Lipid peroxidation within different amaranth cultivars

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Abstract

In natural environments, plants are exposed to biotic and abiotic stresses during their whole life circle. Moreover, lipid peroxidation is a physiological indicator of the above stress responses, hence is often used as a biomarker to assess stress-induced cell damage or death. This study evaluated the lipid peroxidation of base and stress leaf discs for nine amaranth cultivars. The feasibility of optical density with $\lambda = 532$ and $\lambda = 600$ nm was investigated, and the malondialdehyde (MDA) concentration intensity was determined using the TBA method, especially the Thiobarbituric acid (TBA) extinction coefficient to detect its content. Furthermore, MDA values were ranging from 0.007 ± 0.001 mM/g^{-1} to 0.013 ± 0.002 mM/g^{-1} and from 0.016 ± 0.002 mM/g^{-1} to 0.035 ± 0.008 mM/g^{-1} for base and stress conditions respectively. This study represented high MDA content under water stress and low MDA content detection in leaves of *A. caudatus* L., *A. hypochondriacus* L., *A. cruentus* L., and *A. hybridus* L. cultivars. This indication defines the better antioxidant activity of these cultivars.

Keywords: lipid peroxidation, leaf discs, MDA content, amaranth cultivars.

Peroxidação lipídica em diferentes cultivares de amaranto

Resumo

Em ambientes naturais, as plantas estão expostas a estresses bióticos e abióticos durante todo o seu ciclo de vida. Além disso, a peroxidação lipídica é um indicador fisiológico das respostas de estresse, portanto, é frequentemente usado como um biomarcador para avaliar danos ou morte celular induzida por estresse. Este estudo avalia a peroxidação lipídica dos discos foliares de base e estresse de nove cultivares de amaranto. A viabilidade da densidade óptica com $\lambda = 532$ e $\lambda = 600$ nm foi investigada, e a intensidade da concentração de malondialdeído (MDO) foi determinada usando o método TBO, especialmente o coeficiente de extinção do ácido Tiobarbitúrico (TBO) para detectar seu conteúdo. Além disso, os valores de MDO variaram de 0,007 mM/g^{-1} e 0,013 mM/g^{-1} e de 0,016mM/g^{-1} e 0,035 mM/g^{-1} para condições básicas e de estresse, respectivamente. Este estudo representou detecção de alto teor de MDO sob estresse hídrico e baixo teor de MDO nas folhas das cultivares *A. caudatus* L., *A. hypochondriacus* L., *A. cruentus* L. e *A. hybridus* L. Essa indicação define a melhor atividade antioxidante dessas cultivares.

Palavras-chave: peroxidação lipídica, discos foliares, teor de MDA, cultivares de amaranto.

1. Introduction

Lipid peroxidation is a deleterious process in plants, which affects membrane properties, causes protein degradation, and limits the capacity of ionic transport, ultimately triggering the cell death process (Awasthi et al., 2018). It degrades the lipids that occur because of oxidative damage (Vasilaki; McMillan, 2011) and is a useful marker for oxidative stress (Ito et al., 2019). It may contribute to the pathology of many diseases including atherosclerosis, diabetes, and Alzheimer's (Negre-Salvayre et al., 2008).

Amaranth is a source of several thousands of polyphenols (natural antioxidants) that decrease oxidative stress in the human body (Ayala et al., 2014). Polyunsaturated lipids are susceptible to an oxidative attack, typically by reactive oxygen species (ROS), resulting in a well-defined chain reaction with the production of end products such as malondialdehyde (MDA) (Tao, 2015).

This present study evaluated the level of lipid peroxidation in terms of MDA contents in nine different amaranth cultivars to prove or estimate the presence of their antioxidant defense systems and adaptive stress response.

2. Materials and Methods

2.1 Plant material

Seeds of amaranths for our investigations were obtained from different Botanical Gardens through an international seed exchange. The species and cultivars used were listed in (Table 1).

Table 1. Amaranth species and cultivars studied.

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

Source: Authors, 2022.

2.2 Experiment design

In nine cultivars of amaranth, three biological replications (plants) for each cultivar were selected with fully expanded healthy leaves from each seedling. Ten leaf discs were prepared and weighed from each seedling. They were weighed again after imbibed in distilled water for two hours in two separate parts (5 leaf disks for one part); one part was fixed in liquid Nitrogen for the assessment of the base lipid peroxidation level. Another part was desiccated for one hour in *Petri* dishes. And after that, the dried leaf disks were weighed before fixing them in liquid Nitrogen for the lipid peroxidation assessment of stressed leaf disks.

