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# Growth Response and Gene Expression Analysis of Chili Pepper (*Capsicum annuum* L.) Plant Dehydrin Against Salt Stress and Drought *In vitro*

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## ABSTRACT

The need for plants resistant to abiotic stress now and in the future is predicted to be very high. It is related to extreme climate change and converting agricultural land into residential and industrial land. As Indonesia's national strategic commodity, chili peppers require special attention when assembling chili peppers resistant to salinity and drought stress. New varieties of chili pepper plants resistant to saline and drought can be obtained through unconventional breeding (overexpression of the dehydrin gene). As a first step in assembling saline and drought-resistant chili plants, growth response and dehydrin gene expression tests were carried out from explants of chili pepper plants of the TM999 variety in vitro on salt and drought treatment media. This study aims to obtain information on the initial response to the growth and expression of the dehydrin gene from chili pepper plants of the TM999 variety before further research is carried out to increase the expression of the dehydrin gene through a molecular approach. The method used in this study is a complete randomized design with two treatments: Sodium chloride (NaCl) and polyethylene glycol (PEG-6000). The results obtained in this study showed that chili pepper varieties TM999 were more tolerant of drought stress than salinity based on several growth response data in both treatments. The analysis of dehydrin gene expression in both treatments showed that the gene expression was strongly influenced by the two strokes given. NaCl and PEG-6000 treatments increased the dehydrin gene expression of chili pepper plants grown *in vitro*.

*Keywords*: Chili pepper, dehydrin, drought stress, morpho-physiology, salt stress

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## INTRODUCTION

Chili pepper plants, as a national strategic commodity with an increasing demand every year in Indonesia, often cannot be met by the amount of national chili production, which is relatively low compared to the potential yield. The scarcity of chili peppers and the sudden high price are often found yearly, especially when extreme abiotic stress exists. This condition is exacerbated because agricultural lands have narrowed due to land conversion for residential and industrial needs. Suboptimal land use can be a promising alternative to overcome these problems. However, suboptimal land is known to have several limiting factors for plant growth, including poor nutrients, lack of water, and salinity (Sirappa & Titahena, 2014). Therefore, saline- and drought-resistant chili pepper plants are needed for suboptimal land quickly through unconventional plant breeding (overexpression of dehydrin gene by gene editing method).

Dehydrin is known as subgroup D-11 of the late embryogenesis abundant (LEA) protein that was first identified as expressed during seed maturation and desiccation and was found to be also expressed in vegetative tissues in response to abscisic acid (ABA), drought, salinity, low temperature, and heavy metals (Yuxiu et al., 2007). The utilization of the dehydrin gene to increase abiotic resistance has been proven by several studies (Brini et al., 2007; Meng et al., 2021; Puhakainen et al., 2004). However, experiments on overexpression of the dehydrin gene in chili pepper plants have not been widely reported. Information or knowledge of the dehydrin (DHN) gene in chili pepper plants is still little studied (Jing et al., 2016). Therefore, the novelty of this research will be of considerable value because it will add information related to the expression of the dehydrin gene from chili pepper plants.

The initial stage can be testing the resistance of sampled chili pepper plants (TM999 varieties) under salt stress and drought conditions *in vitro* before the dehydrin gene is modified through gene editing to obtain new varieties of chili pepper plants resistant to salt stress and drought. The aim is to obtain preliminary data on how much the increase in dehydrin gene expression is produced from chili pepper plants when treated with salt stress and drought. The data will then be useful as a baseline or comparative data on the increase in the amount of dehydrin gene expression from sample plants that have received gene modification to prove whether it is true that gene editing can increase the expression (overexpression) of the dehydrin gene of sample plants grown on salt stress and drought treatment media. Therefore, this study aims to study the growth response and expression of the dehydrin gene of chili pepper plants of the TM999 variety *in vitro* with various salt stress (NaCl) and drought stress (PEG-6000) concentration levels.

