Tolerance response of ten chili genotypes under the limited watering condition

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Abstract. Ten chili genotypes of germplasm collection had been evaluated under limited watering conditions. The objective of the research was to know the mechanism response of each treated genotype under limited watering conditions. The research was conducted from February to December 2017 at Indonesia Vegetables Research Institute, Lembang (1,250 m sal). A randomized complete design (RCD) was used as a research design with three replications. There were 20 treatments which combination between 10 chili genotypes with limited (interval per six days) or unlimited watering (per daily). The seedlings were planted in plastic pots with a mixture of media soil:sand = 1:2, then be applied treatments. The population number of each genotype per treatment was 15 plants/replication. The results showed that AN and 238 were a better tolerance level than other genotypes under-treated limited watering conditions by some mechanisms, i.e. the smallest change in total chlorophyll, but increase in proline content, and reduction in leaf area index and stomata number. Moreover, genotype 238 also developed effectiveness water absorption by root elongation at untreated stress conditions. Hence the results could determine a good strategy for each genotype to be improved as a new tolerant variety in water deficiency conditions.

1. Introduction

Chili has high economic value due to many benefits and functions. Chili is used not only for seasoning but also for raw materials of some food industries, pharmaceuticals and cosmetics. Chili contains protein, fat, carbohydrates, vitamins (A, B1, C), and minerals [1]. In general, the national chili productivity in Indonesia is around 8.35 t ha\(^{-1}\). This condition is still low if compared with average world chili productivity (16.11 t ha\(^{-1}\)) [2].

One strategy to increase chili’s production in Indonesia is through extensification outside of Java. This is intended also to support the national program of food security and food self-sufficient [3, 4]. However, several areas in Indonesia are marginal land with limited water. Dryland in Indonesia was estimated at around 12.90 million hectares spread across Sumatra, Kalimantan, Sulawesi, and Papua [4].

Limited water conditions can cause drought stress. In the stress situation, soil water content is at a minimum for plant growth and production [5]. Plants in drought stress will reduce water absorption rate by plant roots. Its effect is growth disorders, especially in meristematic tissue [6]. One effort to anticipate drought impact is by cultivating drought-tolerant genotype because indicates the ability to survive with low water potential [7]. This case is always related to changes in morphology and physiology of plants.

The use of a new variety with the local genetic background can be done to support food security in areas with limited water conditions. Therefore, it requires the availability of germplasm that can be
focussed to solve these conditions [8]. The first step that should be conducted is to select germplasm collection genotypes through a screening method. The response of each genotype to the screening method varies because each genotype has a self mechanism. Tolerance to water-limited conditions can occur if one genotype is able to use a mechanism to avoid or challenge the stress situation. Hence it is needed to study the tolerance response of each chili germplasm collection genotype by several screening methods.

The ability of plants to maintain high water potential condition in the tissues indicate a drought tolerance mechanism. Plants can maintain highly potential by increasing water absorption capacity or by decreasing water loss out of the tissue. In this mechanism, plants have the ability to increase the root system, regulate stomata, reduce the absorption of solar radiation by forming a waxy layer or hairy leaves, and reduce evapotranspiration through narrowing leaves or decreasing leaf area index [9, 10]. Thus, a screening can be carried out based on these parameters.

The objective of the study is to know the mechanism of each treated genotype to respond under limited watering conditions.

2. Materials and methods
The research was conducted from February to December 2017 at Indonesian Vegetables Research Institute, Lembang (1,250 m sal). The research activity was carried out at the screen house and laboratory. The research used a randomized complete design (RCD) with three replications. There were 20 treatments in which several combinations between 10 chili genotypes with limited (watering with interval 6 days) or unlimited (watering per daily) condition. The seedling with 2-4 true leaves was transplanted to plastic pots (Ø 10 cm) containing mixture media: soil:sand = 1:2, then be applied treatments. The population number of each genotype per treatment was 15 plants/replication. Also, be observed temperature and humidity inside the screen house as long as research.

2.1. Pollen viability
Pollen viability was done by taking pollen in the morning at anthesis, then stained by aceticarmine [11]. After 30 minutes carried out observation under a microscope with 10x magnification. If pollen’s color is red, that indicates viability. Observation of the viable pollen number was done three times in several point views then averaged, be calculated using a formula:

\[
Pollen\ viable = \frac{\text{The stained pollen number in a point of view}}{\text{Total observed pollen in a point of view}} \times 100\% \quad (1)
\]

2.2. Total chlorophyll content
Content of total chlorophyll (chlorophyll A & B) was carried out using Hendry and Grime Method [12, 13]. 0.1 g of leaf samples were crushed, then filtered. The resulting filtrate was diluted with 90% alcohol to reach a volume of 25 ml and centrifuged at 4,000 rpm for 10 minutes. The green liquid in the upper centrifuge tube was transferred to the cuvet and its absorbance was measured using Optical Density (OD) at a wavelength of 663 nm and 645 nm.

