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Physiological Screening for Drought Tolerance Traits in Vegetable Amaranth (*Amaranthus tricolor*) Germplasm

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Abstract: Amaranth (*Amaranthus tricolor*), an underutilized climate smart crop, is highly nutritious and possesses diverse drought tolerance traits, making it an ideal crop to thrive in a rapidly changing climate. Despite considerable studies on the growth and physiology of plants subjected to drought stress, a precise trait phenotyping strategy for drought tolerance in vegetable amaranth is still not well documented. In this study, two drought screening trials were carried out on 44 *A. tricolor* accessions in order to identify potential drought-tolerant *A. tricolor* germplasm and to discern their physiological responses to drought stress. The findings revealed that a change in stem biomass was most likely the main mechanism of drought adaptation for stress recovery, and dark-adapted quantum yield (Fv/Fm) could be a useful parameter for identifying drought tolerance in amaranth. Three drought tolerance indices: geometric mean productivity (GMP), mean productivity (MP) and stress tolerance index (STI) identified eight drought-tolerant accessions with stable performance across the two screening trials. The highly significant genotypic differences observed in several physiological traits among the amaranth accessions indicate that the amaranth panel used in this study could be a rich source of genetic diversity for breeding purposes for drought tolerance traits.

Keywords: drought tolerance indices; plant physiological parameters; relative water content; chlorophyll a fluorescence parameters; photosynthetic leaf gas exchange; photosynthetic rate; stomatal conductance; intercellular CO_2 concentration; transpiration rate

1. Introduction

Drought is a major abiotic stress that hampers crop yields worldwide [1,2]. This affects the food and nutrition security of more than 1.1 billion people in South Asia and Sub-Sharan Africa who are largely dependent on the agricultural sector for income generation [3–5]. Recently, a paradigm shift in agriculture systems toward greater sustainability has offered utilization of a wider range of crop species that match prevailing climates [6,7]. In this context, amaranth, an ancient climatic smart crop, offers an alternative cheap source of nutrition for future food and nutrition security. Amaranth belongs to the Amaranthaceae family and is composed of 60–70 diverse species ranging from cultivated pseudo-cereal and vegetable crops to the world's worst weeds [8]. The leafy vegetable amaranth species, *Amaranthus tricolor*, is widely cultivated in Southeast Asia and Africa, providing an excellent source of macro- and micronutrients such as protein, lysine vitamin C and iron, greater than other leafy vegetables such as lettuce and spinach [9].

A. tricolor possesses important traits such as resistance to drought, is highly nutritional and is very genetically diverse [10–12]. Partly because of its C_4 photosynthetic pathway, which is more common in grasses but rare in dicots [13], amaranth can maintain CO_2 fixation and water use efficiency during drought stress [12,14–16]. Amaranth is phenotypically plastic and has the capacity to change its phenotype in response to drought stress, for example increasing chlorophyll and betacyanin content [17], exhibiting an indeterminate flowering habit and growing an extensive lateral root system [18]. Other studies have reported increasing proline content that activates antioxidizing enzymes to scavenge reactive oxygen species in amaranth under water stress [16,19,20]. Several studies have generated valuable data on the growth and physiology of the plants upon drought stress [14,15,21–27], nevertheless, suitable rapid screening protocols for drought tolerance in amaranth germplasm are still not well documented.

Understanding the genetic basis of phenotypic differences in vegetable amaranth in response to water deficit is crucial if new cultivars are to be developed. It is important to explore multiple factors that are involved in drought stress before establishing a reliable screening method for a large-scale selection or for breeding stock. This is due to the requirement for large space, time-consuming selection, and expensive and inadequate seed availability of certain genotypes in early generations [28]. The key criteria for the development of rapid screening methods are that the technique used must be capable of evaluating plant performance at critical stages of development, must use a small amount of plant material and must be able to screen large number of plant varieties in as short a time as possible [29]. It is well known that development of tolerant cultivars is difficult, primarily due to environmental variations which make stress highly variable to observe [30]. Several studies have suggested a complementary strategy for selection of tolerant genotypes through correlations between yield and yield-related traits as a surrogate measure [31]. Drought tolerance indices are used to quantify the level of drought tolerance of a genotype based on yield loss under drought condition compared to normal conditions [32–34]. Typically, selection should target genotypes with relatively high yield under normal and drought stress conditions [32].

The principal approach for identifying *A. tricolor* with superior drought tolerance traits is to exploit the diverse genetic resources available within the amaranth germplasm. Although ex-situ conservation of *A. tricolor* has been improved and genetic variability in agronomical traits has been characterized [35–40], the utilization mainly depends on resources available in the selected germplasm. Therefore, this study aimed to identify potential drought-tolerant *A. tricolor* germplasm from diverse geographical origins and to discern their physiological attributes to drought stress. Forty-four *A. tricolor* accessions were screened in two screening trials to discern a rapid screening protocol for drought stress. From this, suitable drought tolerance indices and possible surrogate traits were elucidated. Furthermore, eight *A. tricolor* accessions were identified with superior drought tolerance traits.

2. Materials and Methods

2.1. Plant Materials

In total, 44 *A. tricolor* accessions were selected for trait phenotyping (Table 1). Twenty-one accessions were obtained from World Vegetable Center Genebank, Taiwan (AVRDC), nineteen accessions from the United State Department of Agriculture Genebank (USDA) and four commercial varieties were included as checks, of which two local varieties were from Malaysia and two African varieties were from East-West Seed, Thailand. Two accessions entries, 22 and 23, were excluded from the analysis as the plants died at an early stage of drought stress.

Table 1. List of plant materials used for drought tolerance in two screening trials.

Entry	Accessions	Germplasm	ID	Origin Country
1	AV-TRI 2	AVRDC	VI055356	Bangladesh
2	AV-TRI 18	AVRDC	VI044446	India
3	AV-TRI 26	AVRDC	VI049006	Thailand
4	AV-TRI 33	AVRDC	VI050610-A	Viet Nam
5	AV-TRI 34	AVRDC	VI050609-A	Viet Nam
6	AV-TRI 39	AVRDC	VI054572	Philippines
7	AV-TRI 40	AVRDC	VI054571	Philippines
8	AV-TRI 44	AVRDC	VI048286	Bangladesh
9	AV-TRI 49	AVRDC	VI047504	Bangladesh
10	AV-TRI 51	AVRDC	VI057270	Cambodia
11	AV-TRI 53	AVRDC	VI042979	Indonesia
12	AV-TRI 54	AVRDC	VI042978	Indonesia
13	AV-TRI 56	AVRDC	VI058498	India
14	AV-TRI 57	AVRDC	VI044426	Malaysia
15	AV-TRI 58	AVRDC	VI055139	Malaysia
16	AV-TRI 68	AVRDC	VI050111	Taiwan
17	AV-TRI 69	AVRDC	VI049431	Taiwan
18	AV-TRI 3	AVRDC	VI055353	Bangladesh
19	AV-TRI 11	AVRDC	VI047795	Bangladesh
20	AV-TRI 24	AVRDC	VI044396-A	Pakistan
21	AV-TRI 31	AVRDC	VI050615-A	Viet Nam
22	AV-TRI 20	AVRDC	VI043725	Malaysia
23	AV-TRI 21	AVRDC	VI043724	Malaysia
24	US-TRI 3	USDA	Ames 29505	Brazil
25	US-TRI 6	USDA	PI 478310	China
26	US-TRI 13	USDA	Ames 2039	Indonesia
27	US-TRI 14	USDA	Ames 5354	Madagascar
28	US-TRI 15	USDA	Ames 2029	Malaysia
29	US-TRI 16	USDA	Ames 29034	Malaysia
30	US-TRI 19	USDA	Ames 2199	Taiwan
31	US-TRI 21	USDA	PI 607446	Thailand
32	US-TRI 24	USDA	PI 632237	USA
33	US-TRI 25	USDA	Ames 5110	West Africa
34	US-TRI 29	USDA	Ames 26216	China
35	US-TRI 39	USDA	Ames 2132	India
36	US-TRI 46	USDA	Ames 5118	Puerto Rico
37	US-TRI 47	USDA	Ames 1993	Taiwan
38	US-TRI 20	USDA	Ames 2024	Thailand
39	US-TRI 30	USDA	Ames 5102	Hong Kong
40	US-TRI 48	USDA	Ames 1998	Taiwan
41	US-TRI 49	USDA	Ames 5134	USA
42	US-TRI 51	USDA	PI 633591	Unknown
43	Local Red	LOCAL (Check)	var. BBS027	Malaysia
44	Local PR	LOCAL (Check)	var. BBS014	Malaysia
45	Thida	E-WEST (Check)	Thida	Tanzania
46	Zeya	E-WEST (Check)	Zeya	Tanzania