## MATERIALS AND METHODS

This study was conducted at Plant Tissue Culture and Agrotropica Learning Center (AGLC), Universitas Gadjah Mada,

Daerah Istimewa (D.I.) Yogyakarta, from August to October 2023. The experimental design used in this study was a complete randomized design (CRD). The treatment consists of two factors: Salt stress and drought stress. The chili pepper plant variety used is TM999 (Menteri Pertanian Republik Indonesia, 2005), considering that the variety is most widely planted by farmers in the D.I. Yogyakarta area and has a tolerant nature in lowland planting areas. The salt treatment level in Murashige and Skoog medium with NaCl (MS-NaCl media) consisted of 0, 20, 40, 60, 80, and 100 mM. Meanwhile, the drought treatment level (MS-PEG-6000 media) consisted of 0, 0.5, 1, 2, 4, and 6%. Each level of treatment was repeated as many as 10 repetitions (10 culture bottles), and every culture bottle consisted of 3 plants. So, the total plants used for both treatments were 360 plants.

**Planting**

Explant planting was carried out by preparing treatment media and sterilizing explants for salt stress treatment media consisting of MS media plus NaCl with a composition per liter of media, namely: 4.43 g MS (Phygenera, Germany), 30 g sucrose (Gulaku Murni, Indonesia), 7 g agar (Swallow Globe Brand, Indonesia), and NaCl (Sigma-Aldrich, USA) for the concentration treatments of 100 ml (20 mM), 200 ml (40 mM), 300 ml (60 mM), 400 ml (mM), and 500 ml (100 mM), respectively. In comparison, the drought stress treatment media consists of MS media plus PEG. The composition of 1 L of media (MS-PEG) consisted of 4.43 g MS (Phygenera, Germany), 30 g sucrose (Gulaku Murni, Indonesia), 7 g agar (Swallow Globe Brand, Indonesia), and PEG-6000 (Merck-Germany) for the treatments of 5 g (0.5%), 10 g (1%), 20 g (2%), 40 g (4%), and 60 g (60%), respectively. The explant sterilization procedure was as follows: chili pepper seeds of the TM999 variety were soaked with sterile aqueous for 30 min to help with imbibition; then, in laminar air flow, seeds were soaked with 70% alcohol (Rachma Sari, Indonesia) for 30 s; then the alcohol was removed and continued by soaking at 20% Clorox (Bayclin, Indonesia) for 30 min while shaking on the shaker. Next, the seeds are soaked in sterile aqueous three times, each for 3 min; The seeds were drained and placed on a Petri dish (filled with water) to ensure the seeds did not dry out during culture. After sterilizing the seeds (explants), they were planted in each treatment medium.

**Parameters Observed**

***Growth Parameters***

***Plant Height.*** Plant height was measured six times during the study: 7 days after planting (1 week after planting [WAP]), 2, 3, 4, 5, and 6 WAP. Measurements were made by measuring from the stem's base to the plant's growing point.

***Plant Leaves Number.*** The number of true leaves was calculated by the time the plant was 6 WAP.

***Plant Roots Length and Number.*** The primary root was measured to obtain the root length data by the time the plant was 6 WAP. For root number, the primary and lateral roots were calculated by the time the plant was 6 WAP.

***Plant Stem Diameter.*** The diameter of the stem was measured at 2-3 cm from the base of the stem using a caliper when the plant was 6 WAP old.

***Physiological Parameters***

Physiological observations were made by measuring the diameter and length of stomata and calculating the density or number of stomata. Stomata morphophysiology was observed using the stomata printing method (Sari & Harlita, 2018). The underside of the leaves was coated with transparent nail varnish. The dried layer of nail varnish was peeled off using sellotape and then stuck onto a glass object. The stomatal openings were observed under a microscope using an ocular micrometer at 10× magnification. The ocular micrometer was calibrated with the objective lens at 40× magnification.

