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MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES OF MANGO (*Mangifera indica* L.) TO DROUGHT STRESS AND DROUGHT ALLEVIATION STRATEGIES DURING EARLY SEEDLING STAGE

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ABSTRACT – The effects of drought stress and selected drought alleviation strategies on the morphological and physiological responses were investigated on seedlings of mango (*Mangifera indica* L.) cv. 'Manila Super Mango.' Two weeks before drought stress imposition, the seedlings were treated with 40 mM acetic acid, 1 mM hydrogen peroxide, 2 ppm potassium silicate, and 0.5 mM salicylic acid. Drought stress was imposed by withholding water for 15 days. Morphological and physiological responses measured before and after drought imposition were plant height, stem diameter, number of leaves, stomatal aperture length, width and area, stomatal density, relative water content, and chlorophyll a content. Drought arrested the growth of stem diameter and induced leaf abscission. Stomatal density increased but with a concomitant decrease in stomatal aperture length, width, and area. Relative water content decreased while chlorophyll a content increased. As for the effect of drought alleviation treatments, comparable responses were observed for the plant growth parameters, stomatal responses, and chlorophyll a content. However, for relative water content, seedlings treated with 40 mM acetic acid and 2 ppm potassium silicate exhibited significantly higher relative water content than the other treatments. Furthermore, it was observed that mango seedlings can tolerate 15 days of drought. It is recommended that additional drought-alleviating measures be tested and that a more prolonged drought period be imposed to observe the stress-alleviating effects of the different treatments.

Keywords: drought alleviation, drought stress, mango, morphological responses, physiological responses, seedling stage

INTRODUCTION

Drought stress is considered the main environmental limiting factor for plant growth and crop productivity worldwide. It can vary from light to severe, from a few days to several months, and can occur at any developmental stage of a specific crop (Jones and Corlett, 1992). Some studies on the effects of

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water deficit had been observed and established on annual crops but only few on perennial crops like mango (Bukhari et al., 2019; Seleiman et al., 2021; Rasheed and Malik, 2022). It was hypothesized that certain perennial plants are drought resistant, but it is necessary to investigate the mechanisms by which these plants endure drought. In addition, perennial plant species have developed mechanisms to cope with an inadequate water supply (McDowell et al., 2008; Cai et al., 2022; Chen at al., 2022). Much less information is available for the occurrence of adaptive strategies of these crops, especially during the first phase of life after seed germination, with seedling establishment being one of the critical phases in the life of a plant.

A wide variety of molecular, cellular, tissue, organ, and organismic level responses are brought about by drought stress. These responses are exhibited in various ways in different species and varieties within species. These morphological and anatomical responses could either enhance or reduce the capability of the plants to survive and grow during these stressful periods. Previous studies on the effect of water deficit have focused on annual crops and very little research had been conducted on perennial crops particularly fruit crops at the seedling stage. In wheat plants, drought stress decreased the leaf water potential, relative water content and transpiration rate (Siddique et al., 2000). In melon plants, water stress reduced fruit yield, total dry matter, chlorophyll content and relative water content, but increased proline accumulation (Tuna et al., 2010). In another study on *Hibiscus rosa-sinensis,* relative water content, turgor potential, transpiration, stomatal conductance, and water-use efficiency decreased under drought stress (Egilla et al., 2005). In the case of papaya, Bunya-atichart et al. (2014) observed that stem height, stem diameter, canopy width, and leaf area were reduced at three to nine days of water stress. On the other hand, Luvaha et al. (2007) revealed that leaf chlorophyll content slightly increased in mango rootstock seedlings when subjected to water stress.

Effects of drought stress can be managed using acetic acid, salicylic acid, hydrogen peroxide, and potassium silicate. Kim et al. (2017) recorded 70% survival on wheat, rice, corn, and rapeseed seedlings treated with acetic acid after drought stress. They explained that exogenous acetic acid stimulated the jasmonate signaling pathway to confer drought tolerance. In like manner, the data obtained by Sun et al. (2022) showed that acetic acid enhanced the drought tolerance of apple trees by influencing the abscisic acid and jasmonic acid-induced signaling pathway. Also, plants treated with salicylic acid stimulated growth, photosynthetic pigments, and accumulation of soluble and insoluble carbohydrates and proteins as observed by Azooz and Youssef (2010). Hydrogen peroxide, on the other hand, alleviates water loss by inducing and increasing antioxidant activities. Furthermore, the use of potassium silicate increases growth and physiological parameters (Hafez et al., 2021). Karvar et al. (2023) observed that spraying of potassium silicate on the leaves of sweet corn decreased the adverse effects of water limiting conditions and improved the physiological features and grain yield of the plants. According to Mubarak et al. (2016), potassium silicate as source of potassium affects osmotic adjustment and enhances assimilate translocation and maintenance of osmotic charge. As source of silicon, it mitigates drought stress by enhancing water uptake and transport thus maintaining whole plant-water balance (Wang et al., 2021).

