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Original Article

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Effects of Prior Heat Stress on the Growth and Phytochemical Contents Accumulation of *Amaranthus hybridus* (Linn.)

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Abstract

The present study aimed at investigating the impact of abrupt heat stress on growth and phytochemical contents accumulation in *Amaranthus hybridus*. The treatments were as follows: control without heat treatment, seedlings subjected to heat at 45 °C for two hours and seedlings subjected to heat at 45 °C for four hours. After the stipulated time for each category, plants were removed from the Gallenkamp oven and were transplanted into other sets of thirty six pots (of 21 cm deep and 24 cm in diameter), as well as the control. The seedlings were kept in a screen house to minimise extraneous factors such as pests and rodents. They were watered daily with 200 mL of tap water in the morning and 200 mL of tap water in the evening until they were fully established. The phytochemical contents were determined at vegetative, flowering and fruiting stage using ethanolic extracts from the dried leaves of plant samples. From the results obtained, it was observed that leaf, shoot and root fresh and dry weights of the stressed plants were lower than the control plants. Exposure of the plants at different durations of heat treatment enhanced and inhibits the quantities of phytochemicals at different growth stages. From the present study it can be concluded that heat stress, on the basis of global warming in the future, will likely have overall negative effects on the growth of *Amaranthus hybridus* that will become more severe as the time of exposure increases and and might cause variation in the level of phytochemical constituents of *Amaranthus hybridus* at different growth stages.

Keywords: biochemical; early stage; growth; heat; phytochemicals; stress

Introduction

Physiological processes of plants are largely affected by alteration of surrounded environmental factors such as heat, light, rainfall, heavy metal, pathogenicity, nutrient deficiency, etc. Plants respond to these factors by acclimatization, avoidance and resistance. Most acclimatization strategies ranges from morpho-anatomical to physiological and biochemical strategies (Zhu, 2001). Plants select the sort of acclimatization by additive or synergistic effects of genetic constitution and resistance to be able to withstand and resist the effects of the environmental stress factors (Bray *et al*., 2000); avoidance involves the prevention or decreasing the impact of the stress on the plant, such as minimizing water loss and maximizing water uptake in case of drought (Chaves *et al*., 2003) or exclusion of salt ions (Munns *et al*., 2006).

Like other abiotic stresses, heat stress brings about a range of conditions that might positively or negatively influences the growth, development and function of an organism. Heat stress is a great modulator of growth and productivity (Zhang *et al*., 2000). Prevailing high temperature reduces crop yield and affect plant growth from germination up to maturity. The mechanism leading to the survival of a crop under heat stress entail changes in physiology and accumulation of osmolytes like proline, glycinebetaine, soluble sugars and proteins (Wahid and Close, 2007).

The stability of life processes in most plants is comparatively wide, whereas temperature ranges from several degrees above 0 °C to around 35 °C. The increase of temperature up to a certain level increases plant growth, photosynthesis, respiration and enzyme activity. After this temperature, such parameters tend to decline (Mirza *et al*., 2003).

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Amaranthus hybridus and other species of *Amaranth* have been used by various clans in African countries for a long time as indispensable constituents of human diets. *Amaranthus hybridus* has been shown to contain large amount of phytochemicals that have both health and industrial benefits (Rao and Newmark, 1998; He *et al*., 2003). These phytochemicals are alkaloids which function in the defence of plants against herbivores, glycosides and phenols which play an important role in the control of human pathogenic infections (Firn, 2010), flavonoids which are used as antioxidants, saponin which are extremely poisonous as they cause haemolysis of blood and can be used as soap, terpenes which are commonly found in essential oils or oleoresins (Firn, 2010). Others are essential oils, steroids and antraquinones are also noted. They are therefore mainly consumed for their nutritional and medicinal values with much consideration on their medicinal importance and their phytochemical contents which have been scarcely explored (Knekt *et al*., 1997).

Despite the vast use of this vegetable, there is paucity of information on the effect of some environmental factors which can disrupt the growth and phytochemical constituents of this species. The present work therefore aimed at documenting the effect of heat stress on the growth and phytochemical contents of *A. hybridus* in a bid to provide information on the limiting factor which can affect the growth and phytochemical constituents.

Materials and Methods

Collection of seeds

Seeds of *Amaranthus hybridus* were utilised for the present experiment. The seeds were obtained from Nigeria Horticulture Research Institute (NIHORT), Ibadan, Nigeria.

