Comparison Waterlogging Tolerance Potential of Cassava

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Abstract. This experiment was conducted to evaluate the waterlogging tolerance of cassava. Five cassava genotypes from CIAT: CM9912-167, GM214-62, GM1263-6, GM1406-13 and GM1521-10, were used to assess the impact of artificial waterlogging conditions on growth and development of cassava. Cassava plantlets (in vitro) were cultivated in soil pots, and after 3 months of growth, they were waterlogged for 12 days. After three days of waterlogging, it was found that all 5 genotypes of cassava were affected by the waterlogged conditions. Notably, the variety of CM9912-167 clearly showed physiological changes such as chlorosis of leaves, wilting leaves and some plants even died within 6 days. Other varieties of cassava showed a belated onset of symptoms. Genotypes GM1406-13 and GM1512-10 showed the highest percent of chlorosis leaves, they resisted the effects of waterlogging for 12 days which was the longest time period for the genotypes tested. Through this experiment, cassava has two types of response to flooding. The first was cassava showing early yellow leaf, starting from old leaves and losing those yellow leaves. The other was withered whole the plant, then leaves dries and fall off, only a few young leaves on the top. In conclusion, cassava with yellow leaves is more resistant to flooding.

1. Introduction
Cassava (Manihot esculenta. Crantz) is an important crop in world agriculture due to it’s simple planting requirements and ability to grow in nutrient-poor soils. In Vietnam, demand for cassava is increasing and therefore requires more field area. This expansion has pushed growth into fields which are prone to flooding and erratic weather due to climate change. In the future, it would be beneficial to develop varieties of flood-resistant of cassava, which would add diversity of choice to the crop structure in flood prone areas. For this reason, the following experiments were conducted to assess the waterlogging tolerance of cassava and to identify which varieties are most tolerant to flooded conditions.

2. Materials and methods
2.1. Materials
Five cassava genotypes (CM9912-167, GM214-62, GM1263-6, GM1406-13 and GM1521-10) of in vitro plantlets were sourced from CIAT and provided by the Agricultural Genetics Institute in Vietnam. Plants were potted in containers measuring 30x28x20 cm. Each pot used 5 kg of soil supplemented with 10 g of P2SO5 to stimulate root formation. After planting for a month, each of the pots were supplemented with urea and potassium at 20 g to stimulate leaf growth and increase photosynthesis capacity of the plant.
2.2. Method

20 plantlets of each of the 5 genotypes were provided by the Agricultural Genetics Institute in Vietnam, but 15 of those plants were randomly chosen for flooding treatment. The remaining 5 plants of each species were retained as a control. Each plant was spaced 50 cm apart.

2.3. Artificial flooding method

Three months after planting, the pot was transferred to a larger bucket with dimensions: length x width x depth = 30x34x30 cm. Then the bucket was filled with water until all plant roots are submerged. When all varieties showed signs of wilting, the water was drained to observe the plant's regeneration. The following indices were measured every 3 days during the experiment:

- Number of leaf, number of node and height of plants (Counting directly on the plants).
- Leaf chlorophyll index (SPAD): Measured using a portable chlorophyll Konica Minolta SPAD 502 plus. 5 measurements were taken from the top of each plant, excluding the 3 newest emerging leaves. Control plants were also measured.

Determination of total chlorophyll content by SPAD-502

Chlorophyll a content:

\[ Y = 1.56 \times 10^{-6} + 3.33 \times 10^{-3}X + 9.03 \times 10^{-4}X^2 \ (r^2 = 0.952) \]

Chlorophyll b content:

\[ Y = 5.46 \times 10^{-4} + 6.89 \times 10^{-3}X + 3.37 \times 10^{-4}X^2 \ (r^2 = 0.964) \]

Chlorophyll a+b content:

\[ Y = 5.52 \times 10^{-4} + 4.04 \times 10^{-3}X + 1.25 \times 10^{-4}X^2 \ (r^2 = 0.960) \]

Where: \( Y = \) Chlorophyll content (mg/cm²); \( X = \) SPAD value

Number of wilted plants (percentage): Number of wilted plants is the number of plants showing stress through wilting leaves during the experiment.

\[
\frac{\text{Number of wilted plants}}{\text{Total plants}} \times 100
\]

The percentage of leaves with chlorosis (percentage):

\[
\frac{\text{Number of chlorosis leaves}}{\text{Total leaves on plant}} \times 100
\]

According to Vietnam's national technical regulation on testing value of cultivation and use of cassava varieties, the evaluation scale is as follows:

- Very good tolerance: plants grow normally.
- Fair tolerance: leaves slightly yellow.
- Medium tolerance: leaves turn yellow, no leaf loss.
- Slightly poor tolerance: leaves turn yellow and lose 1/3 of the leaves.
- Very poor tolerance: leaves turn yellow and lose 2/3 of the leaf.