***Dehydrin Gene Expression Analysis.***

The mRNA from the dehydrin gene was isolated using the Total RNA Mini Kit (Plant) w/DNase Set (RPD100, Geneaid Biotech Ltd., Taiwan). The RNA isolation results were then quantified by NanoDrop (Biochrom, United Kingdom). The cDNA synthesis was carried out with the help of Toyobo FSQ-101 ReverTra Ace® qPCR RT kit (Japan). The synthesis of cDNA began with a dilution of extracted RNA according to the RNA quantification value: the formula used was 500 ng/known RNA concentration (ng/µl). Next, a cDNA synthesis cocktail was made with the following composition: 5× RT Buffer (2 µl), primer mix (0.5 µl), enzyme mix (0.5 µl), RNA (depending on the results of quantification calculations needed for dilution), and nuclease-free water adjusted the amount of RNA used with the final volume of the cocktail, which was 10 µl. Furthermore, incubation was carried out with the help of a polymerase chain reaction (PCR) machine with the following reaction: temperature 37ºC for 1 hr, 98ºC for 5 min, and the final temperature of the reaction was 10ºC (∞). After cDNA was successfully synthesized, the next step was the PCR process using a real-time PCR machine (QuantStudio™ 3, Thermo Fisher Scientific, USA). Before PCR, PCR cocktails are prepared using Bioline BIO-94005 SensiFAST SYBR Lo-ROX Kit (Thermo Fisher Scientific, USA) with cocktail composition: 2× SensiFAST SYBR Lo-ROX (10 µl), 10 µM forward primer (0.8 µl), 10 µM reverse primer (0.8 µl), cDNA (1 µl), $H_2O$ (7.4 µl), so that the total final volume was (20 µl). Each sample was to be amplified with both specific primers and control primers and was repeated 3×. The specific primer sequences to amplify the dehydrin gene used (Chen et al., 2015) are (1) forward primer (qCaDHN1-F) 5'-AGTGATCATTCTTTGCTTTTTATTC-3', and (2) reverse primer (qCaDHN1-R) 5'-TTAACTTTCTCACCAAACTCAGA-3'. While the primer control sequence

used (Chen et al., 2015) was (1) forward primer (qCaUbi3-F) 5'-TGTCCATCTGCTCTCTCTCTTG-3', and (2) reverse primer (qCaUbi3-R) 5'CACCCCAAGCACAATAAGAC-3'. The PCR programs used were denaturation for 5 s (95ºC), annealing for 10 s (54 ºC), and extension for 5 s (72ºC). The total number of cycles used was 40. After the PCR results were obtained, the data was analyzed using the Livak method (Schmittgen & Livak, 2008).

### *Data Analysis*

The obtained data were analyzed using analysis of variance with a significance level of 5%, followed by a post hoc honestly significant difference (HSD) Tukey's test to find the significant differences between treatment groups.

## RESULTS AND DISCUSSION

### Growth Response of Chili Pepper Plants in Salt and Drought Treatment Media

The growth response of chili pepper plants in salt stress and drought treatment media (Figures 1A-1B) showed that salt stress treatment had a more significant effect on plant height growth than drought stress treatment in each observation week. The trend graph in both treatments shows a decrease in plant height in accordance with the higher concentrations of NaCl and PEG. The data in Figure 1A also showed that the decrease in plant height at salt treatment (NaCl) with high concentration (100 mM) could reach about twice the height of control plants (0 mM). Unlike the data in Figure 1B, although all plants had reduced PEG concentration levels compared to controls, the decrease was not as significant as that of salt treatment. The height difference between the control and highest PEG concentration plants (6%) was only about 0.56 cm apart. According to Xiao and Zhou (2023), soil salinization is one of the most detrimental environmental stresses, severely restricting plant growth and development and threatening agricultural production worldwide. So, it is not surprising that from the two choking comparisons given in this study, a more significant growth reduction response was shown in plantlets in salt stress media than plantlets in PEG media. According to van Zelm et al. (2020), salt stress causes inhibition of plant growth, abnormal development, and metabolic disorders. Generally, plants that experience salt stress will inhibit water absorption in their body tissues due to the viscosity of tissue fluid. According to Ludwiczak et al. (2021) as well as Yang and Guo (2018), the adverse effects of high salinity in plants are (1) osmotic stress as sodium (Na) accumulation in the soil and (2) ionic stress. Furthermore, osmotic stress is caused by hyperosmotic soil fluid disrupting plant cell pressure. In contrast, ionic stress is characterized by disrupting the sodium or potassium balance in cells, thus disrupting most metabolic and physiological processes (Zhang et al., 2018).

