Research Article

Morphological and Physiological Responses in Red Amaranthus (*Amaranthus tricolor* L.) and Green Amaranthus (*Amaranthus dubius* Mart. ex Thell.) Under Progressive Water Stress

Chithra Santhakumari Amma*, Radhakrishnan Rajalakshmi

Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram, Kerala, INDIA.

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ABSTRACT

Aim: Drought is a major constraint to the sustainable production of crop plants around the globe. *Amaranthus tricolor* (var. Arun) and *Amaranthus dubius* (var. CO1) are the leafy vegetables commonly cultivated in India, especially in Kerala. To date, the morphological and physiological changes with respect to drought stress and its mechanisms are not known thoroughly. **Materials and Methods:** The current research attempted to instigate the morphological and physiological changes of two Amaranth varieties under drought stress. The plant varieties were subjected to different levels of soil water treatments such as mild (4 days), moderate (6 days), and severe (8 days) stress, along with the control. After 15 days of transplanting, the water supply was limited to impose stress. **Results:** Morphological responses showed that only severe stress inhibited Amaranthus growth. Therefore, the stress at different stages of growth can reduce yield possessing a threat to agronomic activity. Even though there was a visible morphological change, the plants physiologically adjust the water stress condition by increasing their relative water content, decrease in electrolyte leakage, and chlorophyll stability index. The results also showed that the green variety was more tolerant than the red, even in severe water stress conditions. **Conclusion:** The present study helps in the future crop improvement programme of the green leafy vegetable, Amaranth.

Keywords: *Amaranthus tricolor*, *A. dubius*, Water stress, Leaf area, Relative water content, Electrolyte leakage.

Correspondence:
Mrs. Chithra Santhakumari Amma,
Research Scholar, Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram-695 581, Kerala, INDIA.

Email id: schithra121@gmail.com

INTRODUCTION

Research reports have suggested that the drier regions of the world will shortly suffer from water stress due to the varying environmental changes, and hence this can be a problem for future generations. This, in turn can affect the primary producers of the food chain, especially the plants on which life is dependent. Plants on exposure to drought possess both morphological and physiological changes that occur drastically.[1] The changes in morphology and physiology are characterized by a series of responses including drought avoidance, tolerance, escape, and subsequent recovery.[2,3] Findings suggest that morphological responses considerably affect vegetative growth and biomass accumulation and partition.[4] In comparison with morphology, physiological parameters can greatly affect the plant's survival. This is because physiological parameters are, in fact related to the overall production of primary and secondary metabolites. This is more evidence in distinguishing the control and drought-treated plants. In addition, drought reduces the relative water content along with its water potential. When grown in dry, semi-arid regions, foods with high nutritional content that can withstand drought have the potential to enhance diets.

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The most well-known green vegetable in Kerala, and one of those that may be produced all year round, is Amaranthus, which is a member of the Amaranthaceae family. It is a unique source of betalain and a cheap supply of natural antioxidants such as vitamins, phenolics, and flavonoids (betacyanin and betaxanthin). Apart From this, minerals and vitamins are all present in significant amounts in the leaves and succulent stems. Although Amaranthus may grow in a variety of soil types, it prefers sandy soil that is somewhat acidic. Currently, it is grown and consumed in large quantities throughout Southern and Eastern Africa, the entirety of Central America, China, Indonesia, Malaysia, and the Philippines. The family Amaranthaceae, subfamily Amaranthoideae, and order Caryophyllales all contain the genus Amaranthus.

Amaranthus is a member of the C4 plant group, which outperforms C3 plants in terms of photosynthesis and water use efficiency in hot climates. Due to its great genetic diversity and wide range of phenotypic plasticity, this genus is also well suited to agriculture on the marginal ground and has significant potential for further breeding improvement. Amaranthus is a very intriguing crop for difficult growing conditions, especially in light of the effects of climate change, due to its innate resilience to high temperatures, drought, poor soil conditions, and lack of severe illnesses.[5] To date, there are only a few reports related to the effect of drought stress on the growth and physiological activities of leafy vegetables are available. Therefore, the present study was conducted on two leafy vegetables of Amaranthus, aiming to assess the vegetative growth and physiological changes when subjected to water stress.

MATERIALS AND METHODS

Materials selected for the present study include two commonly edible species of Amaranthus viz *Amaranthus tricolor* (Var. Arun) and *Amaranthus dubius* (Var. CO1). Seeds of both varieties were collected from Agriculture College, Vellayani, Thiruvananthapuram.