The research data was processed by SPSS 16.0 software with One-Way ANOVA application with Duncan test at the significance level of 0.05.

3. Results and discussion

3.1. Effect of waterlogging conditions on leaf.

In the artificial waterlogging conditions, we found that all five genotypes of cassava had a response to flooding conditions. By the third day, leaves began to exhibit chlorosis, and plants began to wilt, especially CM9912-167 (Figure 1B). By the 6th day, the remaining genotypes had clearer physiological symptoms in response to the flooding conditions exhibited by the increase in the number of chlorosis leaves, the number of wilted plants and the decrease in the number of leaves. By the 9th day and 12th day, there was a clear difference between 5 genotypes with good and weak flood resistance. According to table 1.

After the 3rd day of waterlogging, the percentage of leaves lost among all 5 genotypes, revealed no significant change, while the number of chlorosis leaves was the highest at 36.93% belonging to GM1521-10, followed by GM1263-6, CM9912-167, GM1406-13 and GM214-62 (14.84%, 13.96%, 13.04% and 12.93%, respectively), however only CM9912-167 and GM1406-13 had wilted plants (22% and 14.28% respectively). This suggests that CM9912-167 should be classified as “slightly
poor resistance” because the variety had the highest the number of wilted plants, although the number of chlorosis leaves is not high. Next was the GM1406-13 variety with medium tolerance. Finally, the remaining 3 genotypes were graded with fair resistance because they still grew normally despite the few chlorosis leaves. (Figure 1B, 2B, 3B, 4B, 5B).

Table 1. Effect of waterlogging conditions on plants.

| Indicator                      | Variety of cassava | Before | After 3rd day | After 6th day | After 9th day | After 12th day | Control       |
|--------------------------------|--------------------|--------|---------------|---------------|--------------|---------------|---------------|
| Number of leaves (leaf/plant)  | CM9912-167         | 19.56±3.74a | 19.89±3.79a  | 15.44±9.05a   | 0.33±1.00b   | 0.56±1.13b   | 15.56±4.41a   |
|                                | GM214-62           | 16.29±2.05a | 16.57±2.07a  | 16.00±2.16a   | 4.71±5.90b   | 2.00±2.76b   | 14.88±1.80a   |
|                                | GM1263-6           | 18.86±1.86a | 18.29±1.79a  | 18.29±1.79a   | 8.00±5.44b   | 1.43±1.81a   | 18.38±2.32a   |
|                                | GM1406-13          | 18.50±3.77a | 18.40±3.37a  | 17.40±3.20ab  | 11.90±6.70b  | 10.30±5.51b  | 18.33±2.87a   |
|                                | GM1521-10          | 22.00±2.44a | 22.20±1.31a  | 18.40±3.27b   | 13.00±3.12c  | 9.70±1.70d   | 18.50±4.19b   |
| Percent of chlorosis leaves (%) | CM9912-167         | 0.56   | 13.96        | 39.56         | 71.42        | 40            | 0.75          |
|                                | GM214-62           | 2.63   | 12.93        | 28.57         | 66.67        | 57.14         | 2.75          |
|                                | GM1263-6           | 0.75   | 14.84        | 40.62         | 64.28        | 0             | 1.45          |
|                                | GM1406-13          | 0.54   | 13.04        | 22.41         | 31.09        | 43.68         | 2.64          |
|                                | GM1521-10          | 0      | 36.93        | 43.47         | 36.15        | 58.76         | 3.93          |
| Percent of wilted plant (%)    | CM9912-167         | 0      | 22.22        | 44.44         | 88.89        | 100           | 0             |
|                                | GM214-62           | 0      | 14.28        | 42.85         | 57.14        | 100           | 0             |
|                                | GM1263-6           | 0      | 0            | 14.28         | 57.14        | 71.42         | 0             |
|                                | GM1406-13          | 0      | 0            | 10            | 40           | 60            | 0             |
|                                | GM1521-10          | 0      | 0            | 0             | 70           | 80            | 0             |