2.2. Water Treatments, Experimental Design and Growing Conditions

Single-seed descent of 44 *A. tricolor* accessions were grown in two separate trials: Trial I and Trial II under shade-house conditions at University of Nottingham Malaysia (latitude 2.940° N, longitude 101.8740° E). Seedlings were sown in black peat moss (Holland, Malaysia) in 14 × 10 cell trays (54 cm × 36 cm), and after 14 days of emergence plants were transplanted into plastic pots (16 cm × 12.5 cm × 14.5 cm), with one plant per pot. The soil used was a mixture of 40% clay, 50% sand and 10% silt, and was first dried out and sieved (0.5 cm × 1 cm) to obtain a 2 kg uniform soil bulk density. The plants were watered daily and five days after transplanting, 5 g of fertilizer (15N:15P:15K) was applied once during the establishment period.

Two water treatments, controlled (water-sufficient, WS) and drought stress (water-deficient, WD), were imposed at the vegetative growth phase once the plant had 5–7 mature leaves, in a split-plot randomized design with three replications in Trial I and four replications in Trial II. Each replicate (block) consisted of two whole-plots (WS and WD treatments) and within the whole-plot there were sub-plots (44 *A. tricolor* accessions). The drought treatments were imposed at 25 days post emergence in Trial I and 28 days after emergence in Trial II. The WS plants were watered daily throughout the experimental period to maintain maximum water holding capacity (100% WHC), while WD plants were subjected to progressive soil drying with an additional 100mL watered at a one-day interval to maintain consistent soil moisture. Soil moisture content was measured at the beginning of the water treatment (0 DAT) and every 3 days in Trial I and every 5 days in Trial II until the soil water reduced to terminal drought stress at 20% WHC. The volumetric soil water content was determined using a portable soil moisture sensor (ML3-ThetaProbe, Delta-T Device, Cambridge, England) (Figure 1). The minimum and maximum temperature, humidity and photosynthetic active radiation during drought treatment were recorded from 7 a.m. to 7 p.m. using a data logger (HOBO ® U30 Weather Station, MA, USA). The averaged PAR and temperature were PAR: 309.8–616.9 µM/m2s; temperature: 30.3–33.1 °C, and RH: 61.0–74.8% for Trial 1 and PAR: 147.8–531.09 µM/m2s; temperature: 26.6–32.7 °C; and RH: 62.2–88.5% for Trial 2. The onset of drought and the rate of soil depletion were more rapid in Trial I (6 days) compared to Trial II (10 days).

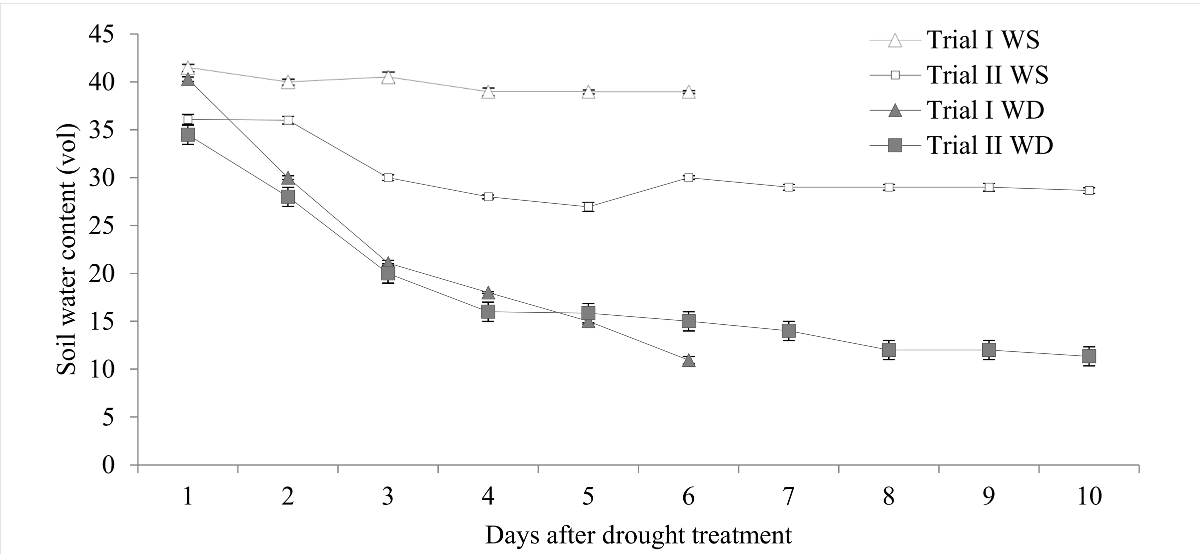

Figure 1. Soil volumetric content (% vol) of water-sufficient (WS) and water-deficient (WD) treatments in Trial I and Trial II. Values represented are the mean of 44 *A. tricolor* accessions in Trial I (N = 3) and Trial II (N = 4), and error bars represent the standard error of the mean (SEM).

An additional three biological replicates from each accession were arranged in a completely randomized design and subjected to rewatering assessment. These plants were subjected to progressive soil drying without irrigation until they reached terminal wilting point (10% WHC) before being rewatered to full soil water capacity for five days.

2.3. Plant Physiological Parameters

The water status of the plants was evaluated by measuring relative water content (RWC) at 100% WHC and 20% WHC in Trial I (n = 3) and 100% WHC, 50% WHC and 20% WHC in Trial II (n = 4). Two fully expanded leaflets were excised (2 cm × 2 cm sections) and fresh weight (FW) was recorded immediately. The leaf sample was then immersed in distilled water for 24 h in the dark at room temperature to obtain turgid weight (TW).

The leaf sample was then oven dried for 24 h at 80 °C and weighed to determine the dry weight (DW). RWC was calculated using formula as described in [41]:

$$RWC\ (\%) = [(FW\text{-}DW)/(TW\text{-}DW)] \times 100$$

Chlorophyll a fluorescence parameters, dark-adapted Fv/Fm (maximum quantum efficiency of PSII photochemistry), and Fv′/Fm′ (the maximum efficiency of PSII photochemistry, in the light, if all centers are open) were recorded using a handheld fluorometer FluorPen FP100 (Photon Systems Instruments, Czech Republic). The leaves were dark acclimated for 20 min using blackout paper before Fv/Fm measurements were taken on the third most fully expanded leaflet, avoiding the midrib section (Trial II: 100%, 50%, 20% WHC) at pre-dawn (7 a.m.). Fv′/Fm′ measurement was determined on the same leaf forTrial I: 100%, 20% WHC (n = 3) and Trial II: 100%, 50%, 20% WHC (n = 4) between 10 a.m. and 11 a.m. in sunny conditions (average PAR 450–500 nm. Two readings were taken per leaf, with two leaves per plant averaged to give a final reading. The equation for quantum yield under light and dark adaptations were calculated based on [42] as follows:

$$Fv'/Fm' = (Fm' - Fo')/Fm'$$

$$Fv/Fm = (Fm - Fo)/Fm$$

where Fv′ is variable fluorescence, Fm′ is maximal fluorescence and Fo′ is minimal fluorescence under light adaptation. Fv is variable fluorescence, Fm is maximal fluorescence and Fo is minimal fluorescence under dark adaptation.