Experimental design

The study was conducted between August 2016 and May 2019 in Greenhouse, Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram. In plastic trays containing a mixture of sand, dirt, and cow dung, the seeds were planted. Twelve days after germination, selected the seedlings with the same height and number of leaves for each variety, and transplanted individually into polythene bags. The chemical properties of the soil were analyzed to find out the impact of pH, and micro and macro elements. The plants were divided into two groups test and control; control plants were irrigated regularly, whereas the test groups were subjected to withholding irrigation (2-day, 4-day, 6-day and 8-day). Each treatment included three replications and five seedlings per replication. All the plastic bags were randomly arranged inside the greenhouse and maintained a temperature range of 22°C to 32°C and a relative humidity range of 50-80%. During the experimental period, a thermo-hygrometer (WSB-2, China) was used to record the mean temperature and relative humidity.

Morphological Parameters

To determine the status of plant growth in the two varieties of Amaranthus under different levels of water stress (2, 4, 6 and 8 days), different morphological and growth parameters were recorded, and the data were statistically analyzed using the ANOVA program in Statistics 8 software.

Physiological Parameters

Various physiological parameters such as leaf-water relations, leaf-relative water content, electrolyte leakage, membrane integrity, and chlorophyll stability index were determined based on standard protocols.[6-9]

RESULTS

Several species of Amaranthus are known to grow in extreme water deficit conditions. Therefore, an attempt was made to analyze the morphological and physiological parameters of two varieties of Amaranthus species. At the time of harvest, morphological measures were taken, including root-to-shoot ratio based on dry weight and fresh weight and dry weight of the shoot and root (after drying at 60°C). In the present work, the response of two species of Amaranthus towards different days intervals of water stresses slightly affects the root length (Table 1). In both varieties, when comparing the three different water-stressed plants with the control, the root length decreases on the 6-day stressed plant, but an increase in the root length was noticed in the 8-day stressed plant. That is, on the 8th day of stress, the plant reached the root length, which was the same as that of control. The root length was maximum in the red variety when compared with the green variety in control as well as in later stages of stress. At the end of the drought period, neither species' secondary roots differed in length from one other. At the height of the stress, the tap root length of the drought-treated plants was considerably (*P* 0.01) longer than that of the controls. Most notably, at the end of the drought, the

Table 1: Morphological changes of two varieties of *Amaranthus* during different days of water stress.

Plants	Root Length(cm)	Shoot Length (cm)	Petiole Length (cm)	Leaf Length (cm)	Leaf Breadth (cm)	Leaf Area (cm^2)	Lateral Root Length (cm)	Internodal Length (cm)	Leaf Number
RC	6.8±0.25^{a}	35.4±0.79^{a}	7.2±0.33^{a}	11.5±0.27^{b}	8.24±0.28^{b}	63.13±2.21^{b}	4.86±0.37^{a}	3.7±0.25^{a}	13.8±0.86^{a}
R4day	6.3±0.33^{a}	33.7±0.53^{a}	7±0.35^{a}	10.7±0.60^{bc}	7.62±0.25^{bc}	54.38±3.47^{bc}	5±0.35^{a}	3.76±0.11^{a}	13±0.83^{ab}
R6day	5.1±0.43^{b}	33.4±0.73^{a}	7±0.31^{a}	10.1±0.43^{cd}	6.80±0.46^{cd}	46.15±4.58^{cd}	5.16±0.38^{a}	3.46±0.12^{a}	11.4±0.40^{c}
R8day	6.5±0.44^{a}	29.4±0.62^{bc}	6±0.15^{b}	8.6±0.62^{e}	6.32±0.23^{de}	36.61±3.93^{d}	5.46±0.47^{a}	3.3±0.12^{a}	8.8±0.20^{d}
GC	6.74±0.21^{a}	44.4±0.57^{a}	6.7±0.20^{ab}	15.6±0.36^{a}	9.40±0.29^{a}	97.78±3.54^{a}	4.86±0.37^{a}	3.6±0.29^{a}	12.2±0.37^{ab}
G4day	6.54±0.24^{a}	43.7±0.30^{a}	6.66±0.18^{ab}	11.2±0.25^{bc}	7.20±0.25^{cd}	54.72±3.72^{bc}	4.7±0.30^{a}	3.64±0.21^{a}	11.8±0.73^{bc}
G6day	5.96±0.27^{ab}	41.0±0.85^{b}	6.56±0.16^{ab}	10.7±0.43^{bc}	6.64±0.27^{cd}	53.22±1.11^{bc}	4.86±0.37^{a}	3.5±0.27^{a}	10.2±0.58^{cd}
G8day	6.1±0.33^{a}	35..3±0.48^{c}	6.4±0.18^{ab}	9.2±0.33d^{e}	5.70±0.30^{e}	39.59±4.91^{d}	4.8±0.33^{a}	3.46±0.20^{a}	9±0.70^{d}
Main effect	2.815*	15.950***	2.317*	23.702***	14.942***	27.682***	0.423	0.509	8.302***