Notes: 1. mean±SE, Control measurement after 12 days of experiment.  
2. Different letters in the same row mean statistically different (ANOVA Duncan at 0.05)

On the sixth day after waterlogging, the plants had showed more stress signals with an increase in the number of chlorosis leaves and in the number of wilted plants. Although there are many chlorosis leaves and many wilted plants, no leaves were shed from the plants. Regarding the number of chlorosis leaves, GM1521-10 variety were still the highest at 43.47%, followed by GM1263-6, CM9912-167, GM214-62 and GM1406-13 (40.62%, 39.56%, 28.57% and 22.41% respectively). The highest number of wilted plants was still the CM9912-167 variety with 44.44%, followed by GM214-62, GM1263-6, GM1406-13 (42.85%, 14.28%, 10% respectively), only the GM1521-10 variety no wilted plants. Although the number of leaves had no significant change, for CM9912-167 variety there was a very high standard deviation (15.44±9.05) because when some plants withered, all mature leaves died. So, CM9912-167 had very poor tolerance, next was GM214-62, which is slightly poor tolerance, then GM1263-6 has medium tolerance and the remaining 2 genotypes are classified as “fair tolerance”. After 9th day of artificially waterlogged conditions, the cassava varieties had clear changes in morphology, and became a major indicator of flood tolerance. The number of leaves among all 5 genotypes decreased dramatically. The lowest number of leaves is CM9912-167, followed by GM1263-6, GM214-62, GM1406-13 and GM1521-10 (0.33±1.00, 4.71±5.90, 8.00±5.44, 11.90±6.70 and 13.00±3.12 respectively). Due to the high death rate
of mature leaves, the number of chlorosis leaves and drought tolerance ranking changed greatly on the 9th day of waterlogged conditions. The highest number of chlorosis leaves was CM9912-167 at 71.42%, followed by GM1263-6, GM214-62, GM1521-10 and GM1406-13 (66.67%, 64.28%, 36.15% and 31.06% respectively). In regard to the number of wilted plants, the highest number of wilted plants was CM9912-167 with 88.89%, followed by GM1263-6, GM214-62, GM1521-10 and GM1406-13 in order (57.14%, 57.14%, 70% and 40% respectively). So, after 9 days of waterlogged conditions, the genotypes CM9912-167, GM1263-6, GM214-62 demonstrated very poor tolerance while GM1521-10 and GM1406-13 displayed slightly poor tolerance.

![Figure 1. Symptoms of cassava in artificial waterlogging conditions. CM9912-167 (1), GM214-62 (2), GM1263-6 (3), GM1406-13 (4) and GM1521-10 (5), before (A), after 3 days (B), and after 9 days (C).](image-url)

By 12th day, the results were similar to the 9th day, the genotypes CM9912-167, GM1263-6, GM214-62 had lost all mature leaves with the exception of only a few young leaves on top. While the GM1521-10 and GM1406-13 genotypes still had a lot of mature leaves, the number of chlorosis leaves and the number of wilted plants increased, so they were graded at very poor tolerance after the 12th day of the experiment. The results of this experiment are not well matched to Vietnam’s technical regulation on testing cassava flood tolerance. The results show that chlorosis in leaves is actually an indicator of good flooding tolerance, as a plant was more likely to survive after the flooded conditions if it lost a few leaves.
3.2. Effect of waterlogging conditions on chlorophyll content