Mango (*Mangifera indica* L.) is one of the most popular and economically important crops in countries of Southeast Asia, particularly in the Philippines. Documented studies on how water deficit affects the early seedling growth of this major fruit crop are wanting. Thus, it is important to investigate the morphological and anatomical responses of the seedlings to drought stress and how different drought alleviation strategies will affect these responses. The results of this study will help mango researchers, breeders, farmers, and nursery managers in formulating crop production strategies during adverse conditions like drought. This study aims to determine the morphological and physiological responses of mango seedlings to drought stress and how these responses will be affected by drought alleviation strategies.

MATERIALS AND METHODS

Growing of Seedlings and Preparation of Test Plants

Seeds of 'Manila Super' mango, locally known as 'Carabao' mango, were sourced from the San Marcelino Public Market in Zambales. These were sown in 15.24 cm x 17.78 cm black polyethylene plastic bags filled with 1 kg of growing media composed of 3 garden soil:1 coir dust. After one month, seedlings were thinned to one seedling per plastic bag and placed inside the greenhouse. Watering and fertilizer application were done regularly until the start of treatment.

Imposition of Drought Stress and Drought Alleviation Treatments

Five-month-old seedlings of mango of uniform size were selected. These were treated with drought alleviation measures two weeks prior to imposition of drought stress. Treatments included T1/ 40 mM acetic acid, T2/ 1mM hydrogen peroxide, T3/ 2ppm potassium silicate, and T4/ 0.5 mM salicylic acid. The control-well watered (C1/ Well-watered) group was composed of seedlings with no drought-alleviation treatment and watered every other day while the seedlings with no drought-alleviation treatment and were subjected to drought stress served as the control-drought (C0/ Drought) group. Drought stress was imposed by withholding water for 15 days.

Data Collection and Statistical Analysis

Morphological and physiological characters such as plant height, stem diameter, number of functional leaves, stomatal density, stomatal aperture length, width and area, relative water content, and chlorophyll a content were measured.

Morphological characters

Plant height was measured using a meter stick while the stem diameter was measured using a vernier caliper. The number of functional leaves included those leaves which were fully expanded and were $\geq$ 25% green.

Stomatal density and stomatal aperture were measured by making epidermal impressions of the third or fourth leaf from the apical portion of the seedling. A thin layer of clear nail polish was applied on the abaxial side of the leaf surface. A clear adhesive tape was pressed over the dried nail polish which was then peeled off and placed on a glass slide. The slide was viewed under the microscope under 400x magnification and images of the fields of vision were captured. The images were analyzed using Image J v1.52j software (Schneider et al., 2012) to measure stomatal aperture length and width. The stomatal aperture area was computed by multiplying the stomatal aperture length and the stomatal aperture width. For the stomatal density, the number of stomates was counted per field of vision which was 0.45 mm^2.

Physiological characters

The relative water content (RWC) of the third leaf was measured using the method described by Barr and Weatherley (1962). A 100 mg leaf sample was obtained from the third leaf and cut to 1x7 cm length. The samples were weighed (fresh weight, FW) and imbibed in distilled water for four hours in a Petri dish at room temperature. The turgid weight (TW) of the samples were measured and recorded. These were then oven-dried at 65°C for 24 hours and the dry weight (DW) was measured. RWC was calculated as follows:

$$RWC\ (\%) = \left[\frac{(FW - DW)}{(TW - DW)}\right] x\ 100$$

Chlorophyll a content determination was done following the method of Wintermans and De Mots (1965). Thirty mg leaf sample was cut into smaller segments, suspended in 5 ml of 96% ethanol, and incubated in the refrigerator under dark conditions for 24 hours or until plant tissue becomes colorless. Absorbance was measured using a spectrophotometer. The following formula was used to measure the chlorophyll a content:

$$Chl\ a\ content = (13.70\ x\ A_{665}) - (5.76\ x\ A_{649})$$

where: A_{665} = absorbance reading at 665 nm; A_{649} = absorbance reading at 649 nm

All the parameters were recorded at two periods: (1) on the day before the imposition of drought stress (BD); and (2) on the day after the imposition of drought stress (AD). The experiment was laid out in a randomized complete block design with three replications per treatment and five plants per replicate. Data were subjected to analysis of variance (F-test) at α=5%. Tukey's test was used to determine differences across treatment means. Data were analyzed using the Jamovi statistical software (Ver. 2.3) 2022.