2.3. Proline content
Proline content refers to the method used by Bates [14, 15]: the roots of chili plants at the age of 60-65 DAP (first harvest) were taken, cleaned, and weighed as much as 0.5 g. The roots were pounded with a mortar in 10 ml of 3% sulfosalicylic solution, filtered with Whatman filter paper No. 1. Then, 2 ml of filtrate was taken and put in a test tube, and 2 ml of Ninhydrin acid was added. Ninhydrin acid was prepared by heating 1.25 g of Ninhydrin in 30 ml of glacial acetic acid and 20 ml of phosphoric acid. Heating was carried out in a bath at 100°C until dissolved. The filtrate and 2 ml of Ninhydrin acid plus 2 ml of glacial acetic acid were then heated at 100°C for 1 hour. The reaction was ended by inserting a test tube containing the filtrate into a beaker filled with ice. The mixture of filtrate, ninhydrin, and
glacial acetic acid was added with 4 ml of toluene and shaken for 15-20 seconds with a stirrer to form two layers of different colored liquid. Red toluene containing proline was taken with a pipette, inserted in a cuvette, and read the optical density (OD) at a wavelength of 520 nm. Proline content was calculated by making a standard proline solution first, i.e: 2.5 μM of the main solution diluted with 3% sulfosalicylic acid. Dilution was intended to obtain variations in the proline concentration. Furthermore, the solution was reacted with ninhydrin acid and glacial acetic acid, then the solution was put in a cuvette and read the optical density (OD) at a wavelength of 520 nm. The results of the absorbance of the standard solution were made a linear regression equation in order to obtain the equation: Y = ax + b. Then the absorbance of the sample was entered as the Y value so that the x value (μg mol$^{-1}$) = μ mol g$^{-1}$.

2.4. Stress Intensity (SI) and Stress Susceptibility Index (SSI)
Stress Intensity (SI) and Stress Susceptibility Index (SSI) [16]: weight of fruit per plant from a genotype under optimal was compared with stress conditions using the following equations:

\[
SI = [1 - /MYs/MYp] \quad (2)
\]

\[
SSI = [1 - Yp/Ys] / SI \quad (3)
\]

Note: Yp is the mean values for the investigated trait under normal conditions, whereas Ys is under stress conditions, MYp is the mean trait value of all investigated genotypes under normal conditions, whereas MYs is under stress conditions. Categories: Tolerant (SSI < 0.5), Moderate (SSI 0.5 – 1.00); Sensitive (SSI > 1.00). All data were collected and analyzed statistically. Analysis of variance was carried out using F test and DMRT test at the 5% level.

3. Results and discussion

3.1. Pollen viability
In table 1 can be seen that there was a significant difference in pollen viability of limited watering treated genotypes with untreated. Generally, under normal conditions, pollen viability was in the range of 88.36-94.25%, but in the stress condition becoming decrease in the range of 52.60-72.45%. AN genotype had the smallest decrease (21.80%), while HT genotype was highest (44.19%). This caused in limited watering conditions can induce hydrogen peroxide that affects microspore abortion. Water deficiency can also cause high levels of glucose in the tapetum and loculus as a natural response to oxidative stress by increasing cell turgor. This condition causes many tapetum abnormal, thus pollen to become sterile [17].

3.2. Total chlorophyll content
Total chlorophyll consists of chlorophyll a and b. Chlorophyll a (C55H72O5N5Mg) is a green leafy substance that causes dark green leaves, while chlorophyll b (C55H70O6N4Mg) causes light green leaves. Drought stress could increase the chlorophyll a / b ratio because decreasing chlorophyll b is greater than chlorophyll a [18]. Drought would inhibit chlorophyll synthesis in leaves because the increase in temperature and transpiration could cause chlorophyll disintegration [19]. Thus, sensitive genotypes to drought stress will respond to a greater decrease in total chlorophyll content than tolerant genotypes. In table 1 can be known that AN genotype shows the smallest difference in terms of decreasing total chlorophyll content. This indicates that AN genotype is a tolerant genotype under limited watering conditions.