Photosynthetic leaf gas exchange was only measured in Trial II (100% and 50% WHC, n = 4), using a portable photosynthetic system (LI-6400, LI-COR, Inc., Logan, NE, USA) coupled with a standard red/blue LED broadleaf cuvette (6400-02B, LI-COR, Inc., Logan, NE, USA) and a CO_2 mixer (6400-01, LI-COR, Inc., Logan, NE, USA). Readings were taken on the third most fully expanded leaf with the leaf chamber (2 cm × 3 cm) set to 400 μmolmol^{-1}CO_2 concentration, 1500 μmolphotonm^{-2}s^{-1} photosynthetically active radiation (PAR), 35 °C leaf block temperature, 400 μmolmol^{-1} flow rate and 50–70% relative humidity to keep the vapor pressure deficit (VPD) in the leaf chamber at approximately 1–1.5 kPa. Photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci) and transpiration rate (E) were recorded once a steady state was obtained (around 2 to 4 min). The measurements were used to calculate instantaneous water use efficiency (WUE) [43], intrinsic water use efficiency (WUEi) and stomatal limitation value (Ls) [44] as follows:

$$WUE\ (\mu molmol^{-1}) = Pn\ (\mu molCO_2m^{-2}s^{-1})/E\ (mmolH_2Om^{-2}s^{-1})$$

$$WUEi\ (\mu molmmol^{-1}) = Pn\ (\mu molCO_2m^{-2}s^{-1})/(Gs\ molH_2Om^{-2}s^{-1})$$

$$Stomatal\ limitation = 1 - [Ci\ (\mu molCO_2mol^{-1})/Ca\ (\mu molCO_2mol^{-1})]$$

where Ca is ambient CO_2 concentration.

2.4. Total Yield and Leaf and Stem Biomass Partitioning

Plant yield was obtained once the soil water status of WD plants fell to 20% WHC, at 6 DAT in Trial I (n = 3) and 10 DAT in Trial II (n = 4). Plants were harvested and separated into leaves and stems, and leaf and (LFW) stem fresh weights (SFW) were recorded. Total leaf area (TLA) was measured using a LI-3100 Area Meter (LICOR, Lincoln, Nebraska, USA). Dry weights were determined after drying at 80 °C in an oven for 72 h, and dry weight of leaf (LDW) and stem (SDW) were recorded. Yield and specific leaf area (SLA) were then calculated using the following formula:

$$SLA = TLA\ (cm^2)/Leaf\ dry\ weight\ (g)$$

$$Yield = Leaf\ fresh\ weight\ (g) + Stem\ fresh\ weight\ (g)$$

2.5. Rewatering Assessments

Days to flowering (DTF) and days to wilting (DTW) were recorded, pre-dawn from day 1 of soil drying (n = 3). All the plants were rated for leaf wilting scoring (LWS) and drought tolerance symptoms (DS) (Supplementary Figure S1) at the same time as DTW was recorded. The LWS and DS scoring on a scale of 0–5 and 0–6, respectively, with 0 = healthy and higher score being more severe. Days to recovery (DTR) were obtained after the plants were rewatered at 10% WHC for 5 days. DS were recorded twice, first during the day of DTR were observed and second on the 5th day of recovery.

2.6. Drought Tolerance Indices

Seven stress indices were evaluated to identify a suitable criterion for screening drought-tolerant accessions based on yield comparisons under WS and WD conditions. The drought tolerance indices were calculated using the following formula:

Stress susceptibility index [45],

$$SSI = [1- (Ysi/Ypi)]/SI$$

Stress tolerance index [32],

$$STI = (Ypi \text{ x } Ysi)/Yp2$$

Drought resistance index [46],

$$DI = [Ysi \text{ x } (Ysi/Ypi)]/Ys$$

Tolerance index [47],

$$TOL = Ypi - Ysi$$

Geometric mean productivity [32],

$$GMP = \sqrt{}(Ypi \text{ x } Ysi)$$

Mean productivity [47],

$$MP = (Ypi + Ysi)/2$$

Yield stability index [48],

$$YSI = Ysi/Ypi$$

where Ysi is yield of accession in WD condition, Ypi is yield of accession in WS condition, SI is stress intensity = 1 − (Ys/Yp), Ys is total yield mean in WD condition and Yp is total yield mean in WS condition.

2.7. Statistical Analysis

The interaction of accessions and water treatments across the two trials on the growth and plant physiological responses of amaranth accessions were analyzed using restricted maximum likelihood (REML). The data obtained from the two trials were averaged, and combined analysis of variance was performed using Wald-statistics following a test of homogeneity of variances by keeping water treatments x accessions (WT × A) as fixed effects and Trials × WT × A as random effects.

One-way ANOVA with a split plot design was used to investigate the effect of plant growth and physiology under WS and WD conditions and rewatering assessment. Pearson linear correlations, principal component analysis (PCA) and biplots were performed to analyze the significant correlations between trait parameters. Hierarchical clustering was generated using Manhattan distance to classify tolerance ranking via average linkage method. All data were generated using Genstat Software for Windows 18th edition (VSN International, 2015).

3. Results

3.1. Combined Analysis of Water Treatment, Accession and Trial Environments on Growth and Plant Physiology

The data in Table 2 summarizes the estimates of treatment effects from restricted REML combined analysis for the growth and physiological traits of amaranth accessions evaluated across the two trials. Significant interactions between water treatment and accession were observed across the two trials for yield ($p < 0.001$), TLA ($p < 0.001$), RWC at 20% WHC ($p < 0.05$) and Fv′/Fm′ at 100% WHC ($p < 0.01$). There were significantly higher relative reductions observed for yield and physiological traits in Trial I than Trial II as the severity of drought stress increased. The mean of each accession for yield and physiological parameters are presented in Supplementary Table S1.

3.2. Genotypic Variations in Growth and Plant Physiology in Response to Drought Stress

3.2.1. Yield and Biomass Partitioning

The mean yield of amaranth accessions was higher in Trial II with an average yield of 48.11 g under WS condition and 33.24 g under WD condition compared to Trial I with 15.78 g and 4.21 g, respectively, however both trials showed a significant reduction under WD (Table 3). There were significant genotypic differences observed in fresh weight biomass partitioning between the leaf and stem among the amaranth accessions in both WS and WD conditions, and drought stress had shifted the fresh weight allocation primarily into stems ($p < 0.01$). Meanwhile, the SDW was not affected by the drought stress in Trial I or Trial II, although significant changes were observed in SFW in WS and WD conditions.

3.2.2. Relative Water Content

RWC expressing leaf turgor significantly reduced with soil drying ($p < 0.001$) (Table 4). RWC reduced rapidly from 90% to 61% at terminal drought stress in Trial I, while reductions of RWC were slightly slower in Trial II, with the leaf water status declining from 90% to 83% to 76% at terminal drought stress. Significant genotypic differences were observed among amaranth accessions at 100% WHC in Trial I, and at 50% WHC ($p < 0.01$) and 20% WHC ($p < 0.001$) in Trial II.

3.2.3. Chlorophyll Fluorescence

The efficiency of PSII photochemistry, Fv/Fm and Fv′/Fm′ were not affected by soil drying (Table 4). Nevertheless, genotypic differences were observed among amaranth accessions for Fv/Fm and Fv′/Fm′ ($p < 0.05$) under drought stress in both Trials. The Fv′/Fm′ was significantly higher during Trial I (0.65) compared to Trial II (0.54), and the value declined as the plant increased in size. The decreasing trend also was also observed in Fv/Fm value from 100% to 50% and 20% WHC under WS (0.72, 0.67, 0.64, respectively) and WD conditions (0.72, 0.67, 0.64, respectively) throughout the experimental period.