RC: Control Red; R4DAY: Red 4 Day stress; R6DAY: Red6 Day stress; R8 DAY; Red 8day stress.
GC: Green Control; G4DAY: Green 4day stress; G 6DAY; Green 6day stress; G8DAY: Green 8day stress.

drought-treated plants' taproots were longer than those of the control plants. However, neither species' LRL nor lateral root numbers have significantly changed. Red amaranth has somewhat longer lateral roots and more of them overall when compared to green amaranth. Under drought conditions, the root architecture changes and makes it easier for a lot of long lateral roots and root hairs to grow, potentially increasing the total surface area available for water uptake. Growth reduction is considered an important and established visible effect in plants. In amaranth the shoot length was negatively affected by all stages of water stress when compared to the control. Drought stress reduced plant growth in both red and green varieties at all stages except for 4-day stressed plants. The plant height of the red variety was noticed to be the shortest of the two varieties in all three treatments (Table 1). Stem growth was considerably reduced at 8-day stressed plants.

The physiological parameters assessed included, Relative Water Content (RWC), Water Saturation Deficit (WSD), and Relative Saturation Deficit (RSD). An essential factor in determining the metabolic activity and survival of leaves is Relative Water Content (RWC). Despite being the same, LRWC in all treatments was high, demonstrating a high level of water maintenance in the face of a water shortage. There was no discernible difference in RWC between the two varieties of well-watered plants. However, under water-stressed conditions, the green variety showed the highest RWC value (Figure 1). The value of relative water content in control plants was 50.94 ±7.04 (red variety) and 45.41 ± 2.59 (green variety). In the red variety, RWC significantly increases in the initial and middle stages of

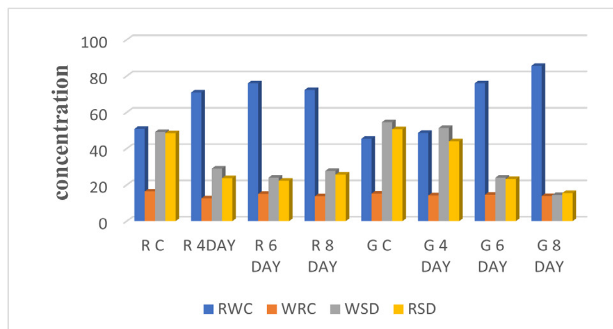

Figure 1: Changes in the leaf water relation in the two varieties of *Amaranthus* during the water deficit period.

RC: Control Red; R4DAY: Red 4 Day stress; R6DAY: Red6 Day stress; R8 DAY; Red 8 day stress.

GC: Green Control; G4DAY: Green 4day stress; G 6DAY; Green 6day stress; G8DAY: Green 8day stress.

RWC: Relative water content; WRC: Water retention capacity; WSD: Water saturation deficit; RSD: Relative saturation deficit.

stress and slightly decreases at the late stages of severe stress. But in the case of the green variety, there was a rapid increase in RWC when compared with the control. The results are shown in Figure 2.

In the red variety, the RWC of the control plant was 50.94%, whereas at the late stage of stress, RWC increased by 72.29%. But in green, the RWC of the controlled plant was 45.41% whereas at the late stage of stress, it increased by 85.66%. The well-watered plants showed lower RWC, while under stressed conditions, the green Amaranth showed the highest RWC value (Figure 1). The high tolerance of this variety to drought is also supported by the percentage of injury to the cell membranes. Although showing the highest RWC value, the percentage of increase in RWC in relation to control was slightly higher in the green variety, than in red. RSD,

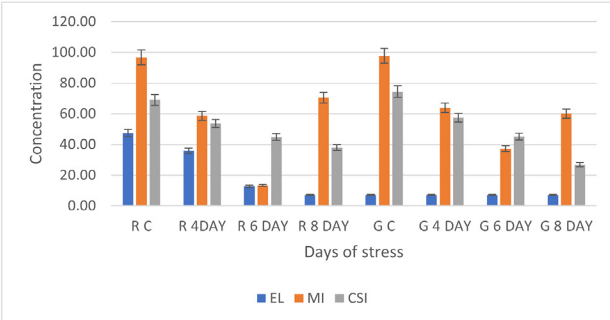

Figure 2: Changes of Physiological parameters in the two varieties of *Amaranthus*.