2.3 Lipid peroxidation assay

The lipid peroxidation level was determined by quantifying the malondialdehyde (MDA) content using the thiobarbituric acid assay (TBA test) according to Heath & Packer (Heath; Packer, 1968). The solution (reaction mixture) of 200 mL was prepared from 20 g of 10% Trichloroacetic acid (TCA) + 0.5 g of 0.25% Thiobarbituric acid (TBA). 100-150 mg of frozen plant tissues were ground in a mortar using a pestle with glass powder and were homogenized with 1 mL of the reaction mixture. The homogenate was transferred from the mortar into a 15 mL conic tube, washing off the homogenate with a reaction mixture twice (0.5 mL + 0.5 mL). The final volume of the homogenate is 2 mL. The homogenate was heated in a water bath (95 °C) for 30 min, then the homogenate

was treated in cold water for two hours. The homogenate was centrifuged for 15 min and filtered to pick up 300 µL of the supernatant and pour it into the well of the plate.

The optical density was recorded by UV-*Vis* spectrophotometer at λ = 532 and λ = 600 nm. MDA concentration intensity was calculated using TBA extinction coefficient: TBA-RP (mM/g^{-1}) = (OD_{532}-OD_{600})/(155*m), TBA-RP – TBA reacting products/malondialdehyde (MDA) m – fresh biomass, g and 155 – TBA extinction coefficient, mM/cm^{-1}.

2.4 Analysis statistical

Data are expressed as mean ± standard errors. The regression statistics and ANOVA within Microsoft Excel 2010 were used to provide Statistical analysis of the data.

3. Results and Discussion

In cultivars of *A. hypochondriacus* L., *A. cruentus* L., *A. caudatus* L. and *A. hybridus* L. leaves; MDA (mM/g^{-1}) values were obtained and presented as 0.007 ± 0.001 for *A. caudatus* L. *cv.* Edulis and *A. caudatus* L. f. Yellow-brown; 0.008 ± 0.000 for *A. cruentus* L. *cv.* Nodoja; 0.008 ± 0.001 for *A. caudatus* L-R-124, *A. hypochondriacus* L. *cv.* Black leaved and *A. cruentus* L. *cv.* Hopi & Red Dye; 0.012 ± 0.001 for *A. hybridus* L. and *A. cruentus* L. *cv.* Pygmy & Torch and 0.013 ± 0.002 for *A. hypochondriacus* L. (Table 2).

In stress conditions, the leaves MDA values were 0.016 ± 0.002 for *A. caudatus* L. f. Yellow-brown; 0.020 ± 0.001 for *A. hypochondriacus* L. *cv.* Black leaved; 0.027 ± 0.003 for *A. caudatus* L. *cv.* Edulis; 0.028 ± 0.004 for *A. cruentus* L. *cv.* Nodoja; 0.030 ± 0.006 for *A. cruentus* L. *cv.* Hopi Red Dye; 0.032 ± 0.004 for *A. hypochondriacus* L.; 0.033 ± 0.001 for *A. caudatus* L. R-124; 0.033 ± 0.005 for *A. hybridus* L. and 0.035 ± 0.008 for *A. cruentus* L. *cv.* Pygmy & Torch (Table 2).

The comparative observation of our data between two different conditions, the normal or base conditions, and the stress conditions showed that the MDA values under stress conditions were higher than those under normal conditions. These observations are similar to reports by Lukatkin et al. (2020); however, a great significance was the increase in lipid peroxidation intensity at the highest concentration of heavy metals in the medium. The MDA values of stressed leaves ranged from 0.016 ± 0.002 mM/g^{-1} to 0.035 ± 0.008 mM/g^{-1} whereas for the leaves without stress but after imbibition in distilled water, the MDA values ranged from 0.007 ± 0.001 mM/g^{-1} to 0.013 ± 0.002 mM/g^{-1}.