RESULTS AND DISCUSSION

Table 1 shows the different mean plant heights, stem diameters, and the number of leaves of mango seedlings treated with different drought alleviation treatments before drought imposition. Comparable mean plant heights among the different treatments were observed before and after drought imposition. Before drought, the mean plant height ranged from 55.67- 59.13 cm while after drought, the mean plant height ranged from 56.80- 59.93 cm. In mango seedlings subjected to water stress for 15 days, plant height was unaffected. Regarding stem diameter, before drought imposition, the mean stem diameter ranged from 6.43-7.15 cm while after drought imposition, the mean stem diameter ranged from 5.97- 6.73 cm. Though stem diameter decreased after drought imposition, there were no statistically significant differences among the drought alleviation treatments. On the other hand, significant differences were observed in the mean number of leaves of mango plants before (p=<0.01) and after (p=0.004) drought imposition. The seedlings with the drought alleviation treatments had a lesser number of leaves than the well-watered control seedlings. This indicates that at 15 days of drought, treatments with 40 mM acetic acid, 1 mM hydrogen peroxide, 2 ppm potassium silicate, and 0.5 mM salicylic acid were not sufficient to maintain a high number of leaves.

The results of the present study corroborated the findings of Luvaha et al. (2007) who observed comparable plant heights and stem diameters of mango seedlings subjected to drought. They observed that only at severe water deficit did mango seedlings exhibit reduced increment in height and that mango plants were able to withstand certain levels of dehydration through solute accumulation in the leaves and some osmotic adjustments. In the same study, the authors observed significant differences in the mean number of leaves after drought imposition. They explained that the reduction in the number of leaves under extreme water deficit could be due to the reduction of leaf formation and the abscission of lower leaves. In species like *Citrus*, it was observed by Gomez-Cadenas et al. (1996) that leaves did not abscise during water stress. In mango, however, abscission took place during the period of water deficit as observed in this study. In terms of drought alleviation, the different treatments did not perform as well as the well-watered control.

Table 1. Plant height, stem diameter, and number of leaves of mango seedlings treated with different drought alleviation strategies and subjected to 15 days of drought stress.

Treatment	Plant Height (cm)		Stem Diameter (mm)		No. of Leaves	
	BD	AD	BD	AD	BD*	AD*
C0/ Drought	55.67 ± 8.71	56.80 ± 9.25	6.97 ± 0.87	6.51 ± 0.75	12.93 ± 2.25 b	10.04 ± 3.24 ab
C1/ Well-watered	58.77 ± 10.88	59.57 ± 10.80	7.14 ± 0.78	6.68 ± 0.81	17.27 ± 3.94 a	13.87 ± 2.17 a
T1/ 40mM Acetic Acid	56.73 ± 8.01	57.23 ± 8.00	6.78 ± 0.97	6.47 ± 0.99	12.33 ± 2.38 b	9.53 ± 2.92 b
T2/ 1 mM Hydrogen Peroxide	56.27 ± 11.51	59.10 ± 11.59	6.43 ± 1.04	5.97 ± 1.01	12.13 ± 3.29 b	9.00 ± 4.41 b
T3/ 2 ppm Potassium Silicate	58.67 ± 8.82	59.27 ± 8.82	7.15 ± 0.74	6.73 ± 0.90	12.67 ± 3.20 b	9.60 ± 4.64 b
T4/ 0.5mM Salicylic Acid	59.13 ± 9.25	59.93 ± 9.30	6.59 ± 0.75	6.15 ± 0.68	12.60 ± 2.13 b	9.60 ± 3.40 b
p-value	0.87	0.90	0.13	0.12	<0.01	0.004

*Means with same letters within a column are not significantly different at α=5%. Data are means ± SE and each value was determined in five different plants with three replicates per treatment (n=15). BD- Before Drought. AD- After Drought.

Table 2 shows the mean stomatal density, and stomatal aperture length, width, and stomatal aperture area of mango seedlings treated with different chemicals before drought imposition. It was observed that well-watered seedlings had longer stomatal aperture compared to drought-imposed seedlings. An overall increase in stomatal density and a decrease in stomatal aperture length, width, and area were observed after 15 days of water stress. As for the drought alleviation treatments, these were found to be comparable to both drought-imposed and well-watered control.