3.2.4. Leaf Gas Exchange

The efficiency of photosynthetic activity was not influenced by the drought stress, but significant interactions between water treatment and accession were observed for all photosynthetic parameters ($p < 0.01$) (Table 5). This may imply that amaranth accessions have different adjustments in photosynthetic activity in response to drought stress, as shown by the significant genotypic differences exhibited among amaranth accessions at 100% WHC and at 50% WHC under WS and WD conditions. As the water treatment progressed, the majority of the photosynthetic activity was significantly reduced, including Pn (from 29.13 at 0 DAT to 19.07–19.30 $\mu molCO_2 m^{-2} s^{-1}$ at 6 DAT), Gs (from 0.19 to 0.12–0.13 $molH_2Om^{-2}s^{-1}$), Ci (from 110.4 to 95.78–108.5 $\mu molCO_2 mol^{-1}$), E (from 4.51 to 3.17–3.48 $mmolH_2Om^{-2}s^{-1}$) and WUE (from 6.65 to 6.13–6.35 $\mu mol\ mol^{-1}$), while WUEi and Ls significantly increased (from 163.3 to 170.3–176.5 $\mu mol\ mmol^{-1}$, from 0.71 to 0.72–0.75 respectively) in both WS and WD conditions.

Table 2. Restricted maximum likelihood (REML) combined analysis for yield, leaf fresh weight (LFW), fresh weight of leaf (LFW) and stem (SFW), dry weight of leaf (LDW) and Genotypic variations in growth and plant physiology in response to drought stress stem (SDW), total leaf area (TLA), specific leaf area (SLA), relative water content (RWC) and Fv′/Fm′of 44 *A. tricolor* accessions under two water treatments evaluated across the two screening trials. Significant difference at $p < 0.05$, <0.01, <0.001 (*, **, *** respectively) and non-significant (ns).

	Yield (g)	LFW (g)	SFW (g)	LDW (g)	SDW (g)	TLA (cm^2)	SLA ($cm^2 g^{-1}$)	RWC		Fv′/Fm′	
								100% WHC	20% WHC	100% WHC	20% WHC
					Estimated variance						
Trial (T)	468.6	93.83	142.76	0.88	0.83	64,634	4491	1.01	0	0.0049	0
T × Water treatment (WT)	2.4	1.02	0.23	0.06	0	2645	1213	2.08	7.1	0.0001	0.0003
T × Accession(A)	23.5	9.06	10.32	0.04	0.11	12,758	2052	0.58	0	0.0001	0
T × WT × A	−4.3	−1.46	−0.72	0.02	−0.01	−193	1121	−0.05	0	−0.0007	0
Error Trial I	19.82	6.316	4.75	0.14	0.1	4554	2042	32.01	227.1	0.003	0.0075
Error Trial II	123.3	35.52	41.82	0.54	0.41	39,889	33,123	39.91	153.5	0.0066	0.0077
					Wald tests for fixed effects						
WT	63.39 ***	51.98 ***	90.02 ***	1.51 ns	22.46 ***	12.64 ***	5.62 *	0.77 ns	76.65 ***	0.01 ns	5.44 *
A	59.06 *	65.23 *	68.06 **	102.68 ***	92.78 ***	62.99 *	30.44 ns	67.57 *	51.01 ns	71.5 **	78.19 ***
WT × A	132.96 ***	204.03 ***	116.47 ***	48.86 ns	55.82 ns	91.3 ***	26.51 ns	37.86 ns	61.2 *	67.73 **	42.92 ns

Table 3. Mean and the analysis of variance of water treatment on yield and biomass partitioning: fresh weight of leaf (LFW) and stem (SFW), dry weight of leaf (LDW) and stem (SDW), total leaf area (TLA) and specific leaf area (SLA) in 44 *A. tricolor* accessions evaluated in Trial I (n = 3) and Trial II (n = 4). Significant difference at $p < 0.05$, <0.01, <0.001 (*, **, *** respectively) and non-significant (ns).

	Yield and Biomass Partitioning						
Trial I	**Yield (g)**	**LFW (g)**	**SFW (g)**	**TLA (cm^2)**	**LDW (g)**	**SDW (g)**	**SLA (cm^2g^{-1})**
Mean							
Water-sufficient	15.78	8.42	7.37	265.49	1.15	0.94	239.62
Water-deficient	4.21	1.81	2.40	129.29	1.09	0.78	118.78
SEM	3.96	2.58	2.50	79.82	0.34	0.30	41.82
***p*-values**							
Water treatment (WT)	8838 *	2882 *	1626 *	12,224,197 **	0.19 ns	1.76 ns	963,703 **
Accession (A)	28.88 ***	12.52 ***	13.07 ***	17,104 **	0.47 ***	0.32 ***	4008 **
WT × A	20.54 ***	8.28 **	6.90 ***	7713 **	0.15 *	0.08 ns	2807 ns
Subplot error	9.4	3.25	2.54	3677	0.1	0.06	1515
Trial II							
Mean							
Water-sufficient	48.11	23.2	24.90	682.32	2.48	2.27	305.06
Water-deficient	33.24	14.5	18.73	440.83	2.00	2.02	257.44
SEM	11.27	6.89	7.42	248.69	0.83	0.71	106.28
***p*-values**							
Water treatment (WT)	19,453 ***	6690 **	3327 *	5,132,472 **	20.34 **	5.48 ns	199,527 *
Accession (A)	6673 **	234.29 ***	289.25 ***	318,207 ***	4.59 ***	3.42 **	80,039 ***
WT × A	115.11 ns	42.42 *	41.01 ns	46,112 *	0.41 ns	0.36 ns	41,261 ns
Subplot error	94.9	26.7	36.19	31,761	0.49	0.39	33,114

3.3. Genotypic Variation in Drought Adaptability and Recovery

Amaranth displayed significant variation in drought adaptability and recovery during rewatering assessment, as shown in DTF ($p < 0.001$) and DTR ($p < 0.001$) (Table 6). The plants exhibited tolerance through longer days to wilting (range 1–8 days) while initiating early flowering (range 1–4 days) and recovered instantly once rewatered (range 1–6 days). Although there was no significant difference in LWS, the majority of accessions wilted into a V-shape or U-shape leaf and displayed symptoms such as slight tip drying in early response to drought stress (Figure 2). Upon rewatering, most of the amaranth accessions had at least five leaves senescence, and more branches grew along the stem while accessions 12, 14, 15 31 and 32 died five days after rewatering. The mean of each accession for rewatering assessment is presented in Supplementary Table S2.

3.4. Interrelationship between Growth, Physiological Traits and Drought Adaptability

PCA biplot analysis was used to identify a combination of indices that may provide a useful criterion for improving drought resistance in amaranth (Figure 3a,b). In Trial I, PC1 accounted for 64% of the variations in Yp, Ys, GMP, MP and STI and PC2 accounted for 30% total variations in DI and YSI (Figure 3b). In Trial II, PC1 accounted for 45% variations in Ys, DI, GMP, MP and STI and PC2 accounted for 39% of variation in YSI (Figure 3b). Smaller angles between dimension vectors in the same direction indicate high correlation of the variable traits in terms of discriminating accessions. Therefore, high yielding accessions in both WS and WD conditions can be obtained based on positive and high PC1 on the biplot (such as accession entries 8, 19, 31, 42 in Trial I and 18, 19, 35 in Trial II). Meanwhile, high yielding accession in WD but low in WS conditions can be obtained based on positive and high values of PC2 (such as accession entry 5, 20, 37 in Trial I; and entry 25 and 2 in Trial II).

Table 4. Mean and the analysis of variance of water treatment on relative water content and chlorophyll fluorescence in 44 *A. tricolor* accessions (A) at different water holding capacity (WHC) evaluated in Trial I (n = 3) and Trial II (n = 4). Data represents mean, standard error of mean (SEM), mean square with probability significantly different at $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) or non-significant (ns) and mean square of error.

