Physiological and ethylene accumulation responses of cassava under drought stress

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Abstract. Drought is a major production constraint of cassava worldwide. The objective of this study was to evaluate the chemo-physiological and phytohormone responses of cassava under water stress conditions. Five cassava (Manihot esculenta) varieties were grown in greenhouse conditions under different water regimes (field capacity (FC), 2/3 and 1/3 available water (AW)). The physiological responses to drought were monitored. Plant height, relative water content (RWC) and SPAD Chlorophyll meter reading (SCMR) were recorded at 3, 4 and 5 months after planting. Ethylene accumulate were recorded at 1, 2 and 3 weeks after withholding water at 2 months after planting. The result revealed that plant height and RWC decreased significant difference under water deficit. Plant height and RWC under 1/3 AW had lowest followed by 2/3 AW and FC conditions. Under 1/3 AW, ethylene and SCMR increased significant difference followed by 2/3 AW and FC conditions. Thus, plant height, RWC, SCMR and ethylene accumulation could be used as a criterion in breeding programs to improve drought tolerance in cassava.

1. Introduction
Cassava scientific name is Manihot esculenta. Origin from South America [1]. Cassava an important industrial crop for starch, biofuel production and animal feed [2]. Cassava is classified as a drought-resistant crop, extensively cultivated as an annual crop in tropical and subtropical regions. The planted area for cassava is mainly under rain-fed conditions. An indirect effect of global warming can higher plant water demand due to increased transpiration at water limitation making drought conditions more severe. [3, 4]. Cassava as resistance to drought tolerance and low nutrient soil. It can grow in the area receiving 300 mm of rain annually [5]. Abiotic stress, such as drought, heat, cold and salinity are the cause of reducing plant growth and crop yields quality. Drought is serious threats to plant, in addition to physiological hormones are rapidly occurring responses to drought stress [6]. Drought is multidimensional stress, affect cell organs until the whole plant [7].

Several hormones, such as ethylene has been reported to associate with stress signaling [8]. Ethylene gas is a plant hormone produced by plant tissue and effect to physiological metabolism including fruit ripening leaf senescence and senescence [9, 10]. Under water deficit also increases ethylene accumulation [11] and leaf abscission occurs as the result of hormone signaling by ethylene consequently reduced water loss [12].

Measuring leaf water content is a simple way to evaluate the water status of leaf and important parameter to the determination of plant drought tolerance [13]. Leaf water content is an attribute for discriminating tolerance and sensitive genotypes. Hight relative water content is a resistant mechanism
to drought stress and more osmotic regulation [14]. RWC has been proposed as a selection criterion for drought tolerance in many crops such as wheat [15] and potato [16].

The chlorophyll content is closely linked with photosynthetic systems and drought is one of the most important factors constraining plant photosynthesis, stomatal and metabolic limitations [17]. Recent research has shown that the chlorophyll content can be measured using the SPAD chlorophyll meter reading [18]. SPAD-502 chlorophyll meter reading is a tool that measures the greenness of leaves by the leaf transmission of red light and infrared light [19]. SCMR is easy to use, stable and low cost [20]. Therefore, this study aimed to verify the effect of genotypes, water regimes and plant age on parameters related to cassava breeding program. Understanding of tolerance mechanisms under drought stress in cassava is a key in developing tolerance varieties and increased yield.

2. Materials and methods

2.1. Plant materials experimental design

The experiment was arranged in 3 X 5 Factorial in randomized complete block design (RCBD) with four replications. Five cassava varieties including Rayong 9, Rayong 90, Kasetsart 50, Huay Bong 80 and Hanatee were assigned as factor A. 3 different water levels [field capacity (FC), 2/3 Available Water (AW) and 1/3 AW] were arranged as factor B. The experiment was conducted under greenhouse conditions at Suranaree University of Technology Farm, Nakhon Ratchasima province, Thailand. The Cassava were grown in 20-L plastic plots. The plant spacing was 1m x 1m between plots. The drought conditions in the greenhouse were created by soil moisture content. In FC treatment, fully irrigated conditions were applied throughout the experiment for maintaining soil moisture levels at 28.06 %.

In 2/3 and 1/3 AW conditions, soil moisture content was maintained at 16.84 % and 11.22 %, respectively.

2.2. Leaf retention

Leaf retention was monitored by counting the number of leaves on the ground. Data collected at 1, 2 and 3 weeks after withholding water at 2 months after planting.

2.3. Ethylene accumulation

Ethylene gas on fresh leaves was collected weighted and placed into 70 ml. tube. After incubating at room temperature for 48 h. 1 ml. of the gas sample was injected into gas chromatography (GC). Data collected at 1, 2 and 3 weeks after withholding water at 2 months after planting.