In general, this study showed altered stomatal responses of mango seedlings under water deficit. The data indicate that with drought, stomatal density in mango seedlings increased but the stomatal aperture size decreased. This is congruent with the studies by Spence et al. (1986) and Martinez et al. (2007) that showed an increase in stomatal density and a decrease in cell size under water-limiting conditions indicating the occurrence of drought adaptation. Likewise, Bosabalidis and Kofidis (2002) stated that changes such as an increase in stomatal density and a decrease in stomata size are adaptations of the plants to drought conditions. Furthermore, the results of the study by Xu and Zhou (2008) suggested that stomatal density may have an important role in the net photosynthetic rate under drought stress.

According to Franks and Farquhar (2007), an increased stomatal density and reduced stomatal size could improve total pore area resulting in improved gas exchange. However, the inverse relationship between stomatal density and stomatal aperture size as observed in this study does not hold in all plants, as stated by Sun et al. (2014). Changes in stomatal density and stomatal size in response to water deficit seem to be species-specific and is dependent on environmental conditions.

Table 2. Stomatal density, length, width and area of stomatal aperture of mango seedlings treated with different drought alleviation measures and subjected to 15 days of drought stress.

Treatment	Stomatal Density (stomata/mm^2)		Length of Stomatal Aperture (mm)		Width of Stomatal Aperture (mm)		Area of Stomatal Aperture (mm)	
	BD	**AD**	**BD***	**AD****	**BD****	**AD****	**BD****	**AD****
C0/ Drought	56.22 ± 7.84	91.36 ± 24.51	3.441 ± 1.16 bc	2.896 ± 0.65 b	1.51 ± 0.49 a	1.15 ± 0.37 a	5.56 ± 3.27 a	3.38 + 1.16 ab
C1/ Well-watered	58.60 ± 12.15	88.89 ± 13.33	3.182 ± 0.764 a	3.684 ± 0.77 a	1.53 ± 0.346 a	1.11 ± 0.31 a	4.90 ± 1.80 ab	4.17 + 0.30 a
T1/ 40 mM Acetic Acid	72.10 ± 20.52	80.99 ± 13.11	3.326 ± 0.802 b	3.142 ± 0.69 ab	1.22 ± 0.24 b	0.98 ± 0.33 ab	4.08 ± 1.32 abc	3.20 + 1.59 ab
T2/ 1 mM Hydrogen Peroxide	68.15 ± 20.95	67.66 ± 10.95	3.160 ± 0.971 bc	2.774 ± 0.58 b	1.37 ± 0.41 ab	0.82 ± 0.24 b	4.56 ± 2.52 abc	2.30 + 0.81 b
T3/ 2 ppm Potassium Silicate	52.10 ± 23.68	75.31 ± 11.89	2.567 ± 0.837 c	2.616 ± 0.71 b	1.18 ± 0.40 b	1.00 ± 0.38 ab	3.17 ± 1.70 c	2.81 + 1.82 b
T4/ 0.5mM Salicylic Acid	53.33 ± 21.83	80.99 ± 23.12	3.011 ± 0.747 bc	2.628 ± 0.78 b	1.29 ± 0.26 ab	1.16 ± 0.40 a	3.94 ± 1.44 bc	3.25 + 1.81 ab
p- value	0.15	0.055	0.005	<0.001	<0.001	0.001	<0.001	<0.001

*Means with same letters within a column are not significantly different at α=5%. Data are means ± SE and each value was determined in five different plants with three replicates per treatment (n=15). BD- Before Drought. AD- After Drought.

To determine the effectiveness of the drought alleviation treatment imposed on the mango seedlings, relative water content and chlorophyll a content were also determined before and after drought imposition. Table 3 shows the mean relative water content, and chlorophyll a content of leaves collected from mango plants that were subjected to different treatments before drought imposition. Significant differences were observed in the relative water content of leaves after drought imposition. Seedlings treated with 40 mM acetic acid and 2 ppm potassium silicate performed similar to the well-watered control and better than the other treatments. Soltys-katina (2016) stated that leaf relative water content is an important indicator of water status in plants, reflecting the balance between the water supply in leaf tissues and the rates of transpiration. Also, relative water content is a common method of quantifying water deficit stress (Ogbaga et al., 2020). Under severe and prolonged drought conditions, leaf relative water content is decreased by an average of 20-40% (Jenkins and Pallardy, 1995).