Osmotic stress is also found in plants that experience drought stress. Rao et al. (2006) stated that drought causes plants to experience an increase in osmotic pressure

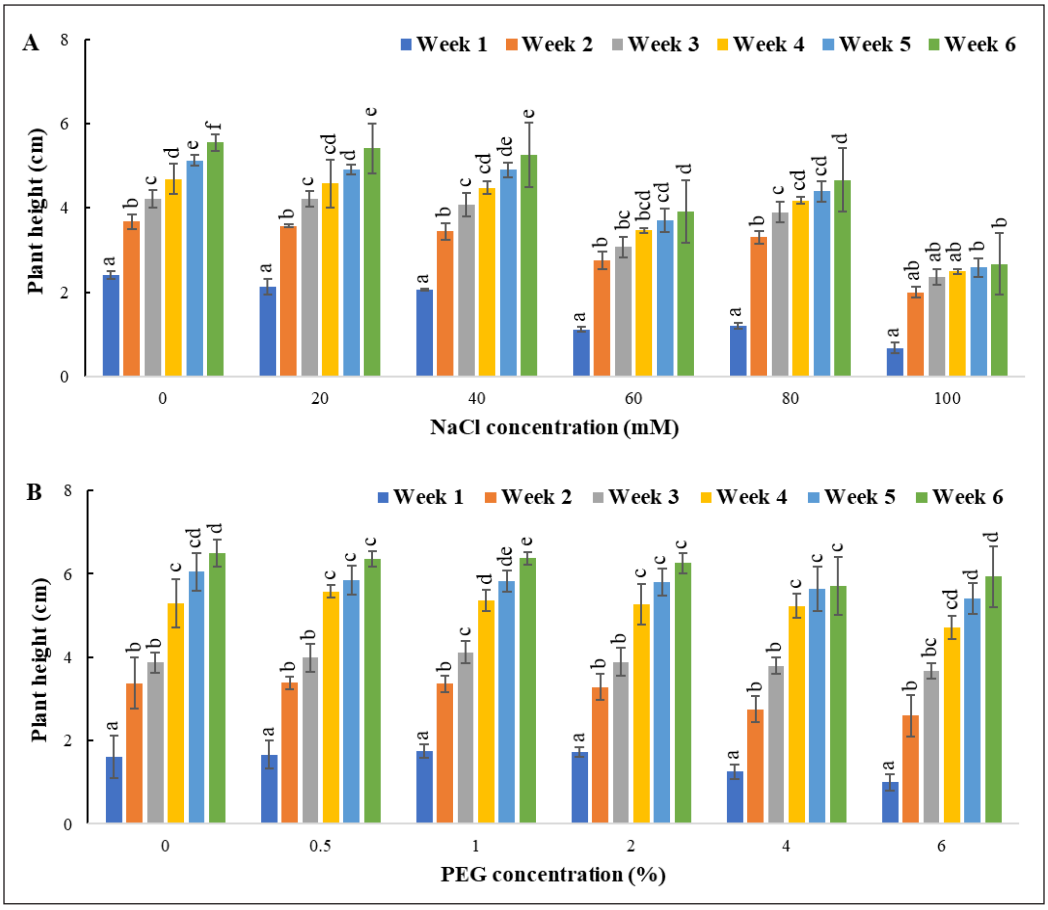


*Figure 1*. The average chili pepper plants' heights under (A) sodium chloride (NaCl) and (B) polyethylene glycol (PEG) exposure using various levels of concentration, respectively
*Note*. Bars showing the same letters at the same concentration level are insignificantly different based on honestly significant difference Tukey's test at α = 5%

and a decrease in cell turgor pressure. Therefore, the response of sample plants in this study, both in salt and PEG stress media, showed a decrease in plant height, which was predicted to occur due to osmotic stress events experienced by these plants. Cushman (2001) supported this, stating that salinity and drought cause osmotic stress inhibiting plant growth. Osmotic pressure or stress is a growth inhibition in the form of a decrease in external water potential as a physiological response of plants to salinity and drought. Lowering the external water potential triggers several major events in plant tissues. For example, at the macroscopic level, osmotic stress inhibits the increase in cells due to decreased turgor pressure (Zhao et al., 2020), so plant cell walls wrinkle and sag (Ma et al., 2020). Poorter et al. (2012) report that when plants experience severe drought stress, they can decrease their biomass by up to 50% compared to control crops, accompanied by an increase in root mass fraction, which

can largely be attributed to a decrease in stem growth. In the face of osmotic stress, plants maintain root growth and reduce shoot growth, especially in the early stages of growth (Ma et al., 2020).