	Relative Water Content (RWC)					Chlorophyll Fluorescence							
						Light-Adapted Quantum Yield (Fv′/Fm′)					Dark-Adapted Quantum Yield (Fv/Fm)		
	Trial I		Trial II			Trial I		Trial II			Trial II		
WHC	**100%**	**20%**	**100%**	**50%**	**20%**	**100%**	**20%**	**100%**	**50%**	**20%**	**100%**	**50%**	**20%**
Mean													
Water-sufficient	89.99	90.84	90.33	87.09	86.73	0.65	0.56	0.54	0.52	0.54	0.72	0.67	0.64
Water-deficient	87.13	61.12	90.65	82.92	75.98	0.64	0.49	0.54	0.53	0.53	0.72	0.67	0.63
SEM	3.25	3.63	2.08	4.63	3.27	0.02	0.03	0.04	0.03	0.04	0.03	0.04	0.04
***p*-value**													
Water treatment (WT)	539.94 ns	58,296 **	2.84ns	9124 **	40,687 *	0.01 ns	0.36 ns	0.001 ns	0.0002 ns	0.03 ns	0.02 ns	0.02 ns	0.03 ns
Accession (A)	55.63 **	199.30 ns	44.13 ns	226.4 **	199.01 ***	0.002 ns	0.01 *	0.003 ns	0.008 ***	0.01 ***	0.006 ns	0.01 *	0.01 **
WT × A	28.12 ns	229.20 ns	39.60 ns	121.50 ns	181.64 **	0.001 ns	0.06 ns	0.003 ns	0.002 ns	0.005 ns	0.003 ns	0.04 ns	0.01 ns
Subplot error	30.08	209.3	39.98	129.6	94.61	0.002	0.06	0.002	0.003	0.006	0.005	0.004	0.01

Table 5. Mean and the analysis of variance of leaf gas exchange measurements in 44 *A. tricolor* accessions at different water holding capacity (WHC) evaluated in Trial II (n = 4). Data represent mean, standard error of mean (SEM), mean square with probability significantly different at $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) or non-significant (ns) and mean square of error.

Leaf Gas Exchange	Photosynthesis, Pn ($\mu molCO_2m^{-2}s^{-1}$)		Stomatal Conductance, Gs ($molH_2Om^{-2}s^{-1}$)		Transpiration, E ($mmolH_2Om^{-2}s^{-1}$)		Intracellular [CO_2], Ci ($\mu molCO_2mol^{-1}$)		Instantaneous Water Use Efficiency, WUE ($\mu molmol^{-1}$)		Intrinsic WUE, WUEi ($\mu molmmol^{-1}$)		Stomatal Limitation, Ls	
WHC	**100%**	**50%**	**100%**	**50%**	**100%**	**50%**	**100%**	**50%**	**100%**	**50%**	**100%**	**50%**	**100%**	**50%**	**100%**	**50%**
Mean																
Water-sufficient	24.61	19.3	0.16	0.13	4.03	3.48	109.97	108.52	6.17	6.13	164.64	170.25	0.72	0.72
Water-deficient	33.04	19.07	0.22	0.12	4.99	3.17	111.67	95.78	7.05	6.35	160.01	176.45	0.71	0.75
SEM	7.99	6.27	0.06	0.05	1.11	1.34	42.27	50.56	1.35	1.67	29.55	37.53	0.11	0.13
***p*-value**																
Water treatment (WT)	591.57 ns	2.62 ns	0.305 ns	0.010 ns	739 ns	8.49 ns	77.53 ns	16,542 ns	65.33 ns	5.20 ns	1468 ns	4227 ns	0.01 ns	0.09 ns
Accession (A)	327.67 ***	138.83 ***	0.016 ***	0.008 ***	10,371 ***	4.82 ***	6.62 ***	11,560 ***	14.25 ***	10.23 ***	5234 ***	4979 ***	0.07 ***	0.08 ***
WT × A	155.85 ns	119.82 ***	0.007 ns	0.007 ***	4729 ns	4.40 ***	2.41 ns	5523 **	6.27 ns	8.44 ***	2493	3346 *	0.03	0.04 **
Spit plot error	47	55.25	0.003	0.003	1974	1.86	1.36	3248	1.73	2.93	880	2179	0.01	0.02

Table 6. Mean and the analysis of variance of days to the early panicle flowering (DTF), days to wilting (DTW), leaf wilting scoring (LWS), drought stress symptoms scoring (DS) and effects of rewatering on the days to recover (DTR) and DS at first and fifth day of recovery (DS-1R, DS-5R, respectively) in 44 *A. tricolor* accessions. Data represent range, mean, mean square with probability significantly different at $p < 0.01$ (**), $p < 0.001$ (***) or non-significant (ns) and mean square of error, n = 3.

Rewatering Assessment							
	Drought Stress				**Recovery**		
	DTF	**DTW**	**LWS**	**DS**	**DTR**	**DS-1R**	**DS-5R**
Range	0–4	1–8	0–5	0–7	1–6	0–9	0–9
Mean	3	3	3	2	2	4	5
***p*-value**							
Accession	2.67 **	2.87 ns	2.209 ns	2.22 ns	5.73 ***	16.24 ***	29.61 ***
Error	1.39	3.48	2.1	2.24	0.8	1.71	7.12

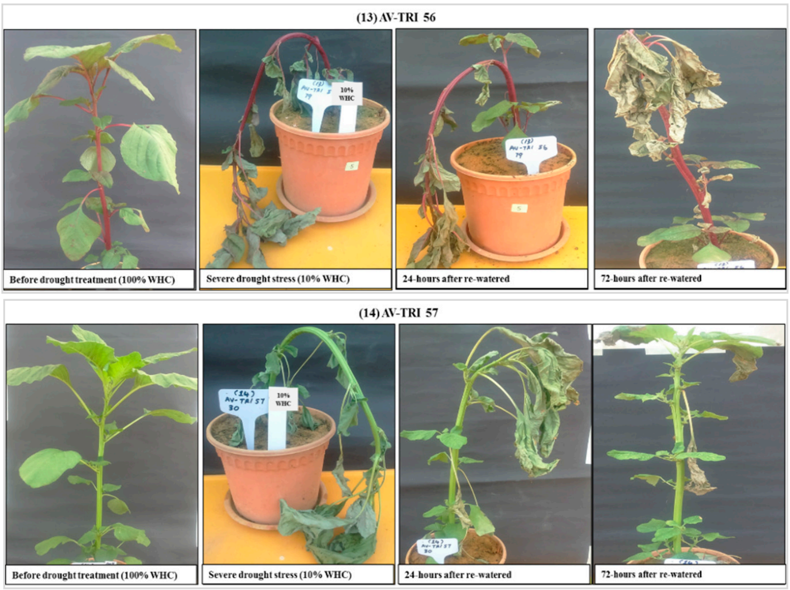

Figure 2. The examples of rewatering assessment on amaranth accessions, entry 13 and 14. The images were captured (i) a day before the imposition of drought stress (100% WHC), (ii) at terminal drought stress (10% WHC), (iii) after 24-h of rewatering and (iv) after 72-h of rewatering.

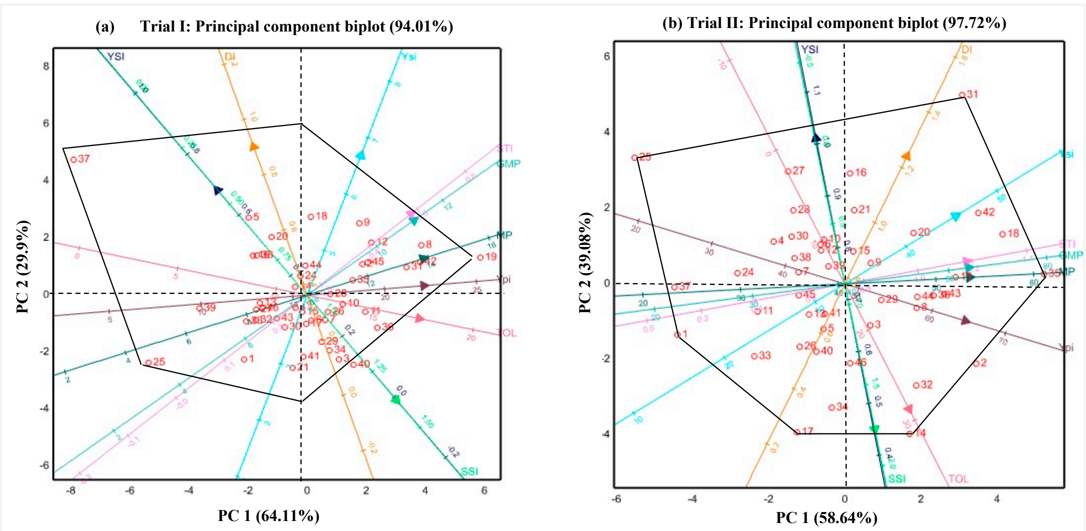

Figure 3. Principal component biplot grouping for drought tolerance indices in (**a**) Trial I and (**b**) Trial II.