During the experiment, regarding the effect of waterlogging conditions on chlorophyll content, we obtained the results of chlorophyll a, chlorophyll b and total chlorophyll content as shown in Table 2 below:

| Variety of cassava | Chlorophyll a | Chlorophyll b | Total chlorophyll (a+b) |
|--------------------|---------------|---------------|------------------------|
| CM9912 -167        | 0.0307±0.003a 0.0272±0.005b 0.0129±0.007c 0.0029±0.005d 0.0034±0.006e 0.0233±0.001f | 0.0097±0.001a 0.0085±0.001b 0.0042±0.002c 0.0013±0.001d 0.0014±0.001e 0.0073±0.001f | 0.0408±0.004a 0.0360±0.007b 0.0172±0.010c 0.0043±0.007d 0.0049±0.008e 0.0308±0.001f |
| GM214-62           | 0.0267±0.004a 0.0251±0.004b 0.0143±0.003b 0.0054±0.007c 0.0052±0.007d 0.0228±0.003e | 0.0083±0.001a 0.0079±0.001b 0.0045±0.001c 0.0020±0.002d 0.0019±0.001e 0.0071±0.001f | 0.0359±0.005a 0.0343±0.006b 0.0190±0.004c 0.0075±0.009d 0.0072±0.009e 0.0303±0.004f |
| GM1263 -6          | 0.0340±0.005a 0.0334±0.004a 0.0241±0.010b 0.0126±0.006c 0.0074±0.009d 0.0329±0.004e | 0.0107±0.001a 0.0105±0.001b 0.0076±0.003c 0.0040±0.001d 0.0026±0.002e 0.0103±0.001f | 0.0452±0.004a 0.0444±0.006b 0.0321±0.014c 0.0168±0.008d 0.0101±0.012e 0.0437±0.005f |
| GM1406 -13         | 0.0323±0.002a 0.0305±0.004a 0.0214±0.009b 0.0155±0.009c 0.0125±0.007d 0.0299±0.003e | 0.0102±0.001a 0.0096±0.001b 0.0067±0.002c 0.0049±0.002d 0.0040±0.002e 0.0094±0.001f | 0.0195±0.001a 0.0190±0.001b 0.0185±0.001c 0.0165±0.001d 0.0161±0.001e 0.0208±0.001f |
| GM1521 -10         | 0.0292±0.002a 0.4831±0.006b 0.3638±0.089c 0.3118±0.086d 0.0161±0.003e 0.5203±0.054f | 0.0091±0.001a 0.0097±0.001b 0.0062±0.001c 0.0055±0.001d 0.0050±0.001e 0.0081±0.001f | 0.0388±0.002a 0.0326±0.005b 0.0264±0.004c 0.0236±0.004d 0.0213±0.004e 0.0346±0.002b |

Notes: 1. Different letters in the same row mean statistically different (ANOVA Duncan at 0.05)

From the Table 2, the content of chlorophyll a, chlorophyll b and total chlorophyll of these cassava genotypes decreased in growth under waterlogging conditions which correlated with the number of leaves and was inversely proportional to the percentage of chlorosis leaves. After 3 days of flooding, the chlorophyll content of all cassava genotypes decreased but there was no statistically significant difference. After 6 days, 9 days and 12 days of waterlogging conditions, the chlorophyll content of five cassava genotypes decreased significantly.

3.3. Effect of waterlogging conditions on height and number of node

After the experiment comparing the resistance of 5 cassava genotypes under artificial waterlogging conditions, it was found that, although the collected data indicated a very clear change, the height and number of nodes had not changed significantly (table 3). All genotypes halted development and did not produce new nodes when exposed to waterlogged conditions.

Table 3. Effect of waterlogging conditions on height and number of node
| Number of nodes (node/plant) | Variety of cassava | Before | After 3 days | After 6 days | After 9 days | After 12 days | Control |
|-----------------------------|--------------------|--------|--------------|--------------|--------------|--------------|---------|
| CM9912-167                  | 25,22±3,15b        | 25,22±3,15b | 25,22±3,15b | 25,22±3,15b | 25,55±3,04a | 21,87±2,64a   |
| GM214-62                    | 23,57±2,76a        | 23,57±2,76a | 23,57±2,76a | 23,57±2,76a | 24,57±2,76a | 22,87±2,94a   |
| GM1263-6                    | 24,28±1,49b        | 24,28±1,49b | 24,28±1,49b | 24,28±1,49b | 25,00±1,73b | 25,37±5,47b   |
| GM1406-13                   | 22,80±2,34a        | 22,80±2,34a | 22,80±2,34a | 22,80±2,34a | 23,40±2,45a | 25,00±5,38b   |
| GM1521-10                   | 24,50±4,94a        | 24,50±4,94a | 24,50±4,94a | 24,50±4,94a | 25,10±4,67a | 22,40±5,18a   |
| CM9912-167                  | 30,66±6,94a        | 30,66±6,94a | 30,66±6,94a | 30,66±6,94a | 30,88±6,95a | 23,88±4,22a   |
| GM214-62                    | 35,00±7,59a        | 35,00±7,59a | 35,00±7,59a | 35,00±7,59a | 35,42±7,48a | 27,62±5,60a   |
| GM1263-6                    | 29,28±3,25a        | 29,28±3,25a | 29,28±3,25a | 29,28±3,25a | 29,57±3,59a | 28,37±8,33a   |
| GM1406-13                   | 26,30±4,94a        | 26,30±4,94a | 26,30±4,94a | 26,30±4,94a | 26,50±4,99a | 27,12±7,64a   |
| GM1521-10                   | 32,20±5,00a        | 32,20±5,00a | 32,20±5,00a | 32,20±5,00a | 32,30±4,96a | 26,80±8,71a   |