Further plant growth data, namely the number of leaves (Table 1), showed a significant difference between the number of leaves of control plants and sample plants in both treatments (salt and drought), especially in the treatment with the highest concentration. In salt treatment, the number of leaves between the control plant and the highest NaCl concentration treatment plant (100 mM) was two leaves, while the difference in the number of leaves of the control plant with the other four NaCl concentration treatments was only one leaf. The results are also the same in plants with PEG treatment. According to Farooq et al. (2009), drought affects plant growth and development with substantial reductions in the speed of growth and biomass accumulation. With this influence, it is not surprising that the number of leaves of the treatment plant is not more than that of the control plant because of the difference in growth speed, as mentioned earlier. The main consequences of drought in plants are reduced cell division and expansion speed, leaf size, stem elongation, and stem proliferation, disrupting stomatal oscillations, linking plant nutrients and water with reduced plant productivity, and efficient water use. In another mechanism, it is known that plants respond to drought conditions by reducing leaves through leaf shedding to reduce excess transpiration (Seleiman et al., 2021). Unfortunately, this study did not observe the number of fallen leaves. It is related to the large number of plants used, making it difficult to make observations simultaneously as observations of other variables. However, the variable number of leaves significantly differs between control and treatment plants, which has adequately illustrated the plant's response to leaf growth as an influence of both treatments.

Observations on the length and number of plant roots in both treatment media showed that both treatments significantly

Table 1
*The number of leaves of chili pepper plant var. TM999 in salt and drought treatments at 6 WAP*

| NaCl treatment (mM) | Leaf number | | PEG treatment (%) | Leaf number | |
|---|---|---|---|---|---|
| 0 | 5.78 ± 0.83 | a | 0 | 6.22 ± 1.20 | a |
| 20 | 5.11 ± 0.60 | ab | 0.5 | 5.22 ± 0.83 | ab |
| 40 | 4.89 ± 0.60 | ab | 1 | 5.11 ± 0.76 | ab |
| 60 | 4.78 ± 0.83 | ab | 2 | 4.78 ± 0.67 | ab |
| 80 | 4.56 ± 0.88 | ab | 4 | 5.22 ± 0.67 | b |
| 100 | 3.67 ± 2.12 | b | 6 | 4.67 ± 0.71 | b |
| CV (%) | 19.78 | | CV (%) | 16.44 | |

*Note*. Means in the same column followed by the different letters are significantly different at 0.005 probability level; WAP = Weeks after planting; NaCl = Sodium chloride; PEG = Polyethylene glycol; CV = Coefficient of variation

affected the size and number of roots (Table 2). Salt and PEG stress treatments have an impact in the form of increasing root length compared to controls. Different results were found in the variable number of roots, where in salt treatment, the number of roots tended to decrease compared to controls. In PEG treatment, the effect tends to increase the number of roots.

Plants respond to salt stress and drought by increasing root length as self-defense. In growth and development, plants depend on water availability for the metabolic processes of cells and bodies. In nature, in conditions of salt stress and drought, plants make modifications by increasing root length, which is useful as an extension tool in obtaining water at a certain depth of soil or outside the header area. This phenomenon also applies to the results obtained in this study. The root length of the treatment plant exceeds the root length of the control plant to assist the plant in obtaining water at a given depth and area of the medium. As the organ that first senses water shortage, roots feel stress immediately after exposure to drought stress and produce a specific response to the drought stress. Physiologically, drought stress causes changes in some metabolic pathways of plants. The allocation of photosynthates in the roots and rhizosphere is inhibited in severe drought conditions. It reduces the absorption of water and nutrients by the roots, stunts growth, and ultimately affects biomass and plant yield accumulation. In drought stress conditions, a well-developed root system architecture can improve the quality of plant drought