The Correlation coefficients for growth and physiological traits in WS and WD conditions for Trial I, Trial II and the rewatering assessment are summarized in Supplementary Table S3a–c. Physiological traits with consistent association with growth traits which include LFW, LDW, SFW, SDW, TLA and SLA under WS and WD conditions and can be considered as possible surrogate traits for drought tolerance. In Trial I, Fv′/Fm′ was positively correlated with TLA (WS: R = 0.27, $p < 0.001$; WD: R = 0.20, $p < 0.05$ at 100% WHC) and SLA (WS: R = 0.26, WD: R = 0.33, $p < 0.001$ at 20% WHC), but negatively correlated with SFW (WS: R = −0.26; WD: R = −0.08, $p < 0.001$ at 20% WHC). This implies that high Fv′/Fm′ efficiency may promote leaf expansion, but restrict stem growth and contribute less to the total leaf biomass.

In Trial II, Fv/Fm exhibited a negative influence on yield in all soil water conditions, although significant negative correlation was only displayed at 50% WHC in WS (R = −0.19, $p < 0.01$) and WD conditions (R = 0.35, $p < 0.001$). This association was influenced by restricted leaf growth as shown by the negative correlation between Fv/Fm at 50% WHC with LFW and TLA in WS (R = −0.25, $p < 0.001$; R = −0.22, $p < 0.01$, respectively) and WD conditions (R = −0.31, $p < 0.001$; R = −0.34, $p < 0.001$). RWC at 50% WHC was positively correlated with LDW (WS: R = 0.23, $p < 0.05$, WD: R = 0.27, $p < 0.01$) and TLA (WS: R = 0.17, $p < 0.05$, WD: R = 0.29, $p < 0.05$, respectively), demonstrating that leaf cell turgor increased with increased leaf size and weight.

In the rewatering assessment, DTW was negatively correlated with stem traits (SDW: R = −0.21, $p < 0.05$), which revealed amaranth accessions with higher stem biomass wilting rapidly with declining soil water. DTW was also significantly influenced by E at 100% WHC (R = 0.25, $p < 0.01$), Gs at 50% WHC (R = 0.22, $p < 0.01$), RWC at 20% WHC (R = 0.24, $p < 0.05$), and WUE at 100% WHC (R = −0.22, $p < 0.05$). Meanwhile, DTF was significantly influenced by leaf traits (LFW, LDW and TLA: R > 0.20, $p < 0.05$), suggesting that higher leaf biomass delayed flowering during drought stress. DTR was negatively associated with both leaf traits (LFW, LDW and TLA: R > −0.27, $p < 0.05$) and stem traits (SFW: R = −0.21, $p < 0.05$), demonstrating that higher yield amaranth accessions under drought stress were able to recover immediately upon rewatering.

3.5. Selection of Drought Tolerance Indices

The stress intensity of Trial I (0.73) was significantly more severe than that of Trial II (0.31), and significant differences were observed for GMP, MP and STI ($p < 0.05$) in Trial I and Trial II (Supplementary Table S4a,b). The mean comparisons between accessions were determined for each tolerance index (Supplementary Table S4a). Correlation coefficients conducted between indices and yield under WS (Yp) and WD conditions (Ys) demonstrated that Yp was not associated with Ys (R = 0.12, $p > 0.05$) in Trial I, demonstrating that high yielding accessions under WS condition did not produce high yield under WD conditions. Meanwhile, Yp was strongly associated with Ys (R = 0.51, $p < 0.01$) in Trial II, indicating that high yielding accessions under WS condition also have high yield under WD conditions. Nonetheless, several indices showed consistent association with Yp and Ys in both trials, in which GMP, MP and STI were positively correlated with Yp and Ys (R > 0.50, $p < 0.001$), demonstrating that these indices could discriminate amaranth accessions with high yield under both WS and WD conditions. Meanwhile, SSI was positively correlated with Yp but negatively correlated with Ys (R > 0.20, $p < 0.001$), indicating that SSI could identify accessions with high yield in WS conditions but low yield in WD conditions. In contrast, YSI was positively correlated with Ys but negatively correlated with Yp (R > 0.20, $p < 0.01$), showing that YSI will provide accessions with low yield in WS condition but high yield in WD conditions.

3.6. Drought Tolerance Ranking of 44 Amaranth Accessions

The ranking for drought tolerance among 44 amaranth accessions was ascertained based on GMP, MP and STI, the most effective and stable criterion in the present study. The dendrogram distinguished amaranth accessions into high tolerance (high yielding under WS and WD conditions), moderate tolerance, low tolerance and high susceptibility

(Figure 4). Accessions 2, 8, 18, 19, 31, 35, 36 and 42 were considered highly tolerant to drought stress with consistent yield performance in both trials, while accession entries 1, 25 and 37 were the most susceptible. The yield performance of the remaining accessions was varied and unstable, depending on the stress intensity. For example, accession entry 11 was considered highly tolerant in Trial I, but became the most susceptible among the 44 amaranth accessions in Trial II.

3.7. Association of Tolerance Grouping with Plant Growth and Physiology

The relationships between tolerance groups and physiological traits with respective principal components (Supplementary Table S5) are further illustrated by the biplots for Trial I (Figure 5a) and Trial II (Figure 5b). In Trial I, the PCA revealed that the first two PCAs accounted for 65.66% and 66.40% of total variations in WS and WD conditions, respectively. High tolerance accessions were clearly discriminated from susceptible accessions in both WS and WD conditions although there was overlap between high, moderate and low tolerance accessions in the directions of biomass partitioning. There was also no difference in the directions of dimension vectors in biomass partitioning, RWC and Fv′/Fm′ between the two water treatments.

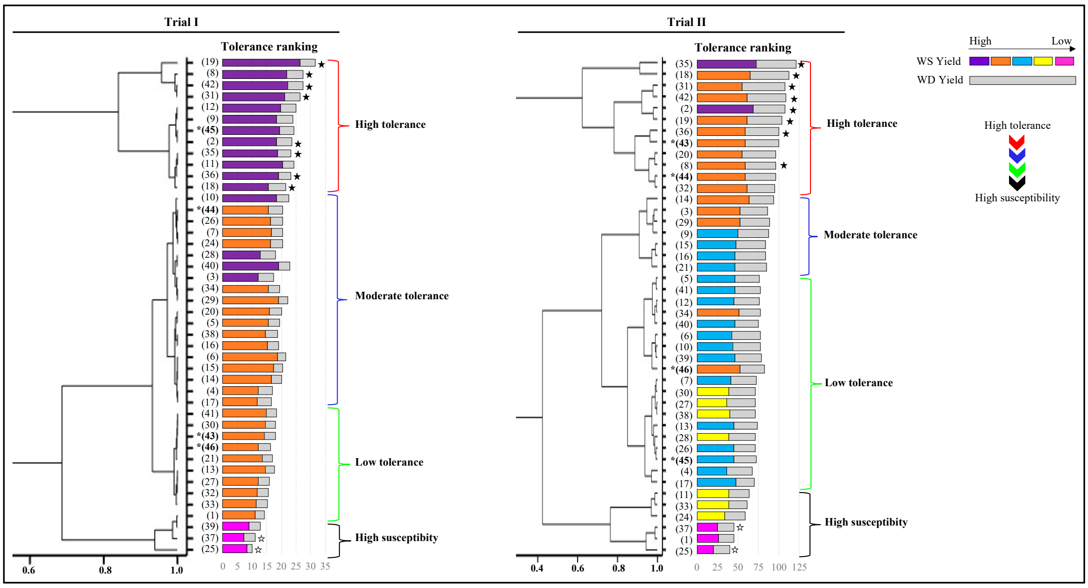

Figure 4. Cluster analysis of drought tolerance ranking for the 44 *A. tricolor* accessions calculated based on the GMP, MP and STI values in Trial I and Trial II. The dendrogram discriminates amaranth into four drought tolerance rankings (high, moderate and low tolerance, and high susceptibility). Data represent mean of yield under water-sufficient (WS) and water-deficient (WD) conditions; *43, *44, *45, *46 accessions check variety. Black star represents tolerant accession and white star represents susceptible accessions, consistent in both trials.