RC: Control Red; R4DAY: Red 4 Day stress; R6DAY: Red6 Day stress; R8 DAY; Red 8day stress.

GC: Green Control; G4DAY: Green 4day stress; G 6DAY; Green 6day stress; G8DAY: Green 8day stress.

EL: Electrolyte leakage; MI: Membrane integrity; CSI: Chlorophyll stability index.

however, decreased in both varieties, the low values of RSD indicate the tolerance of Amaranthus varieties to drought at the growing stage. In the Red variety, there was a slight decrease in water retention capacity was observed and it was 16.37% under non-stressed plants, whereas 12.63%, 14.98%, and 13.75% reduction were noticed at three stages of water stress respectively. In the green variety, there was a reduction in WRC which was noticed, and it was 15.20% in control plants and 14.19%, 14.59% and 13.89% at three stages of water stress. The larger cell structural damage caused by water stress was reflected by, the higher reduction in WRC. The green and red types in the current study both had the lowest decline in WRC, which is a sign of their water stress resistance. The results are shown in Figure 2.

Cell Membrane Stability (CMS) in red and green varieties of Amaranthus decreased significantly during 4 and 6 days of stress. CMS decreased to a great extent but after 6 days of stress, it increases almost to the level of the control plant. In the red variety, there was an increase of 65-70% CMS at eight days of stress. Whereas, in green, the CMS value increases by 55%-60% during the last phase of drought stress. As a result, maintaining the integrity and stability of the cell membrane is a key factor in a plant's ability to withstand drought stress. On the other hand, when a cell membrane is exposed to multiple abiotic stresses at once, its stability quickly depletes. The results are shown in Figure 2. In the case of CSI, both red and green varieties showed a significant decrease in the values. In control plants of the red and green variety, the CSI values were 69.07 ± 3.78 and 74.39 ± 2.13, respectively. In the red variety, CSI decreases by almost 50% of that of control during the eight days of stress. But in green, the CSI decreased by almost 70% to 75%. But when compared with 4 days and 6 days of stress, on the 8th day, the value of CSI increases. So, from the present investigation, it was evident that the chlorophyll stability index decreases during the early stages of stress and later on increases. The increase in CSI during the later stage of stress was high in red when compared to green.

DISCUSSION

Crop diversification to meet the demand of a growing population has become highly essential in a developing populous country like India. Only five species (rice, potato, maize, soy, and wheat) are considered as world food for humans, in this juncture, Amaranthus species can be highly recommended as an alternative due to its high protein content, vitamin and low anti-nutritional content. Similar to this, several plants have reported shorter shoots, and in conditions of water scarcity, soybean stem length decreased.[10] Citrus seedlings that were under water stress had their plant height lowered by up to 25%.[11] Potato stem length was greatly impacted by water stress.[12-16] The leaf length and breadth decreased with the increase in stress level. Both the parameters were highest in the green variety when compared to red. These measurements decrease when days of stress increase. The size of the individual leaves was greater in the green variety. The primary response of plants to water deficit conditions is a reduction in leaf area. The leaf area index is one of the most important growth indicators. Leaf growth was found to be significantly reduced due to stress in both varieties of Amaranthus. About 50% and 75% leaf growth reduction were observed in 8-day stressed red plant and green variety, respectively. The leaf area in the red variety gradually decreases from 63.13 ± 2.21 to 36.61± 3.93 cm^2. But in the green variety, there was a significant decrease in leaf area from 97.78 ± 3.54 to 39.59 ± 4.91cm^2. According to research, drought stress significantly reduced leaf area and had a detrimental impact on grain yield.[17] Restrictive irrigations were found to reduce leaf area because of leaf deterioration and fall.[18] It was shown that Amaranthus cultivars' foliar growth was severely hampered by drought stress. Observations show that when water stress is extended, drought stress directly affects leaf development. In both species, leaf counts decreased by 20 to 60% compared to control in longer-lasting drought (8-day stress). The drop in leaf number was found to be between 0 and 40% in groups that experienced mild to moderate water stress (4-day and 6-day groups). After leaf area, leaf number was the foliar attribute that was most affected. The results of the present study suggest that, Amaranth could resist drought stress, in which the red species is considered more tolerant.