3.3. Leaf area index
Leaf area index is very sensitive to stress conditions. In general, the stress situation will decrease the formation and expansion of leaf, accelerated aging and leaf shedding, or both. Accelerated leaf aging due to limited watering conditions tends to occur in the lower leaves, which part less active in photosynthesis and assimilates supply, thus they had less contribution effect to yield [7]. In table 1 can be seen in some genotypes such as AN, GL, and HT, there was a significant reduction in leaf area index
from treated and untreated genotypes. It is indicated that the three genotypes have a mechanism of reduced leaf area index to respond to limited watering conditions.

**Table 1.** Analysis of pollen viability, total chlorophyll (A + B), and leaf area index in stress and unstress condition of ten chili genotypes.

| Treatments | Pollen viability (%) | Total chlorophyll (A+B) (µ/mg) | Leaf area index (cm²) |
|------------|----------------------|-------------------------------|----------------------|
| ANW1       | 92.65 a              | 35.69 b                       | 560.09 a             |
| GLW1       | 90.40 a              | 46.12 a                       | 475.61 a             |
| HTW1       | 94.25 a              | 37.53 b                       | 517.34 a             |
| SSW1       | 88.75 a              | 42.86 b                       | 234.85 c             |
| YNW1       | 96.20 a              | 37.67 b                       | 128.95 d             |
| 801W1      | 90.35 a              | 48.32 a                       | 186.72 d             |
| 238W1      | 90.65 a              | 44.13 ab                      | 209.50 c             |
| 353W1      | 92.46 a              | 48.84 a                       | 101.00 d             |
| 517W1      | 90.62 a              | 44.46 ab                      | 218.82 c             |
| 113W1      | 88.36 a              | 34.77 b                       | 69.60 e              |
| ANW6       | 72.45 b              | 35.73 b                       | 383.83 b             |
| GLW6       | 64.75 bc             | 48.71 a                       | 215.26 c             |
| HTW6       | 52.60 bc             | 45.29 ab                      | 270.31 c             |
| SSW6       | 64.26 bc             | 37.41 b                       | 200.81 cd            |
| YNW6       | 64.95 bc             | 52.25 a                       | 232.40 c             |
| 801W6      | 66.35 bc             | 45.93 ab                      | 134.19 d             |
| 238W6      | 58.25 c              | 39.46 b                       | 196.06 d             |
| 353W6      | 54.80 c              | 49.00 a                       | 216.12 c             |
| 517W6      | 64.75 bc             | 52.80 a                       | 210.58 c             |
| 113W6      | 58.45 c              | 46.89 a                       | 141.51 d             |

Note: Mean followed by the same letters on the same columns are not significant according to Duncan’s multiply range test at 0.05 level

**3.4. Number of stomata**

In table 2 can be seen that all the treated genotypes with limited watering decreased in stomata number. However, stomata were less sensitive than leaf area index to respond to drought stress [7]. This possibility as causing inconsistent results in the parameter. Contrary to others, some genotypes such as YN, 517, 113 had stomata number much more in stress condition.

**3.5. Root length**

Soemartono [9] stated that one strategy of the plant to challenge drought stress is keeping water potential still high inside the tissue. In this mechanism, plants have the ability to increase the root system. It seems the mechanism occurs in genotypes 801 and 238 which roots become longer under limited watering conditions. The root system of both of them had been functioned to absorb water optimally, thus the high water potential could be maintained properly.

**3.6. Plant height**

In table 2 can also be known that all the treated genotypes under limited watering condition still grew and developed well, but there was difference significantly in plant height size. Generally, all genotypes were shorter under limited watering conditions with the smallest change occurring in AN genotype (31.17%), while the highest was 801 (51.06%). However, plant height was not only influenced genetically but also environmental factors such as improperly plastic pots size could cause plant growth to be limited.
Table 2. Analysis of stomata number, root length, and plant height in stress and unstress condition of ten chili genotypes.