In contrast, in Trial II the PCA revealed that the first two PCAs accounted for 57.26% and 48.31% of total variations on WS and WD conditions, respectively. Under the WS condition, a clear separation was observed between highly tolerant and susceptible accessions, as highly tolerant accessions clustered together in the direction of yield biomass while susceptible accessions were more inclined towards SLA and Fv/Fm. Under the WD condition, the high and moderate tolerance accessions were clustered together towards the positive side of PC1 excelling in E, Gs, RWC, biomass partitioning, Pn, WUE, WUEi and Ls, and clearly separated from high susceptibility accessions which were more inclined towards the negative side of PC1 excelling in Ci and Fv/Fm.

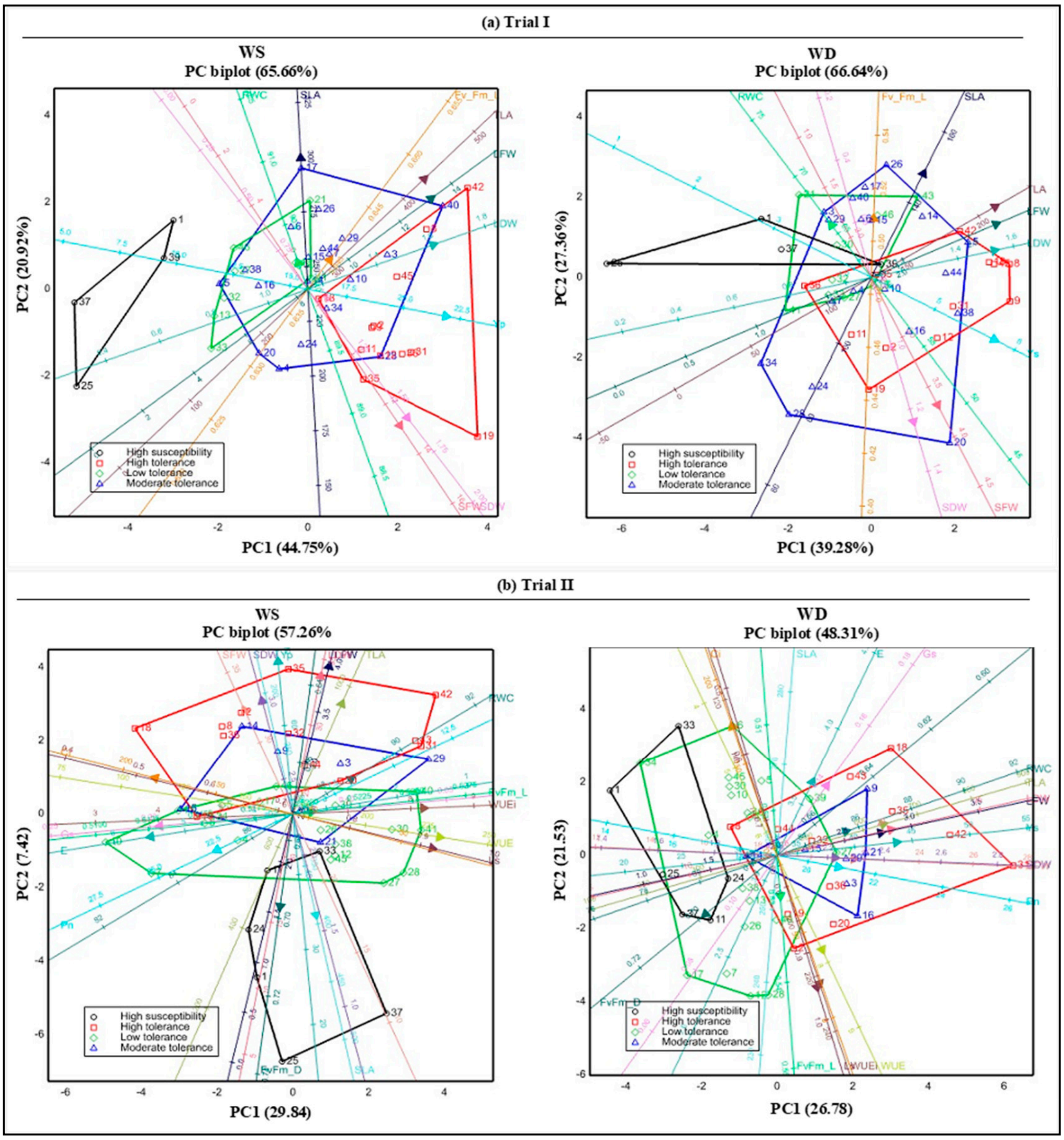

Figure 5. Principal component biplot on tolerance grouping in association with physiological responses under WS and WD conditions (**a**) in Trial I and (**b**) in Trial II.

4. Discussion

Drought tolerance is a complex biological process which involves interactions between morphological traits and physiological and biochemical processes and is dependent on the level of drought severity and timing in relation to the stage of crop development [49–51]. Furthermore, the mechanisms of plant adaptation to drought stress are strongly influenced by the experimental design which might provide different interpretations for the observed effects of water deficit [52,53]. In this experiment, drought stress was imposed on the individual plants so comparative physiological responses could be applied to any accession presents in an environment [53]. The growth of amaranth in irrigated and drought stress conditions was significantly affected by the environmental conditions, as shown by the significant lower yield production in Trial I compared to Trial II and the difference adaptive responses between the two trials. Thus, revealing that environmental stress intensity plays an important role in drought response and adaption in *A. tricolor* accessions. This result has also been observed in maize, grown in multiple environment conditions [54] and wheat, grown in rainfed and irrigated locations [55].

It is possible that the more severe drought stress imposed in Trial I may cause a reduction in physiological and metabolic activity in comparison to the moderate drought stress imposed in Trial II [56,57]. Besides, different stress adaptation mechanisms in amaranth might be determined by the capacity of accessions to adapt to different type of drought stress to enhance their growth and development [58,59]. In this study, drought stress significantly reduced yield of some accessions and several of them had lower yield loss, suggesting that *A. tricolor* was able to grow at reduced soil water availability, although at a slower rate than the fully irrigated plants. The results showed genotypic variations exist in amaranth accessions and these differences may portray the potential success in future improvement for amaranth which may be used to pre-screen lines for further verification under field conditions.

The RWC determines leaf water balance in plants during water deficit periods [60] and estimates the percentage of water in the leaf as a fraction of the total volumetric water that the leaf can hold at full turgor [61]. In this experiment, RWC declined to 61–76%, a range of expected optima for *A. tricolor* (70–80%) [37], which is higher than other amaranth species such as *A. hybridus* and *A. hypochondriacus*, with less than 50% RWC recorded under drought stress [16,62]. Some studies have suggested that high RWC is closely related to drought resistance, as observed in cowpea landraces [63], Arabidopsis [64] and beans [65], and has been successfully used as a screening tool for selecting drought-tolerant potatoes [66]. However, RWC did not appear to be related to drought tolerance in this study but may be serve as indicator for plant water status [67] as higher RWC were correlated with leaf expansion and leaf biomass under WS and WD conditions in this study. Nevertheless, RWC is vital to ensure an accurate assessment of the relative capacity for osmotic adjustment, an indicator of cell turgor through accumulation of organic solutes such as proline [68,69]. Sarker and Oba [37] reported that soil drying increased proline accumulation in drought-tolerant *A. tricolor* cultivar, and RWC was negatively correlated with proline and soluble protein contents, demonstrating that the synthesis of proline and protein was higher as soon as RWC declined. These solutes are considered as a general marker of drought tolerance [16,70] as it helps to protect cellular proteins, enzymes and cellular membrane against cell dehydration [71], and in *A. hypochondriacus* and *A. cruentus*, increase of soluble carbohydrate under drought stress used to regulate water use efficiency [72]. Therefore, further studies are required to quantify compatible solutes on this *A. tricolor* accessions panel to explore the possible plants osmotic adjustment under drought stress, which then can be considered as a tool for effective selection.

