Brainae Journal of Business, Sciences and Technology
ISSN "2789-374X(print)" "2789-3758(online)
Volume 12, Issue 2, August 2022
http://www..brainae.org,
info@brainae.org

EFFECT OF DETACHED LEAVES DESICCATION AFTER IMBIBITION ON PHYSIOLOGICAL INDICATORS OF AMARANTH CULTIVARS

S. Havugimana, I.S. Kiseleva, E.P. Artemyeva

Ural Federal University named after the first President of Russia B. N. Yeltsin, 51, Lenina Ave.,, Institute of Natural Sciences and Mathematics, Department of Experimental Biology and Biotechnologies, 620000, Yekaterinburg, Russia,

Corresponding Author's email: hasylver@gmail.com

Received: 30 June, 2022**; Accepted: 30th** July, 2022**; Published:** 12 August 2022

DOI: https://doi.org/10.53236/23

Abstract

Desiccation is the final result of dehydration and the water status is equilibrated with air. Dehydration implies that whole plants or detached organs encounter a steady water loss and are often kept in the air to lose water. Therefore complete dehydration (desiccation) is used to describe the stressed status of plants. The pattern of changes in the weights of leaves in response to water stress was investigated in nine amaranth cultivars. The leaves were detached, imbibed in distilled water for 2 hours, and desiccated by air in Petri dishes for up to 1 hour, followed by drying in the oven for about 5 hours, a continuous decrease of leaves weight were observed. Therefore, we analyzed leaves' weight changes, water deficits, and transpiration rates under desiccation-drying treatment. Significant differences were detected between transpiration rates and water deficits with time and correlation significant between a decline in leaves weight (increase in water losses) and water deficits with a time of treatment. The results showed high transpiration rates as well as water losses. Transpiration rate plays important role in plant survival under severe water deficit while the water loss rate of detached leaves is an indicator of the ability of a cultivar to maintain yield under field conditions.

Keywords: Water deficit, leaves weight changes, water loss, transpiration rates, amaranth cultivars, imbibition-desiccation–drying treatment.

Introduction

Desiccation tolerance generally refers to the tolerance of further dehydration, when the hydration shell of molecules is gradually lost. Desiccation tolerance includes also the ability of cells to rehydrate successfully. In nature, anhydrobiosis (life without water) often bridges periods of adverse conditions [1]. With an exception of a small group of vascular angiosperm plants, termed resurrection plants can survive desiccation to an air-dried state; others are not [2]. Most of the flowering angiosperm plants where amaranth is included, are drought-sensitive and have relative water contents of around 85–100% under actively growing conditions, and do not survive if the water content falls below 59–30% [2]. In this context, numerous cultivars of amaranth were considered as a model to study the responses of plants toward tolerating moderate water stress. Desiccation tolerance in vegetative tissues is restricted to the unique group of resurrection plants contrary in seeds is common in higher plants[3], and it is found commonly in lower plants such as lichens and bryophytes, but absent in gymnosperms and rarely in pteridophytes and angiosperms[4].

Survival in extreme desiccation conditions (i.e., dehydration) is the reason for the evolutionary success of resurrection plants [5]. Water deficit stress is known to alter a variety of physiological processes such as radiation capture, leaf temperature, stomatal conductance, transpiration, electron transport, photosynthesis, and respiration which ultimately determine yield [6]. The amount of water used by a crop is closely associated with photosynthetic activity, dry matter production, and yield in many species [7]. Desiccation tolerance is controlled by many proteins; therefore, a systems biology approach combining transcriptomics, proteomics, and metabolomics should be informative to understand the mechanism of desiccation tolerance [2]. According to [8], Plants also increase antioxidant defense through a synthesis of antioxidants such as ascorbate and

glutathione and enhance antioxidant enzymes such as peroxidase, superoxide dismutase, and catalase to scavenge the reactive oxygen species (ROS) produced during stress. The detached leaves are useful to investigate desiccation tolerance during dehydration and rehydration [9]. Many studies have proved a continuous increase in temperature (by 3–5°C in the coming 50–100 years) and uneven rainfall the changes of flood and drought is always in consideration [5-6]. Therefore the abiotic stresses (water stress) negatively impact growth, development, yield and seed quality of crop [6]. The leaves as the site of physiological processes of plant were considered. Transpiration process in which varied with weather conditions and loss of water or transpiration plays a vital role in maintaining healthy plant growth, water balance and overall longevity [1]. It is in this sense we conducted desiccation experiments on the detached leaves of nine amaranth cultivars to assess the variability of drought adaptations over time through transpiration rate. Thus the general work's objective was to assess the effect of detached leaves desiccation after imbibition on physiological indicators including water deficits, leaves weight changes and transpiration rates of different nine amaranth cultivars. The study indicated the transpiration rate relation with water deficit as well as water loss rate as an indicator of cultivar ability to maintain yield. The physiological traits that enable amaranths to thrive in harsh conditions, such as drought, and be amenable for cultivation on marginal lands unsuitable for cereals, have been partly uncovered [10].