Raising of seedlings

The experiment was carried out under a screenhouse to minimise extraneous factors such as insects, pests and other materials that may alter the course of this experiment. Germination was carried out in a nursey. Three small bowls (each of 12 cm in diameter and 5 cm high) were used to germinate the seeds. Holes about 2 mm were bored at the bottom to allow for proper drainage of excess water. The bowls were labelled as A, B and C. After three weeks of sowing, two of the three pots (A and B) were put inside a Gallenkamp oven for heat treatment. The two stressed categories were subjected to the same temperature (at 45 °C), but had different durations; pot A was left in the oven for 2 hours and pot B for 4 hours. After the stipulated time for each category, they were both removed and along with the control samples were transplanted into another 36 sets of pots (of 21 cm in deep and 24 cm in diameter) with bored holes at the bottom of each pot to allow proper drainage. The seedlings were divided into three categories containing 12 pots for the stressed plants at 45 °C for 2 hours, 12 pots for 4 hours at 45 °C and the last 12 pots for the control. The seeds were sown at the rate of three seeds per pots.

Measurements of some morphological parameters

Measurements of some morphological parameters were taken: leaf fresh and dry weights, shoot fresh and dry weights and root fresh and dry weights. For fresh weights determination, plants were carefully uprooted and the sand particles that were attached to the roots were washed off with water and then mopped to drain the water. The fresh plants were taken on a weighing balance after which they were dried on a Gallenkamp oven at 80 °C for 72 hours. This procedure was repeated at seven day intervals.

Processing of plant samples for phytochemical screening

The leaves of *Amaranthus hybridus* were harvested, properly washed, oven dried at 40 °C and grinded to a powdered form with mortar and pestle. This process was done separately for the three growth stages and placed in separate, labeled glass bottles.

Preparation of ethanolic extract of plant samples

The aqueous extract of each plant sample at different growth stages was prepared by soaking 5 g of powdered samples in 200 mL of ethanol for two weeks. The extract was then filtered using Whatman's No 1 filter paper.

Phytochemical analysis

Qualitative analysis was carried out to ascertain the presence of the different phytochemicals as described by Edeoga *et al*. (2005).

Test for tannins

Tannin was determined by the Folin-Denis colorimetric method. About 0.5 g of the extracts of each plant were boiled in 20 mL of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added. A brownish green or a blue-black coloration indicated the presence of tannins.

Test for saponins

Two mg of the extracts of each plant were boiled together with 20 mL of distilled water in a water bath, vigorously shaken and noted for froth. The appearance and persistence of frothing before and after warming indicated the presence of saponins.

Test for flavonoids

Five mL of dilute ammonia solution were added to the ethanolic extract of each plant sample in a test tube, followed by addition of concentrated H_2SO_4. A yellow coloration was observed and disappeared on standing. This indicated the presence of flavonoids.

Test for phenol

A sample of 500 mg of the extract was dissolved in 5 mL of distilled water. To this, few drops of neutral ferric chloride solution was added. A dark green color indicated the presence of phenolic compounds.

Test for alkaloids

Five mL solution of the extract and 2 mL of dilute hydrochloric acid were taken in a test tube. Then 1 mL of Dragendroff's reagent was added to this acidic medium. Orange or red precipitate was formed and that indicated the presence of alkaloids.

Test for steroids

Two mL of acetic anhydride were added to 0.5 g ethanolic extract of each sample with 2 mL H_2SO_4. The change of color from violet to blue or green in some samples indicated the presence of steroids.

Test for reducing sugars

About 5 g each of the dried samples in a test tube, equal amount of Fehling's solution A and B were added. The mixture was boiled over a burner. Observation of colour was made. A colour change from deep blue to brick red indicated the presence of reducing sugar.

Test for phlobatannins

When the leaf extract was boiled with 2 ml of 1% hydrochloric acid, formation of red precipitate indicated the presence of phlobatanin.

Statistical analysis

The statistical analysis was performed using Statistical Analytical Software (SAS) version 9.2. A one way analysis of variance (ANOVA) was carried out to investigate the impact of heat stress on the growth and phytochemical contents of *Amaranthus hybridus*. Post hoc setting testing was carried out using Duncan Multiple Range test to separate the significance means at 0.05 confidence limit (alpha level).

Results

Effect of prior heat stress on the growth of Amaranthus hybridus

The leaf fresh weight and leaf dry weights of the stressed plants were lower to the control plants at each course of the experimental period. The plant exposed to heat for four hours had the lowest leaf fresh weight and dry weight compared with those exposed for two hours and the control, while the control plants recorded the highest value (at 45 °C) (Table 1). There was a significant effect of heat stress on this attributes to the control plants.