Low Fv/Fm at 50% WHC was associated with high yield under WS and WD conditions in this study, which may serve as a potential marker for indirect selection for drought tolerance in *A. tricolor*. The value of Fv/Fm did not change under normal and drought stress conditions, 0.72, 0.67 and 0.64 at 100%, 50% and 20% WHC, respectively, and was slightly lower than reported for *A. hybridus* (0.78) and *A. hypochondriacus* (0.80), which was also not affected by drought stress [62]. Meanwhile, Hura et al. [15] reported that Fv/Fm of *A. cruentus* significantly declined in prolonged drought stress at 30% field water capacity. This demonstrates that drought-adaptive response varied between amaranth species, while *A. tricolor*, *A. hybridus* and *A. hypochondriacus* may have the ability to recover from PSII damage at night or because of the PSII protection promoted by the accumulation of total soluble sugar and proline under drought stress [62].

Fv/Fm provides a rapid way to assess plant health, but caution should be used as Fv/Fm is often misinterpreted as a specific indicator of PSII photoinhibition (decrease of CO_2 fixation) due to the damage of PSII core subunit D1 [73–75]. Rather, Fv/Fm represents quantum yield of PSII that will be low not only when the PSII is inactivated but also due to thermal dissipation through slowly relaxing non-photochemical quenching (NPQ) [73]. The used of Fv/Fm to evaluate the drought tolerance of crops is contradictory [76]. For example, Fv/Fm of drought-tolerant barley [77] and tomato varieties [78] were higher than the drought-sensitive varieties. In contrast, drought-tolerant amaranth accessions had lower Fv/Fm than the susceptible accessions in this study. A low Fv/Fm in high yielding accessions might indicate a photoprotective role rather than photoinhibition. This is because at 50% WHC, photosynthesis rate was reduced under WS and WD conditions due to lower stomatal conductance, as shown by positive correlation between Pn and Gs in both water conditions. This may induce an increased light energy and photoinhibition, and therefore lower Fv/Fm in drought-tolerant amaranth was likely an up-regulation mechanism to dissipate excess electrons [59] by limiting light absorption and maintaining the oxidation state of plastoquinone, the electron accepter of PSII [79]. Further study on non-photochemical quenching (NPQ), a process of dissipating excess light energy which plays an important role for photoprotection [73], or other electron dissipation mechanisms such as photorespiration and cyclic electron that are up-regulated under drought stress [59,80,81] are required to strengthen the use of Fv/Fm as trait selection for drought tolerance in amaranth.

In this study, the leaf and stem biomass partitioning among amaranth accessions under normal and drought conditions were phenotypically diverse, and this study revealed leaf to stem biomass allocation as a major mechanism for drought recovery. Liu and Stützel [14] and Jamalluddin et al. [12] also reported that amaranth altered the biomass allocation into stem as the plant increased in size in both normal and drought stress conditions. Similar results were also found in sorghum as the fraction of biomass into leaf and stem changed over time in normal conditions, and stem biomass was less affected by drought stress compared to leaf due to an increase of soluble sugar of the stem internode [82,83]. The reason behind plasticity of amaranth stem biomass in response to drought stress has never been studied. Nonetheless, in the present study, the stem was likely involved in altered source/sink network during drought stress and may be important for a subsequent recovery phase [84].

Amaranth accessions that had relatively high leaf and stem biomass under control condition performed well under drought stress and were identified as drought tolerant. This suggests that these accessions had a capacity to relocate/distribute carbon and nitrogen absorbed by the roots to the leaf and stem for leaf maintenance or growth during drought stress [85], and some of the solutes such as amino acids and carbohydrates are stored in the stem to be used when water is available [86]. Rewatering assessment may reveal the critical role of recovery in drought adaptation [67]. In this study, accessions with higher stem biomass were more sensitive to drought stress as significant wilting was observed at a high threshold soil water capacity. At this threshold, the plants' physiological processes start to decline [14], while allowing the plants to stay alive during drought stress and recovering upon rewatering [87,88]. This could be a strategy of amaranth to alter xylem sap in the stems during drought stress, such as to improve the chances of survival, save resources and serve as recovery after the stress phase [88].

Previous studies have concluded that the effectiveness of selection indices for drought tolerance is dependent on the stress severity [89,90]. Therefore, there is a need to determine whether to use severe or moderate drought stress to evaluate stress tolerance in amaranth germplasm. In this study, under more severe stress intensity in Trial I, yield in normal conditions was not correlated with yield under stress conditions, demonstrating that indirect selection for drought tolerant accessions based on the growth performance under irrigated conditions would not be effective if the stress intensity was severe [91,92]. Furthermore, the PCA biplot revealed that under severe drought intensity, stress indices were less discriminative for the amaranth accessions than the moderate drought stress intensity, suggesting that moderate stress may be more suitable for identifying stress tolerance. Nevertheless, the evaluation of drought stress indices in amaranth accessions at various levels of stress can facilitate plant breeders to identify accessions with stable yields in diverse environments. Thus, there is a need to select accessions with a good combination of agronomically important traits, cumulatively contributing to improved yields under target drought conditions [93]. Accessions that show low fluctuations of yield under various levels of drought stress conditions can be considered drought tolerant and stable [55]. In this study, GMP, MP and STI were the most reliable indicators of accession stability and were able to distinguish tolerant/susceptible amaranth accessions in both severe and moderate drought stress, and furthermore identified eight drought-tolerant accessions with low yield fluctuations under various levels of drought stress.

5. Conclusions

The results obtained in this experiment underline the important role of several mechanisms in protecting plants at specific water-deficit conditions. The differences of aboveground biomass partitioning into leaf and stem under drought stress may be a compromised with plants physiological traits such as the recovery process. Fv/Fm was associated with high yield in amaranth which suggests the need for further investigation of this parameter for use as a target trait during selection. Further studies are required to quantify Fv/Fm value of diverse amaranth accessions at different stress intensity to strengthen the use of Fv/Fm as a trait selection for drought tolerance in amaranth. This could be done using a pool of well characterized drought-tolerant genotypes and a contrasting set of drought-susceptible genotypes. Overall, the results showed that genotypic variations existed in amaranth accessions and these differences may show that the *A. tricolor* germplasm collection in this study could be a rich source of genetic diversity for breeding purposes for drought tolerance traits.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture11100994/s1, Figure S1: (a) Scale for leaf wilting scoring (LWS (b) Scale for drought tolerance symptom (DS). Table S1: (a) Mean of each accession for yield and biomass partitioning under water-sufficient (WS) and water-deficient (WD) in Trial I and Trial II, (b) Mean of each accession for relative water content under water-sufficient (WS) and water-deficient (WD) in Trial I and Trial II, (c) Mean of each accession for chlorophyll fluorescence under water-sufficient (WS) and water-deficient (WD) in Trial I and Trial II, (d) Mean of each accession for leaf gas exchange measurement under water-sufficient (WS) and water-deficient (WD) in Trial I and Trial II. Table S2: Mean of each accession for rewatering assessment. Table S3 (a) Correlation coefficients (R) for traits associated with water-sufficient (WS) in the bottom diagonal and water-deficient (WD) in the top diagonal for the 44 A. tricolor accessions in Trial 1. (b) Correlation coefficients (R) for traits associated with water-sufficient (WS) in the bottom diagonal and water-deficient (WD) in the top diagonal for the 44 A. tricolor accessions in Trial II. (c) Correlation coefficient (r) between drought-adaptive capabilities and physiological responses. Table S4: (a) Mean comparisons of drought tolerance indices among amaranth accession, (b) The analysis of variance and correlation coefficient (R) for stress intensity (SI) drought resistance index (DI), geometric mean probability (GMP), mean productivity (MP), stress susceptibility index (SSI), stress tolerance index (STI), stress tolerance (TOL) and yield stability index (YSI) in Trial I (bottom diagonal) and Trial II (top diagonal). Table S5: PCA biplot.

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