To look at it another way, plants that produce more when under drought stress should have a high relative water content. The cell membrane is prone to alterations such as increased penetrability and decreased sustainability when there is a water deficit.[19] At midday, the relative water content started to decline after being greater in the morning. It was claimed that cultivars with more RWC were drought-resistant.[20] To determine whether the growth reduction in Amaranthus is associated with drought stress, oxidative damage, Electrolyte Leakage (ELR), cell membrane integrity and chlorophyll stability index were measured. This marked increase in ELR coincided with a significant increase in MDA content which was higher under severe stress (131% increase) than under mild stress (82% increase). Oxidative damage is the main cause of growth reduction under stress.[21]

Under environmental stress, plant membranes are frequently prone to alterations brought on by an increase in permeability and a loss of integrity. As a result, a wide variety of tissues are tested for damage using the ability of cell membranes to control the rate of ion transport in and out of cells. Each stress session sees a reduction in electrolyte leakage. In both red and green varieties, EL decreases by around 40% from the control to the late stage of stress. The present study indicates that a water stress-induced membrane injury was less in stress-treated plants of both species.[22,23] Reports show that the varieties that were resistant to drought had more RWC.[24] From emergence until stress-initiation, the green amaranth variety, maintained a higher RWC and a relatively low Relative Saturation Deficit (RSD) as compared with red amaranth, when both cultivars were subjected to drought. These results are consistent with several reports, indicating that drought-tolerant species exhibit significantly higher RWC and lower RSD.[19] Plants grown under a high moisture regime maintains a higher ratio and that might be due to the lower destruction of plant tissues by moisture deficit.[25] RWC and EL are indicators for the selection of drought-tolerant plants.[26] Higher RWC increases leaf ability to maintain more amounts of water and the ability of roots to absorb water in drought conditions, whereas higher EL increases membrane permeability. Environmental stresses such as drought change cellular membranes, often increasing leakage. In the present investigation, extreme accumulation of H_2O_2 in MDS and SDS might have accelerated the Haber-Weiss reaction, resulting in hydroxyl radical (·OH) formation and therefore resulting in serious lipid peroxidation and cell membrane damage The static ROS content from control to LDS (8^{th} week) might be due to inhibition of ROS generation in plant tissues and up-regulates ROS scavenging activity by active accumulation of excessive proline, total carotenoid, ascorbic acid, TPC, TFC, and antioxidant activity that inhibited the increment of MDA and H_2O_2 content. Although the highest accumulation of proline, total carotenoid, ascorbic acid, TPC, TFC, and antioxidant activity was noted in SDS condition compared to any stresses, but MDA and H_2O_2 accumulation was also the highest. This might be likely because the vegetable amaranth fell into severe stress and could not cope with the damage caused by drought. Maintaining a balance between ROS production and scavenging is crucial under stressed conditions.[8]

CONCLUSION

In conclusion, in our experiment, severe water stress-induced different growth responses in both varieties of Amaranthus, which indicates that water stress reduces the yield, so strategies must be taken into consideration for growing any Amaranthus variety. There was no reduction in the taproot length due to water scarcity. This accounts for the fact that if irrigation is made available prior to the onset of stress, the growth of roots would be beneficial. According to the findings, stress had an impact on two Amaranthus types' shoot growth rather than their root development. When the dry periods were over, the drought-treated plants had less shoot growth than the control plants in terms of height, leaf area, and leaf number. Until the end of the drought period, root development did not decline in the drought-treated plants as compared to the controls. Amaranthus cultivars can withstand drought stress by utilizing a variety of avoidance and tolerance strategies. Only extreme drought stress limits the growth of Amaranthus and has a negative impact on its physiological activities, according to morphological and physiological responses. Based on the results of the current study, it may be inferred that Amaranthus cultivars with high water stress tolerance had superior plant-water relations, as evidenced by higher relative water content and water retention capacity. This could show how the physiological and morphological indices and the mechanism of drought tolerance in Amaranthus are properly related. The study emphasizes the morphological and physiological basis of drought tolerance in the two varieties of Amaranthus. Drought tolerance character can be very useful in the future for the development of new tolerant varieties.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

RC: Control Red; **GC:** Green Control; **RWC:** Relative Water Content; **WSD:** Water Saturation Deficit; **RSD:** Relative Saturation Deficit; **ELR:** Electrolyte Leakage.

SUMMARY

The present study provides a baseline data for the development of drought-tolerant varieties of Amarath that can be used for crop improvement programmes. Thereby, new cultivars of Amaranth can be developed. From the present study, it can also be summarized that red amaranth provides a better tolerant variety than green.

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