.82	0.30	94.34	94.04	719.26	3.04	55.49	47.34	
			22	71.23	11.42	36.80	85.57	48.78	130.46	1.29	16.04	72.19	
		First trifoliate	15	29.92	14.13	2.92	70.53	67.60	199.78	1.60	47.23	27.77	
			22	69.39	15.82	16.98	86.49	69.51	250.31	1.79	22.80	73.98	
		New trifoliate	15	16.60	11.76	0.30	48.08	47.78	138.33	1.33	70.84	14.75	
			22	37.50	17.63	0.30	81.59	81.29	310.96	2.00	47.02	37.17	
Healthness (%)	Nonstress	Whole plant	1	94.93	3.11	90.00	100.00	10.00	9.67	0.35	3.28	95.13	
			8	95.00	2.66	90.00	100.00	10.00	7.09	0.30	2.80	95.13	
			15	94.96	2.96	90.00	100.00	10.00	8.75	0.34	3.12	95.00	
			22	95.27	2.99	90.25	100.00	9.75	8.97	0.34	3.14	95.50	
		Stress	1	93.46	4.14	87.50	100.00	12.50	17.10	0.47	4.43	92.63	
			8	87.39	7.25	75.00	99.75	24.75	52.55	0.82	8.30	86.25	
	Stress	Whole plant	15	61.80	14.40	25.00	91.75	66.75	207.32	1.63	23.30	62.50	
			22	48.57	16.07	8.25	79.25	71.00	258.28	1.82	33.09	50.00	
			15	34.87	15.19	5.41	74.81	69.41	2.31	1.72	43.57	33.86	
			22	48.94	16.99	17.45	91.65	74.20	2.89	1.92	34.71	48.12	
		Lodging(%)	Nonstress	1	0.76	1.16	0.00	2.95	2.95	1.35	0.13	152.72	0.00
				8	1.09	1.27	0.00	3.00	3.00	1.61	0.14	115.85	0.00
15	2.20	2.64		0.00	7.62	7.62	6.95	0.30	119.69	0.00			
22	2.79	2.66		0.00	7.94	7.94	7.05	0.30	95.07	2.50			
Stress	Stem	1	1.58	2.39	0.00	8.33	8.33	5.74	0.27	151.66	0.00		
		8	2.12	3.00	0.00	8.33	8.33	8.97	0.33	140.95	0.00		
		15	23.88	9.02	3.33	40.00	36.67	81.42	1.01	37.79	26.67		
		22	56.43	20.53	8.33	100.00	91.67	421.51	2.30	36.39	58.33		
	Lodging Infect Index(%)	Stress	15	22.16	9.38	3.33	40.00	36.67	87.97	1.05	42.33	23.33	
			22	58.50	23.38	5.55	100.00	94.45	546.74	2.61	39.97	64.33	

1, 8, 15, and 22 = days after drought stress (week 0, week 1, week 2, and week 3).