The shoot fresh weights and dry weight tended to decline with heat stress as the duration of exposure increased. The plants stressed for four hours recorded the lowest shoot fresh weights and dry weight, followed by those exposed for two hours, while the control plants had the highest shoot fresh weights and dry weight throughout the entire period of the experiment (Table 2).

Like total leaf fresh weight and leaf dry weights, shoot fresh weights and dry weight it was discovered from the results obtained that the root fresh weights and root dry weights of those plants exposed to heat for four hours were the lowest and that of the control were the highest throughout the experimental period. The results showed that there was a significant difference in the leaf fresh weight and leaf dry weights of the stressed plants to that of the control plants (Table 3).

Effect of heat stress on the phytochemical content of Amaranthus hybridus

The results obtained from the qualitative analysis of *A. hybridus* showed that alkaloids, tannins, saponins and flavonoids were all present in the control, plants stressed for 2 hours and plants stressed for 4 hours. Reducing sugars, terpenoids, phlobatannins were absent in these treatments (Table 4).

The results obtained from the quantitative analysis showed that saponins was highest in the plants stressed for

Table 1. Effect of prior heat stress at the seedling stage, on the leaf fresh and dry weights of *Amaranthus hybridus*

		Weeks after sowing							
	Treatments	1	2	3	4	5	6	7	8
Leaf fresh weights	Control	2. 01b	2.27b	4.10b	5.22c	8. 02b	8.36b	9.46b	10.32b
	2 Hours	2.10a	2.59a	4.45a	8.14a	9.33a	9.57a	10.37a	11.45a
	4 Hours	1.67c	1.79c	3.66a	5.36b	7.47c	7.58c	8.69c	9.01c
Leaf dry weights	Control	0.29a	0.40a	0.66a	0.68a	0.82a	0.98a	1.16a	2.49a
	2 Hours	0.24b	0.38b	0.59b	0.63b	0.81b	0.96b	1.16a	2.41b
	4 Hours	0.24b	0.32c	0.42c	0.57c	0.54c	0.69c	0.94b	2.36c

Means with the same superscript along the same column are not significantly different at P > 0.

Table 2. Effect of prior heat stress at the seedling stage, on the shoot fresh and dry weights of *Amaranthus hybridus*

		Weeks after sowing							
	Treatments	1	2	3	4	5	6	7	8
Shoot fresh weights	Control	3.05b	4.98a	6.68b	8.01b	9.24a	10.53a	11.24b	13.78a
	2 Hours	3.96a	4.46a	7.16a	9.05a	7.52b	10.15b	11.78a	13.71b
	4 Hours	2.42c	2.88c	6.447c	7.31c	7.58c	9.01c	10.85c	12.65c
Shoot dry weights	Control	0.33a	0.53a	0.92a	1.12a	1.60b	1.96a	2.64a	3.14a
	2 Hours	0.31a	0.49b	0.77b	1.10b	1.71a	1.96a	2.53b	3.10b
	4 Hours	0.22c	0.39c	0.52c	0.94c	1.20c	1.67b	2.18c	3.00c

Means with the same superscript along the same column are not significantly different at P > 0.05

Table 3. Effect of prior heat stress at the seedling stage, on the root fresh and dry weights of *Amaranthus hybridus*

		Weeks after sowing							
	Treatments	1	2	3	4	5	6	7	8
Root fresh weights	Control	0.44a	0.73a	1.28a	1.99a	2.00b	2.86b	2.98a	3.78a
	2 Hours	0.31b	0.50b	1.25b	1.89b	2.08a	2.87a	2.71b	3.77b
	4 Hours	0.26c	0.34c	1.03c	1.78c	1.90c	2.67c	2.62c	3.56c
Root dry weights	Control	0.04a	0.06b	0.07a	0/17a	0.19a	0.21a	0.28a	0.44a
	2 Hours	0.03b	0.08a	0.07a	0.12b	0.18b	0.20b	0.28a	0.44a
	4 Hours	0.01c	0.02c	0.05b	0.10c	0.14c	0.18c	0.27b	0.39c

Means with the same superscript along the same column are not significantly different at P > 0.05

Table 4. Effect of heat stress on the presence and absence phytochemical content of *Amaranthus hybridus*

	Treatments		
Phytochemicals	Control	2 Hours stress	4 Hours stress
Alkaloids	+	+	+
Reducing Sugars	-	-	-
Terpenoids	-	-	-
Tannins	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+
Phenolics	-	-	-
Phlobatanins	-	-	-