Review

Amaranth is one of the few non-grasses with potential for becoming a cereal-like grain crop; therefore it is known as pseudo-cereal but the flavour, appearance, and cooking of many species exhibit similarities to grain. It is originated from North America and consumes as a vegetable and/or a grain [21]. However, grain amaranths and many other Amaranthus species show tremendous potential for human consumption and other uses, and are particularly promising as a remedy for hunger and malnutrition in developing countries [20].

The main species for this are Amaranthus caudatus, Amaranthus cruentus and Amaranthus hypochondriacus. The species of annual flowering plant with beautiful inflorescences and is commonly used as an ornamental plant under the names such as "love-lies-bleeding", "red-hot cat-tail", "pendant amaranth", " tassel flower" Etc. [21].

The species are particularly useful for tropical areas, high altitudes, and dry conditions with excellent seed quality and shows the greatest potential for use as a food ingredient. [19] reported that Amaranth grain maintains a high protein content, averaging 15 grams per 100 grams dry weight and more than quantity, amaranth grain is remarkable for its protein quality, maintaining high levels of the essential amino acid lysine, along with uncommon sulfur-containing amino acids like methionine and cysteine. This plant is known to be tolerant to adverse environmental conditions and this tolerance has been associated with their C4 photosynthesis, high water use efficiency, an indeterminate flowering habit, and the ability to develop long taproots and an extensive web of lateral roots [21].

Materials and Methods

The nine cultivars of four *Amaranthus* species were obtained in the botanical garden of the Ural Federal University, which is located in the Sverdlovsk Region (Middle Ural, Yekaterinburg city, Russia) at the 56050′N latitude and 60036′E longitude. No treatment was done to the seeds.

Table 1 Amaranth cultivars used and their identifications

No	Species	Cultivar	Origin	Registration number	Abbreviation
1	*Amarantus caudatus L.*	cv. Edulis	Germany	49406-16	A.ca Ed
		f. Yellow brown	Germany	45378-16	A.ca Yb
		R-124	Austria	28893-95-05-16	A.ca R-124
2	*Amarantus cruentus L*	cv. Hopi Red Dye	France	29844-97-04	A.cru HRD
		cv. Nodoja	Romania	44628-09-10-16	A.cru N
		cv. Pygmy &Torch	Romania	49471-16	A.cru PT
3	*Amarantus hybridus L.*	cv. Oeschberg	Germany	41398-03-08-12-16	A.hyb O
4	*Amarantus hypochondriacus L.*	Unknown	Poland	49785-18	A.hypo P
		cv. Black leaved	Germany	47668-16	A.hypo Bl

The seeds of each species were germinated indoors under semi-controlled light and humidity in normal conditions. They have been sown in a randomized complete pot design. Nine pots (pot of 8cm height, length, and width of its bottom 12cm and 6cm and length and width of its top 16cm and 9cm respectively) were used to germinate the seeds. The substrate (Biomaster Perlite soil) was put on the bottom of each pot and the soil was filled until almost the top of each pot. The soil and soil composition used was the harvest of the fallen soil based on the bio-humus (with nitrogen 1%, phosphorus 0.5%, potassium 0.5%, and pH 6,5-7.5). After this, Water was poured on each pot until the soil became wet. Each cultivar seeds were sown in one pot in an unstructured manner and each pot was covered by microwave foil in two days. On the 15th day, the new plant babies were transplanted into small pots (Diameter's bottom 5cm, height 8cm, and diameter's top 6.5cm).
The same substrate, soil, and same conditions for filling were used. For each cultivar 15 pots were used. In each pot planted 2 plants with a distance of 2 cm.

After a month and five days, five leaves from different plants for each cultivar were cut for further observations. The 5 fresh leaves were weighed separately to fix the weight in milligrams and then imbibed in distilled water using Petri dishes for two hours. The weights after imbibition were recorded. The leaves were desiccated by paper for one hour and the weights were recorded in 5, 10, and 15,20,25,30 minutes respectively, and in 1 hour. Lastly, the leaves were dried in an oven UN 75 at 80^{o}C in 5 hours, and the dry masses were determined.