2.4. Relative Water Content (RWC)

Relative water content (RWC) using the fully expanded leaf from the top of the plant, RWC was accorded by calculated of leaf fresh weight, dry weight, and turgid weight. Mature leave (FW) sample was recorded and soaked overnight in distilled water at room temperature, turgid weight (TW) was recorded and oven dried at 75˚C for 24 h (DW) Collected at 3,4 and 5 months after planting.

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RWC(\%) = \frac{(FW - DW)}{(TW - DW)} \times 100
\]

2.5. SCMR

Collected at 3,4 and 5 months after planting using SPAD-502 meter (Minolta Konica Co.Ltd., Japan). The SCMR measurement was recorded three times at different positions from the expended leaf and average for each plot at 09:00 – 10:00 AM

2.6. Statistical analysis

The physiological and biochemical parameters were statistically analysed for Analysis of variance (ANOVA). The significance of difference among the treatment was performed by Tukey’s HSD (Honestly Significant Difference) test. (p = 0.05) using statistic 8.

3. Results
The averages of soil moisture content field during the experiment at 0–10 cm depth shown in figure 1. It was expected that the soil water content at FC conditions has a higher compared to the 2/3 and 1/3 AW conditions. The water content started to differentiate after no irrigation after 2 months after planting. The FC, 2/3 and 1/3 AW conditions of soil moisture were 26.02, 22.30 and 19.30% respectively.

![Graph showing soil moisture content at different conditions](image)

**Figure 1.** The average value of soil moisture at the FC, 2/3 and 1/3 AW conditions by gravimetric methods

### 3.1. Ethylene accumulation

Water stress also affected to ethylene accumulation under 1/3 AW was an increase when compared to 2/3 AW and FC conditions. Ethylene accumulation under FC conditions was low ranging between 0.75 – 1.30 µmol g⁻¹FW h⁻¹ at 1, 2 and 3 weeks after withholding water. Ethylene accumulation increased to a maximum rate at 3 weeks after withholding water reached 4.56 µmol g⁻¹FW h⁻¹ at 3 weeks after withholding water (figure 2a). The highest ethylene accumulation of 3.62 µmol g⁻¹FW h⁻¹ was recorded for KU50 and the lowest ethylene accumulation of 1.73 µmol g⁻¹FW h⁻¹ for HB50 at 3 weeks after withholding water, but was not a significant difference between Rayong 9, Rayong 90 and Hanatee (figure 2b)
3.2. Leaf retention
At 1, 2 and 3 weeks after withholding water leaf retention of the five-cassava variety was significantly affected by water deficit with the highest (7 leaves) and the lowest (3 leaves) were obtained at 3 weeks after withholding water (figure 3a). Rayong 90 had the highest leaf retention (8 leaves) followed by Huay Bong 80, Rayong 9, Kasetsart 50 and Hanatee, but no significant difference between them. (figure 3b)

3.3. Relative Water Content (RWC)
A significant difference was obtained for RWC among the water regimes. Under the drought stress groups induced significant decreases in RWC in all varieties. RWC at 3, 4 and 5 months after planting under 1/3 AW were higher than 2/3 AW and FC conditions (figure 4a). 3 months after planting was no significant difference in all variety. however, this difference became significant at 4 and 5 months after planting.
planting. Rayong 9 had the highest RWC (86.18 and 87.26 %) follow with Rayong 90 (83.93 and 84.91 %) figure 4b

![Figure 4](image_url)

**Figure 4.** Effect of water on Relative water content (RWC) at 3, 4 and 5 months after planting.

3.4. **SPAD chlorophyll meter reading (SCMR)**

Analysis of variance (ANOVA) showed that under drought stress regimes had a significant effect on trits. Results showed SPAD was increased by increasing water restriction and at 3, 4 and 5 months after planting, SCMR under 1/3 AW conditions have a highest (figure 5a) Rayong 9 has a hightest SCMR follow by HB80 and Hanatee has the lowes. Hanatee had a light green leaf color, but Rayong 9 had a dark green leaf color. (figure 6))

![Figure 5](image_url)

**Figure 5.** Effect of water on SCMR at 3, 4 and 5 months after planting.
4. Conclusion
In a review of the hormonal regulation of abscission [21] concluded that auxin is the only one that has been shown to function in preventing leaf abscission. Drought stress is greatly affected by leaf fall. There was also the possibility that leaf fall was related to an ethylene accumulation. However, water deficit also affected to the SPAD and RWC within the first 3 months after planting under water deficit conditions. Therefore, this study supported the screening period of 5 months for cassava under water deficit.

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Figure 6. Five cassava leaves under 3 water regimes. (A) Rayong 9, (B) Rayong 90, (C) Huaybong 80, (D) Kasetsart 50 and (E) Hanatee
growth, development and senescence: interaction with other phytohormones Front. Plant Sci. 1-19

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