As for the chlorophyll a content, a general increase in chlorophyll a content was observed after drought, though there were no significant differences among the different drought alleviation treatments. There was comparable chlorophyll a content among the treatments which may be due to the short period of drought imposition.

Chlorophyll content affects the photosynthetic process in plants. Syamsia et al. (2008) stated that drought conditions negatively affect chlorophyll content. However, in this study, chlorophyll a content increased slightly after drought. This supported the study of Luvaha et al. (2007) on mango seedlings exposed to different levels of water deficit who observed an increase in chlorophyll a content with increasing water deficit. They stated that chlorophyll a seemed to be resistant to dehydration and that the increase in the chlorophyll content may be an adaptive mechanism of the plant to water stress.

Table 3. Relative water content and chlorophyll a content of mango seedlings treated with different drought alleviation measures and subjected to 15 days of drought stress.

Treatment	Relative Water Content (%)		Chlorophyll a Content (ug/ml)	
	BD	**AD***	**BD**	**AD**
C0/ Drought	44.79 ± 41.16	19.77 ± 0.92 b	2.55 ± 1.01	4.13 ± 1.52
C1/ Well-watered	68.04 ± 21.49	83.58 ± 11.87 a	3.31 ± 0.26	4.90 ± 1.52
T1/ 40mM Acetic Acid	77.58 ± 6.1	51.49 ± 38.84 a	2.61 ± 1.01	6.11 ± 6.59
T2/ 1 mM Hydrogen Peroxide	49.36 ± 3.52	15.51 ± 12.89 b	2.61 ± 1.13	5.23 ± 5.27
T3/ 2 ppm Potassium Silicate	59.90 ± 13.39	43.85 ± 45.76 a	4.12 ± 2.21	3.75 ± 2.04
T4/ 0.5mM Salicylic Acid	89.28 ± 13.17	16.10 ± 1.24 b	4.61 ± 3.02	5.84 ± 3.96
p-value	0.15	0.04	0.56	0.97

*Means with same letters within a column are not significantly different at α=5%. Data are means ± SE and each value was determined in three different plants per treatment (n=3). BD = Before Drought. AD=After Drought.

SUMMARY, CONCLUSION, AND RECOMMENDATIONS

Drought stress is one of the important types of stress that plants may experience across different developmental stages. Scant information is available on how seedlings respond to water deficit stress and the measures to alleviate its effects. This study was conducted to determine the morphological and physiological responses of mango seedlings, particularly “Manila Super Mango” to drought and selected drought alleviation treatments. These included: T1 - 40 mM acetic Acid; T2 - 1mM hydrogen peroxide; T3 - 2 ppm potassium silicate; T4 - 0.5 mM salicylic acid. The control consisted of two groups: C0/drought and C1/well-watered. Morphological responses measured in this study included plant height, stem diameter, number of leaves, stomatal density and stomatal aperture length, width, and area. The physiological responses studied were relative water content and chlorophyll a content. Comparable mean plant height and stem diameter were observed across treatments after 15 days drought. Significant differences were observed for several leaves. After drought, the seedlings with drought-alleviating treatments had significantly lesser number of leaves compared to the well-watered control. Also, significant differences were observed in stomatal aperture length, width, and area across treatments. There was a general increase in stomatal density and a decrease in stomatal aperture length, width, and area after drought. Though no significant differences among treatments were observed for chlorophyll a content, there was a general increase in this pigment after drought. Further research is needed to conclude that there is continued synthesis of this pigment even under drought conditions. As for the relative water content, treatments with 40 mM acetic acid and 2 ppm potassium silicate showed promise in maintaining values comparable to the well-watered control during the 15-day drought.

It was observed that mango seedlings can tolerate 15 days of drought imposition as shown in their morphological and physiological responses. It is, therefore, recommended that more prolonged drought condition be implemented to observe the water deficit stress alleviating effects of the treatments used. Furthermore, additional drought alleviating measures need to be explored to test their effects on drought stress in mango seedlings.

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STATEMENT OF AUTHORSHIP

Sotto RC conceptualized and designed the research, evaluated the data, and finalized the manuscript; Masungsong LA, Estrella FAC, and Telan JAJB conducted the research, summarized the data, and contributed to the first draft of the manuscript; Bautista NS supervised the research and proofread the initial manuscript; San Pascual AO statistically analyzed the data, contributed to the second draft, and provided valuable inputs toward the improvement of the final manuscript.

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