Table 2
*The length and number of roots of chili pepper plant var. TM999 under salt and drought treatment at 6 WAP*

| NaCl treatment (mM) | Root length (cm) | | PEG treatment (%) | Root length (cm) | |
|---|---|---|---|---|---|
| 0 | 6.23 | b | 0 | 8.43 ± 1.87 | b |
| 20 | 9.50 | ab | 0.5 | 5.08 ± 0.75 | c |
| 40 | 9.00 | ab | 1 | 7.39 ± 3.28 | bc |
| 60 | 9.73 | ab | 2 | 8.07 ± 2.32 | bc |
| 80 | 7.94 | ab | 4 | 6.74 ± 2.42 | bc |
| 100 | 10.21 | a | 6 | 13.81 ± 4.24 | a |
| CV (%) | 16.00 | | CV (%) | 16.03 | |
| NaCl treatment (mM) | Number of roots | | PEG treatment (%) | Number of roots | |
| 0 | 9.56 ± 2.79 | a | 0 | 4.67 ± 1.00 | b |
| 20 | 5.22 ± 1.48 | b | 0.5 | 4.89 ± 1.05 | b |
| 40 | 5.00 ± 1.41 | b | 1 | 5.22 ± 1.64 | b |
| 60 | 2.78 ± 0.67 | c | 2 | 6.89 ± 2.03 | ab |
| 80 | 2.56 ± 0.52 | c | 4 | 9.33 ± 3.43 | a |
| 100 | 2.33 ± 0.87 | c | 6 | 6.44 ± 1.67 | ab |
| CV (%) | 14.98 | | CV (%) | 17.39 | |

*Note*. Means in the same column followed by the different letters are significantly different at 0.005 probability level; WAP = Weeks after planting; NaCl = Sodium chloride; PEG = Polyethylene glycol; CV = Coefficient of variation

tolerance and the utilization of limited resources (water and nutrients). Optimized root system architecture can strengthen the properties of plants facing drought stress, including the length and number of roots, to increase the absorption of deeper water sources, ultimately increasing drought tolerance (Kang et al., 2022).

The difference in root count response in salt and PEG treatment plants (Table 2) shows that chili pepper plants of the TM999 variety have different mechanisms in dealing with the two types of stress. In salt stress, plants tend to reduce lateral root growth, although lateral roots are known to be very important for water absorption. It is well known that the effect of salt stress on plants can result in osmotic pressure that inhibits cell division and elongation, so it will automatically also inhibit lateral root growth and reduce their number. In contrast to the number of roots in drought-treated plants that tend to increase compared to control plants, these results show facts that contradict existing theories. In many cases, lateral root growth will be limited to water shortage conditions (Durand et al., 2016). The results of this study suggest that drought stress can increase the number of lateral roots in certain plant species (in this case, chili peppers). In other words, the TM999 chili pepper plant variety used in this study is predicted to have genetic factors that can help its root system deal with drought stress by increasing the number of lateral roots to increase water and nutrient absorption. This phenomenon was also found in another study by Chun et al. (2021) on soybean plants.

Furthermore, this study also found a noticeable influence exerted by both treatments on stem diameter (Table 3). The higher the treatment concentration given, the smaller the diameter of the plant stem. As previously stated, salinity and drought treatment will reduce the weight of plant mass, including inhibition of stem growth through decreasing the number and size of cells.

Stomatal morphophysiological data (Table 4) showed that salt stress treatment had a significant effect on stomatal length

Table 3
*The stem diameter of the chili pepper plant var. TM999 under salt and drought treatment at 6 WAP*

| NaCl treatment (mM) | Stem diameter (cm) | | PEG treatment (%) | Stem diameter (cm) | |
|---|---|---|---|---|---|
| 0 | 0.83 ± 0.05 | a | 0 | 0.81 ± 0.07 | a |
| 20 | 0.72 ± 0.67 | b | 0.5 | 0.83 ± 0.15 | a |
| 40 | 0.68 ± 0.04 | b | 1 | 0.79 ± 0.12 | a |
| 60 | 0.57 ± 0.10 | c | 2 | 0.71 ± 0.12 | a |
| 80 | 0.54 ± 0.10 | c | 4 | 0.69 ± 0.09 | a |
| 100 | 0.42 ± 0.08 | d | 6 | 0.49 ± 0.18 | b |
| CV (%) | 11.92 | | CV (%) | 17.75 | |