Table 2 The formulae for calculating the listed indicators in the table

Indicator	**Formula**	**Notes**	**References**
Water deficit (%)	$\mathbf{WD} = 100 * [\frac{(IW - FW)}{(IW - DW)}]$	**WD**: Water deficit; **IW**: Imbibed leaf weight (common); **FW**: Fresh leaf weight(uncommon) and **DW**: Dry leaf weight	Stocker's whole leaf method [11]
Weight gain (1st scale)	$\mathbf{WGP} = 100 * [\frac{(WVI - FWV)}{FWV}]$	WGP: Weight gain percentage WLP: Weight loss percentage, WVI: Weight values with imbibition, FWV: Fresh weight values, DWV: Desiccated weight values and DMV: Dry mass values.	-
Weight loss in an hour	$\mathbf{WLP} = 100 * [\frac{(WVI - DWV)}{WVI}]$		
Weight loss in 5 hours	$\mathbf{WLP} = 100 * \frac{DWV - DMV}{DWV}$		
Weight loss in 6 hours	$\mathbf{WLP} = 100 * \frac{WVI - DMV}{WVI}$		
Transpiration rate(mg/g.h)	$TR = \frac{M1 - M2}{M1 * T}$	**TR**= transpiration rate in mg/g.h; $\mathbf{M_1}$= initial leaves weight $\mathbf{M_2}$= next leaves weight and T = time.	Weight loss approach[12]

The data of all parameters were analyzed in excel 2010, and within it, further, analyses were conducted using one-way analysis of variance (ANOVA) and regression analysis. The results were expressed as the means ± standard error (SE) for weights of leaves and as the means only for others.

Findings and Discussion

Global results for nine different amaranth cultivars listed in table 3, showed that during the dehydration including desiccation on paper and total drying in an oven, there was a strong correlation with time in weight changes of leaves and water deficit percentages values and there was no correlation between time and transpiration rates values. This weight change of leaves showed a strong correlation with water deficit values and no correlation with transpiration rates values. Thus there was a negative correlation between water deficit values and transpiration rates values.

Our experiments were conducted on amaranth detached leaves sensitive to excessive dehydration after imbibition through the desiccation process followed by drying in the oven and the results have shown a close correlation between leaves weight changes and time of treatment in response to water stress. [13]; reported a similar correlation in cabernet sauvignon leaves placed into a plastic box containing a salt solution, its water loss over an 8h period increased in general with time and for the first hour and a half followed a linear trend followed by a decrease in the amount of water lost. In this study, we have not determined if similar physiological or biochemical changes occur in attached leaves. Imbibition in distilled water- desiccation – drying treatment has been resulted in decreasing of detached leaves weights over time [Table 3].

This decline in weights indicated the weight losses or water losses at each scale from the start of desiccation. Moreover, the weight losses in terms of water ranged from 88.3% to 92.2% in six hours (desiccation and drying), from 82.8% to 86.7% in five hours without the first hour (drying only), and from 31.0% to 49.8% in 1 hour (desiccation only) [Table 4]. Unlike the results were obtained on a scale of imbibition by which the weights increased and ranged from 25.2% to 41.2% [Table 4].

The results obtained in this study were in agreement with the results of [14], who observed a high decrease of the relative water content of R. serbica leaves, during the 13 days of dehydration the RWC continuously decreased from 98% in the fully hydrated plants to 4% in the desiccated ones (Day 4). Present results are in contrast to the findings of [15], in the study of packaged lettuce leaves stored under cold darkness, water losses were close to 5% on day 2 and 10% on day 7. This low amount corresponded to the dehydration during senescence.

Another contrast was concerned in the study of [16] with the succulent leaves of Peperomia magnoliaefolia (Jac) (Piperaceae) during slow desiccation; relative water loss was significantly greater for the hydrenchyma than for the chlorenchyma. When whole leaves had lost 50% of their initial water content. Among factors affecting leaf water loss, a major assumption is that water loss is occurring primarily through the stomata and that they are responding by closing during the dehydration event. Other sources of water loss through the cuticle or cut end of the petiole were considered to be negligible [13]. According to them, leafage is another factor that may influence the amount of water that is lost due to differences in stomatal sensitivity. In common, during the dehydration essay, if leaves failed to respond by closing their stomata, consequently they continued to lose water. Therefore our study explained well this observation.