| Treatments | Number of stomata (cm⁻²) | Root length (cm) | Plant height (cm) |
|------------|--------------------------|----------------|------------------|
| ANW1       | 222 c                    | 13.74 bc        | 40.30 b          |
| GLW1       | 182 d                    | 11.90 c         | 43.80 b          |
| HTW1       | 135 d                    | 16.60 b         | 48.52 a          |
| SSW1       | 221 c                    | 21.94 a         | 50.36 a          |
| YNW1       | 78 e                     | 14.64 b         | 35.40 c          |
| 801W1      | 260 c                    | 10.22 c         | 52.92 a          |
| 238W1      | 590 a                    | 11.06 c         | 46.10 ab         |
| 353W1      | 154 d                    | 10.78 c         | 39.96 bc         |
| 517W1      | 91 e                     | 11.86 c         | 44.50 b          |
| 113W1      | 126 d                    | 19.04 a         | 45.22 b          |
| ANW6       | 125 d                    | 10.96 c         | 27.74 d          |
| GLW6       | 151 d                    | 9.54 cd         | 29.10 d          |
| HTW6       | 113 d                    | 10.40 c         | 28.06 d          |
| SSW6       | 148 d                    | 8.74 d          | 30.70 cd         |
| YNW6       | 375 b                    | 12.30 c         | 24.02 e          |
| 801W6      | 102 d                    | 14.06 b         | 25.90 d          |
| 238W6      | 115 d                    | 14.68 b         | 29.00 d          |
| 353W6      | 88 e                     | 10.10 c         | 25.16 d          |
| 517W6      | 223 c                    | 9.26 cd         | 25.60 d          |
| 113W6      | 189 d                    | 13.36 bc        | 25.18 d          |

Note: Mean followed by the same letters on the same columns are not significantly according to Duncan’s multiply range test at 0.05 level

Figure 1. Proline content in root and leaf of the plant (60-65 DAP).

3.7. Proline content
One of the biochemical compounds produced by plants in response to drought stress and plays a role in osmotic adjustment is proline. Excessive product of this compound can increase tolerance level to limited watering stress [20, 21]. Several results of proline analysis on leaves and roots had proved it. In general, the information revealed there was an increase in proline levels. Likewise in this research known that 517 genotypes increased proline content as much as 1.28 times, whereas on YN genotype was only 0.8 times.
In figure 1 can be seen also that in general root proline levels were higher than leaves. Any two enzymes played an important role in proline biosynthesis, namely dehydrogenase and oxidase [22]. Both enzymes were inhibited under stress conditions, but oxidase proline inhibited more in roots. It means that in chili proline accumulation in the roots is higher than leaves.

3.8. Stress intensity (SI) and Stress susceptibility index (SSI)
Based on the parameter of weight per one fruit stress could determine stress intensity (SI) and stress susceptibility index value [16], thus it could categorize tolerance level of a genotype to limited watering stress (table 3). The results showed that there were two tolerant genotypes i.e. AN and 238; four moderate genotypes i.e. GL, SS, YN, 353, and four sensitive genotypes i.e HT, 801, 517, 113). Both AN and 238 as tolerant genotypes developed mostly the same mechanism such as the smallest change in total chlorophyll content, but increase osmoregulator root proline, and reduce leaf area index and stomata number. But the difference between them occurred in the root system. Genotype 238 had root length was longer under stress conditions, thus indicating the development of an effective mechanism for water absorption through the roots.

| Genotypes | Weight of one fruit (g) | SSI | Categories |
|-----------|------------------------|-----|------------|
|           | W1                    | W6  |             |
| AN        | 12.25                  | 10.90 | 0.30       | Tolerant |
| GL        | 8.60                   | 6.75  | 1.00       | Moderate |
| HT        | 14.45                  | 10.12 | 1.40       | Sensitive |
| SS        | 10.50                  | 9.82  | 0.51       | Moderate |
| YN        | 10.72                  | 8.50  | 0.97       | Moderate |
| 801       | 9.85                   | 6.36  | 1.65       | Sensitive |
| 238       | 8.28                   | 7.72  | 0.32       | Tolerant |
| 353       | 8.52                   | 6.75  | 0.97       | Moderate |
| 517       | 9.66                   | 6.35  | 1.60       | Sensitive |
| 113       | 8.82                   | 6.58  | 1.18       | Sensitive |

Note: W1 = watering per daily; W6 = watering with interval 6 days; SI: Stress Intensity; SSI: Stress Susceptibility Index: Tolerant (SSI < 0.5), Moderate (SSI 0.5 – 1.00); Sensitive (SSI > 1.00).

4. Conclusions
The results showed that genotype AN and 238 were a better tolerance level than other genotypes in treated limited watering conditions by some mechanisms, i.e the smallest change in total chlorophyll, but increase in proline content, and reduction in leaf area index and stomata number. Moreover, 238 genotypes also developed effectiveness water absorption by root elongation at untreated stress conditions. Hence the results could determine a good strategy for each genotype to be improved as a new tolerant variety in water deficiency conditions.

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