This is because MDA is believed to originate under stress conditions and has a high capability of reacting with multiple biomolecules such as proteins or DNA that leads to the formation of adducts and excessive MDA production has been associated with different pathological states (Ayala et al., 2014). And, under physiological or low lipid peroxidation rates (sub-toxic conditions), the cells stimulate their maintenance and survival through constitutive antioxidants defense systems or signaling pathways activation that upregulates antioxidants proteins resulting in an adaptive stress response (Lukatkin et al., 2020). By contrast, under medium or high lipid peroxidation rates (toxic conditions) the extent of oxidative damage overwhelms repair capacity, and the cells induce apoptosis or necrosis-programmed cell death (Ayala et al., 2014).

In both conditions of study, there is no correlation between the masses of leaves and their MDA values because the values calculated from the range of masses and the MDA values range are superior to 0.05 ($p > 0.05$). This is generally, measuring the optical density/absorbance/turbidity is a common method to quantify the concentration of substances (Beer-Lambert law), since the absorbance is proportional to the concentration of the absorbing species in the sample (EloCheck Application Note 1).

MDA levels were significantly enhanced at desiccation stress which is an indication of tissue damage. Thus, lipid peroxidation due to increased water stress has been reported in amaranth cultivars leaves as discussed by Lukatkin et al. (2020). Our results are very lower than the reports of Bashmakov et al. (2012, 2016, and 2017); Sazanova et al. (2012) in three plants where MDA concentration varied from 0.082 to 0.256 $µM/g^{-1}$ FW-1 in maize, from 0.6 to 1.4 $µM/g^{-1}$ FW-1 in wheat, and from 0.38 to 0.91 $µM/g^{-1}$ FW-1 in cucumber (*Cucumis sativus* L.). The same comparison in the study of MDA levels in salvia (*Salvia hispanica* L.) plant species seeds where MDA levels ranged from 0.556 to 0.920 mg/L^{-1} (Sari et al., 2012).

Many researchers reported different results compared to ours like Tandey et al. (2020) in their study of *A. cruentus* responses to the pollution from polycyclic aromatic hydrocarbons from a thermal power plant, mean MDA content was 3.26b ± 0.2 $nmol/g^{-1}$ FW and Lukatkin et al. (2020) in the assessment of *A. retroflexus*

seedlings response to heavy metals, MDA content was ranging from 0.86 to 0.89.

Table 2. Lipid peroxidation level of nine cultivars of amaranth in terms of malondialdehyde (MDA) mM/g^{-1} as a second product of lipid degradation, the imbibed weight and stressed weight of leaf disks in grams, the means, and their standard error (SE) of the base.

Plant material	The base MDA concentration intensity		The stress MDA concentration intensity	
	Imbibed weight	Mean ± SE	Stressed weight	Mean ± SE
A. ca R-124	0.111	0.008 ± 0.001	0.040	0.033 ± 0.001
A. ca Ed	0.105	0.007 ± 0.001	0.035	0.027 ± 0.003
A. ca Yb	0.121	0.007 ± 0.001	0.053	0.016 ± 0.002
A. hypo P	0.116	0.013 ± 0.002	0.049	0.032 ± 0.004
A. hypo Bl	0.122	0.008 ± 0.001	0.056	0.020 ± 0.001
A. hyb O	0.104	0.012 ± 0.001	0.045	0.033 ± 0.005
A. cru PT	0.108	0.012 ± 0.001	0.043	0.035 ± 0.008
A. cru HRD	0.106	0.008 ± 0.001	0.034	0.030 ± 0.006
A. cru N	0.100	0.008 ± 0.000	0.041	0.028 ± 0.004

Notes: *p*-value between fresh masses and their MDA values ($p = 0.848$) and between stressed masses and their MDA values ($p = 0.116$). Source: Authors, 2022.

4. Conclusion

The present study indicates that the malondialdehyde (MDA) concentration intensity was low in both conditions (under stress and without stress) and the MDA contents were higher in stressed conditions because MDA production is associated with pathological states. This low lipid peroxidation shows that all cultivars involved in our study stimulate the antioxidant defense systems and have a good adaptive stress response. The cultivars show resistance to drying stress, and a deep understanding of amaranth's stress tolerance mechanisms is likely to provide valuable input to improve stress tolerance in the cultivars. Our study will be useful in the agriculture and medicine sectors.

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6. Auhors' Contributions

Sylvestre Havugimana: Conceptualization, investigation, methodology, data analysis, writing and, supervision. *Irina Sergeevna Kiseleva*: review and supervision. *Daniel Nsengumuremyi*: original draft and writing.

7. Conflicts of Interest

No conflicts of interest.

8. Ethics Approval

Not applicable.

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