'+' = Present ' -'= Absent

Table 5. Effect of heat stress on the phytochemical content of *Amaranthus hybridus*

		Treatments		
	Phytochemicals	Control (%)	2 Hours stress (%)	4 Hours stress (%)
Vegetative stage	Saponins	0.234b	0.257a	0.193c
	Tannins	0.998a	0.965b	0.908c
	Flavonoids	0.269c	0.275b	0.277a
	Alkaloids	0.164c	0.204a	0.173b
Flowering stage	Saponins	0.327a	0.305b	0.296c
	Tannins	0.879b	0.849c	0.967a
	Flavonoids	0.153c	0.205b	0.239a
	Alkaloids	0.231b	0.212c	0.233a
Fruiting stage	Saponins	0.348b	0.394a	0.305c
	Tannins	1.139a	1.057b	0.882c
	Flavonoids	0.078a	0.072b	0.057c
	Alkaloids	0.348c	0.387a	0.369b

Means with the same superscript along the same row are not significantly different at P > 0.05

2 hours and was lowest in the plants stressed for 4 hours at vegetative and fruiting stage. At flowering stage, it was highest in the control and was lowest in the plants stressed for 4 hours (Table 5).

At vegetative and fruiting stage, tannins accumulation was highest in the control plants and was lowest in the plants stressed for 4 hours. At flowering stage, tannins accumulation was highest in the plants stressed for 4 hours and was lowest in the plants stressed for 2 hours (Table 5).

At vegetative and flowering stage, flavonoids were highest in the plants stressed for 4 hours and lowest in the control. At fruiting it was highest in the control plants and was lowest in the plants stressed for 4 hours (Table 5).

Alkaloids were highest in the plants stressed for 2 hours and were lowest in the control at vegetative and fruiting stage. At flowering stage, it was highest in the stressed for 4 hours and was lowest in the plants stressed for two hours (Table 5).

It was observed that there were significant differences in the level of phytochemical contents of *Amaranthus hybridus* among the treatments ($P > 0.05$).

Discussion

With increased temperature due to global warming, plants are likely to experience increasingly frequent hotter and longer episodes of abrupt heat stress (*i.e.* heat waves) in the future and this will negatively impact plant function (Giri, 2013). The results from the current study showed that exposure of *Amaranthus hybridus* at different duration of heat negatively affects their growth, but the effect on the phytochemical constituents at different stages of growth enhanced the quantity of some phytochemicals and inhibit the quantity of others. As in other previous studies (Heckathorn *et al*., 2013), among roots and shoots subjected to the same high temperatures, roots were more sensitive to heat stress than shoots. In the hereby study, both root and shoot growth as expressed by their fresh and dry weights decreased at high duration of heat stress at 45 °C. This might have contributed to the decrease observed in the leaf fresh and dry weights, consistent with results of a study on abrupt heat stress in a heat-tolerant grass (Mainali, 2007). The probable reason for lower shoot, root and leaf fresh and dry weight in the heat treatments compared to the control is that roots sustained direct damage from this temperature at higher duration, as indicated by heat-related increases in root electrolyte leakage (an indication of membrane damage), as observed in other studies (Liu and Huang, 2000, 2002).

The estimated relative activities of the phytochemicals during heat stress at different stages of growth were variable with the control plants. The phytochemical accumulation detected in this species showed dissimilar responses to heat stress as some of the phytochemicals were enhanced by heat, some were inhibited at vegetative, flowering and fruiting stage. This showed that heat stress at 45 °C for two hours and four hours exposure did not decrease nor increase the relative activities of phytochemical constituents of *Amaranthus hybridus* as obtained at different growth stages though there were variations in the quantities of these phytochemicals at different stages of growth.

Conclusions

The results from the present study indicated that heat stress at different durations impaired growth parameters of *Amaranthus hybridus*. It can be inferred that increase in the duration of exposure to heat negatively affect growth parameters such as leaf fresh and dry weights, shoot fresh and dry weights, as well as root fresh and dry weights. Also, heat stress increases the accumulation of some phytochemical components, while it causes negative effects on some phytochemical attributes of *Amaranthus hybridus* at different stages of growth. It can therefore be concluded that heat stress with global warming in the future will likely have overall negative effects on the growth of *Amaranthus hybridus* and might cause variation in the level of phytochemical constituents of *Amaranthus hybridus* at different growth stages.

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