Notes: 1. Mean±SE, different letters in the same row mean statistically different (ANOVA Duncan at 0.05)
In general, the response of cassava varieties to flooding conditions was similar in terms of the plants showing affected symptoms through the morphology and physiology such as the percentage of leaves lost, the percentages of chlorosis leaves, the chlorophyll content, the height plant and number of the nodes. Cassava varieties differed in the time to symptom onset. Sarohal. (2011) explains that although each plant species is the same, their tolerance to adverse conditions can still differ due to outside factors or individual plant differences [5]. As a result of assessing the tolerance of cassava in waterlogged conditions, we found that the height of the plants and the number of nodes were not significantly different among varieties and did not show clearly indicate tolerance to waterlogged conditions during the experiment. The most obvious indicators were indicators about leaves such as the number of leaves, the percentage of chlorosis leaves, the rate of defoliation, the chlorophyll contents and the wilting plants. These indicators were expressed faster and more clearly, so it is possible to distinguish the difference of tolerance to flooding of cassava varieties more easily.

When the plants was flooded, the roots lack oxygen so that the roots will not be able to respire. This limits the absorption of water and nutrients to other parts of the plants. When the roots had less water, the evaporation rate decreases to ensure no wilting leaves [8], [10]. This was the mechanism of the guard cells, which closes the stomata or shrinks to reduce transpiration. However, when the stomata were closed, there is no loss of water but it is not possible to avoid the impact on photosynthesis rate [7]. When the gas exchange was limited, the amount of CO₂ will be reduced, the productivity of photosynthesis will decrease, which leads to the growth and development of plants to stop or develop slowly [11], [12]. The longer a plant was flooded for, the lower it’s tolerance due to the inability to maintain energy. Once it was no longer flooded, it was quickly able to transition to anaerobic respiration, but the energy generated was not sufficient for the plants, so they would have to respond through changing physiology and morphology [2], [6], [13]. Ekanayake. (1998) suggested that the plant’s mechanism of adapting to flooding serves to increase it’s ability to supply oxygen to the roots. Cassava also has a physiological response when flooded similar to other plant species such as formation the adventitious root. This root sets the direction of the new roots to be free from flooding, and forms the lenticels at the cortex of root and stem sections near the water surface (Figure 2) to enhance oxygen uptake. There are also physiological reactions such as reduced growth, and leaf loss. Biochemical responses such as increases anaerobic respiration and ethylene production are also present [13], [14]. Most plants cannot survive long periods of flooding or soil waterlogging, especially crops and tuberous plants that cannot tolerate hypoxia conditions [9]. Plants in flooded conditions for short or long periods will have a negative impact on plants [15].

![Image](https://via.placeholder.com/150)

**Figure 2.** The lenticels and adventitious roots formations on stem near water surface of GM1521-10 cassava variety

4. Conclusions

The purposes of these experiments was to assess the ability of cassava plants to withstand flooding, and assess cassava varieties abilities and strategies to survive such stressors. The expression of growth stressors were measured by the percentage of leaves lost, the percentage of chlorosis leaves, the number of wilted plants and the chlorophyll content. Based on the timeframe of expression, we found that the best genotypes which were resistant to waterlogging were GM1406-13 and
GM1521-10 with strategy showing early chlorosis leaves on the old leaf and losing those leaves, followed by GM214-62, GM1263-6, and CM9912-167 in that order.

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