Table 3 Physiological indicators of different nine Amaranth cultivars

Nº	Plant material and its character		Phenomena: initiation at 1 minute, imbibition in 2 hours, desiccation in 5, 10,15,20,25,30 minutes, and in 1 hour, and total drying with their corresponding **time in minutes**								
			1	**120**	**125**	**130**	**135**	**140**	**145**	**150**	**180**	**480**
1	*A.ca R-124*	W	383.6±23.5	480.2±34.9	444±32.4	410±30.62	383.8±32.6	347.4±31.4	332.6±27.4	324.2±26.4	275.2±26.2	41.6±7.1
		WD	*	#	20	20	20	30	30	40	50	@
		TR	*	#	918.9	766.8	1138.1	511.2	303.1	1813.7	1697.7	@
2	*A.ca Ed*	W	384.6±22.7	491.6±36.9	442.8±29.8	395.2±26.9	366.4±25.4	354.8±24.7	342.6±24.5	329.6±22.5	304.2±19.9	41.2±3.6
		WD	*	#	10	20	30	30	30	40	40	@
		TR	*	#	1290.0	874.5	379.9	412.6	455.3	924.8	1729.1	@
3	*A.ca Yb*	W	438±41.4	573±44.6	539.8±44.2	504.2±40.5	475.6±38.6	445.4±35.0	423.4±38.3	413.4±37.5	367.6±32.9	48.8±6.6
		WD	*	#	10	10	20	20	30	30	40	@
		TR	*	#	791.4	680.7	762.0	592.7	283.4	1329.5	1734.5	@
4	*A.cru HRD*	W	253.3±47.5	324.0±49.0	298.7±48.6	281.3±43.2	264.7±40.4	245.0±39.3	229.3±37.6	219.3±37.5	172.3±32.5	25.3±6.0
		WD	*	#	10	10	20	30	30	40	50	@
		TR	*	#	696.4	711.0	891.6	767.3	523.3	2571.8	1706.3	@
5	*A.cru* N	W	471.5±18.4	614±34.0	574.8±26.7	548.8±30.0	524.5±30.7	487.5±23.2	468.5±25.4	452.5±23.2	403.3±23.0	57.3±5.7
		WD	*	#	10	10	20	20	30	30	40	@
		TR	*	#	542.8	530.1	846.5	467.7	409.8	1306.1	1715.8	@
6	A.cru PT	W	355.3±42.8	484.2±59.5	426.2±49.2	403±46.1	378±43.7	358.2±43.1	337.2±41.0	321.8±39.4	274.4±35.4	42.6±6.3
		WD	*	#	10	20	20	30	30	40	50	@
		TR	*	#	658.5	744.4	628.6	703.5	548.0	1767.6	1689.5	@
7	*A.hyb O*	W	473.2±36.4	668±54.0	600.6±48.3	569.6±47.5	532.6±46.4	500±45.5	476±43.5	446.8±43.1	335.2±40.6	57.8±6.8
		WD	*	#	10	20	20	30	30	40	50	@
		TR	*	#	619.4	779.5	734.5	576.0	736.1	2997.3	1655.1	@
8	*A.hyp o P*	W	398.4±17.6	502.4±17.3	471.8±18.5	451±17.6	432±18.6	415±20.0	398.6±14.4	369.8±17.3	346.6±16.8	58.6±2.5
		WD	*	#	10	10	20	20	20	30	40	@
		TR	*	#	529.0	505.5	472.2	474.2	867.0	752.8	1661.9	@
9	*A.hyp o Bl*	W	417.4±37.6	585.4±55.0	557.6±51.2	492.2±45.2	473.8±39.1	457.8±38.9	431±35.7	422±34.4	334±26.0	52.4±10.4
		WD	*	#	10	20	20	20	30	30	50	@

NOTE: WD stands for water deficit in percentage at desiccation state; W: weight of leaves in milligrams with their ±SE (standard error) while TR for transpiration rate in milligrams per grams and hour ($mg.g^{-1}h^{-1}$) at desiccation state ***** Initial process getting only fresh weight; **#** Water absorption which differs from transpiration and desiccation process @ Drying process in the oven to get dry mass.

Statistical in function of time variation from desiccation, $P < 0.05$ for both weight variation and water deficit against $P > 0.05$ for transpiration rates values; between water deficit and transpiration rate $P > 0.05$ and lastly $P > 0.05$ and $P < 0.05$ between

weight variation from desiccation with transpiration rate and weight variation from desiccation with water deficit respectively.

Table 4 Water status in percentages under imbibition-desiccation-drying treatment

No	Plant material	Water gain(%) in 2 hours	Water loss (%) under desiccation (1 hour)	Water loss (%) under drying (5 hours)	Water loss (%) under desiccation and drying (6 hours
1	*A.ca L. R-124*	25.2	42.7	84.9	91.3
2	*A.ca L.cv. Ed*	27.8	38.1	86.5	91.6
3	*A.ca L.f.Y-B*	30.8	35.9	86.7	91.5
4	*A.cru L.cv.HRD*	27.9	46.8	85.3	92.2
5	*A.cru L.*cv. N	30.2	34.3	85.8	90.7
6	A.cru L.cv.PT	36.2	43.4	84.5	91.2
7	*A.hyb L.cv.O*	41.2	49.8	82.8	91.4
8	*A.hyp L.*	26.1	31.0	83.1	88.3
9	*A.hyp L.cv.BL*	40.3	43.0	84.3	90.1

The data given in Table 3 indicates that water deficit (%) among amaranth cultivars had a positive significance with a time of desiccation and also indicates that through desiccation, water deficit (%) increases with the decrease of weights of leaves. This hypothesis defines that the increase in water deficit induces an increase in leaf water loss (leaf weight loss). Almost all cultivars showed 10% as minimum water deficit at five minutes, except one cultivar with 20% as minimum water deficit at the same time. On the other side, the maximum water deficit was 50% for five amaranth cultivars and 40% for four amaranth cultivars at 60 minutes [Table 3].

We detected no significant variations in transpiration rates under desiccation treatment. Transpiration was measured by the loss in leaf weight during the period 60 minutes after imbibition and expressed as a percentage of the weight at one hour. For the data in Table 3, our study revealed that the detached leaves of amaranth cultivars (see plant material) had higher transpiration rates but their transpiration intensities were not correlated to their water deficits (%) nor their weight changes through desiccation. It has been shown in several instances that transpiration may be unaffected by a certain degree of water deficit, but that this varies with the plant. [17] Demonstrated with isolated wheat leaves that transpiration was not reduced until the water deficit exceeded 10 percent. The effect of water deficit in minimizing the transpiration rate could not be observed from the present study in all the amaranth cultivars.

The values of transpiration rates are composed of the alternatives of increase and decrease but in general, from the initial treatment time up to the last treatment time, the increase is concerned. The same observations in the study of [18], investigating the transpiration rate of windbreak trees by the method of rapid weighing of detached parts, the values were varied over time, in January the maximum transpiration rate was 439 mg./g.h in Tamarix sp, 408 mg./g.h in Acacia, 330 mg./g.h in Casuarina and 123 mg./g.h in Cupressus whereas in March the maximum transpiration rate reached was 572, 365, 263 and 72 mg./g.h respectively correspondingly. Contrary the values of Rahman are lower than our present study.

The time trend of transpiration rate revealed the alternative changes of increase and decrease trend throughout the water deficit increase with certain constants as well as weights decrease. In our study, some amaranth cultivars showed the same step positions of desiccation for the smallest transpiration rates, others not and this observation pointed again to the highest transpiration rates. Thus the smallest transpiration rates were obtained on the third desiccation step position for the A.ca Ed, A.hypo P., and A.hypo Bl; the fourth desiccation step for A.hyb O and on the fifth desiccation step for A.ca R-124, A.ca Yb, A.cru Hopi HRD, A.cru N and A.cru PT (Table 4). Another side, the highest transpiration rates were resulted in the sixth desiccation step for A.ca R-124, A.cru HRD, A.cru PT, A.hyb O, and A.hypo Bl and on the seventh step for A.ca Ed, A.ca Yb, A.cru N and A.hypo P(Table 4).

Conclusion and Recommendations

The amaranth species are crops with great potential to reduce malnutrition and its attendant diseases as well as tolerant to stressful conditions. We studied the detached leaves dehydration that could be used to detect differences among genotypes within amaranth based on their sensitivity to dehydration. In conclusion, the desiccation effect in amaranth cultivars listed above under stressful water deficit conditions is assessed through a complex of functional adaptations, among which the water loss and transpiration rate plays an important role. And the results showed that all amaranth cultivars had higher leaf water losses, transpiration rates as well as water deficits. We did not investigate the tolerance ability of these cultivars in this present study. These results proved that the detached leaves were desiccated without any resources of water absorption because of the disruption of transport and their stomata failed to close to control the movement of water. Further work is needed in order to assess the desiccation responses through rehydration experiments , evaluate the tolerance ability and its potential evolutionary role in these cultivars and other angiosperm plants.

Acknowledgments

The authors would like to acknowledge the support of providing the seeds of Amaranth cultivars from the Ural Federal University Botanical garden.

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