*Note*. Means in the same column followed by the different letters are significantly different at 0.005 probability level; WAP = Weeks after planting; NaCl = Sodium chloride; PEG = Polyethylene glycol; CV = Coefficient of variation

and had no noticeable impact on stomatal diameter (width) and stomatal density (number). In contrast to drought stress treatment plants, a noticeable influence is not only shown on the length of the stomata but also the diameter (width) of the stomata. Meanwhile, the density (number) of stomata does not influence the provision of drought stress treatment. In order, the three treatment plants with the highest to lowest stomata length in NaCl treatment were 20, 0 (control), and 100 mM concentration, with their respective lengths of 13.97, 9.7, and 5.72 µm. These results indicate that NaCl treatment at a certain concentration (20 mM) can increase the stomata length of chili pepper plants var. TM999. Meanwhile, more than 20 mM NaCl concentrations can decrease stomatal length compared to control plants. Not much different from the size of the average diameter of the stomata of PEG treatment plants, consistently all PEG concentrations tried had an effect in the form of a decrease in stomatal length in line with the higher PEG concentrations used. Based on available data, the length of stomata in PEG treatment plants is quite variable, where the longest stomata are 11.07 µm (0% PEG), and the shortest stomata are found in PEG treatment concentrations of 2% (6.53 µm). However, the stomata diameter length of certain PEG concentration treatment plants (0.5 and 6%) has a longer stomata length when compared to the stomata length of other concentration treatment plants (PEG >0.5%<6%).

Varying yields were also found in the stomata diameter of PEG treatment plants. If

Table 4
*The observation of stomata of chili pepper plant var. TM999 under salt and drought treatment at 6 WAP*

| NaCl treatment (mM) | Stomata diameter (µm) | | Stomata length (µm) | | Stomata density (n/mm$^2$) | |
|---|---|---|---|---|---|---|
| 0 | 12.07 | a | 9.70 | b | 367.00 | a |
| 20 | 14.70 | a | 13.97 | a | 223.50 | a |
| 40 | 12.69 | a | 4.73 | c | 239.00 | a |
| 60 | 14.80 | a | 4.45 | c | 202.50 | a |
| 80 | 11.96 | a | 4.31 | c | 106.50 | a |
| 100 | 12.42 | a | 5.72 | c | 163.50 | a |
| CV (%) | 17.10 | | 20.47 | | 8.13 | |
| PEG treatment (%) | Stomata diameter (µm) | | Stomata length (µm) | | Stomata density (n/mm$^2$) | |
| 0 | 14.10 | a | 11.07 | a | 259.00 | a |
| 0.5 | 15.32 | a | 9.36 | a | 309.00 | a |
| 1 | 9.66 | b | 7.11 | b | 87.50 | a |
| 2 | 13.33 | a | 6.53 | b | 312.00 | a |
| 4 | 10.03 | b | 6.91 | b | 322.00 | a |
| 6 | 9.61 | b | 9.61 | a | 155.50 | a |
| CV (%) | 14.78 | | 18.07 | | 7.03 | |

*Note*. Means in the same column followed by the different letters are significantly different at 0.005 probability level; WAP = Weeks after planting; NaCl = Sodium chloride; PEG = Polyethylene glycol; CV = Coefficient of variation

the stomata diameter range of salt treatment plants is relatively narrow, which is between 11.96 and 14.80, then the stomata diameter range of PEG treatment plants is quite wide, which is 9.61 to 15.32. Variations in stomatal diameter data in PEG treatment illustrate that plants respond in the form of a decrease in stomatal width or diameter compared to controls. The reduction in stomata size (diameter and length) in treatment plants compared to stomata size in control plants indicates that plants respond to salt stress and drought through decreased physiological activity, namely transpiration. The size of stomata that are smaller than their normal size is believed to avoid water loss quickly (Li et al., 2017). However, the size of the stomata is affected by the treatment, not by the number or density of stomata. Stomata are needed plants for gas exchange. Generally, the amount or density is greatly influenced by the amount of carbon dioxide ($CO_2$) available in the plant-growing environment. If enough $CO_2$ is available, the number of stomata is not greater than when there is less $CO_2$ available in the air (Xu et al., 2016). Therefore, this study's salt and drought treatment did not affect stomatal density.

The salt stress and drought treatment results in this study showed that chili pepper plants of the TM999 variety gave a dominant morphological growth response (Figure 2) affected by both treatments rather than their physiological growth response (stomata morphophysiology). It can happen with the suspicion that organ modifications carried out by chili pepper plants of the TM999 variety in the form of an increase in the length and number of roots followed

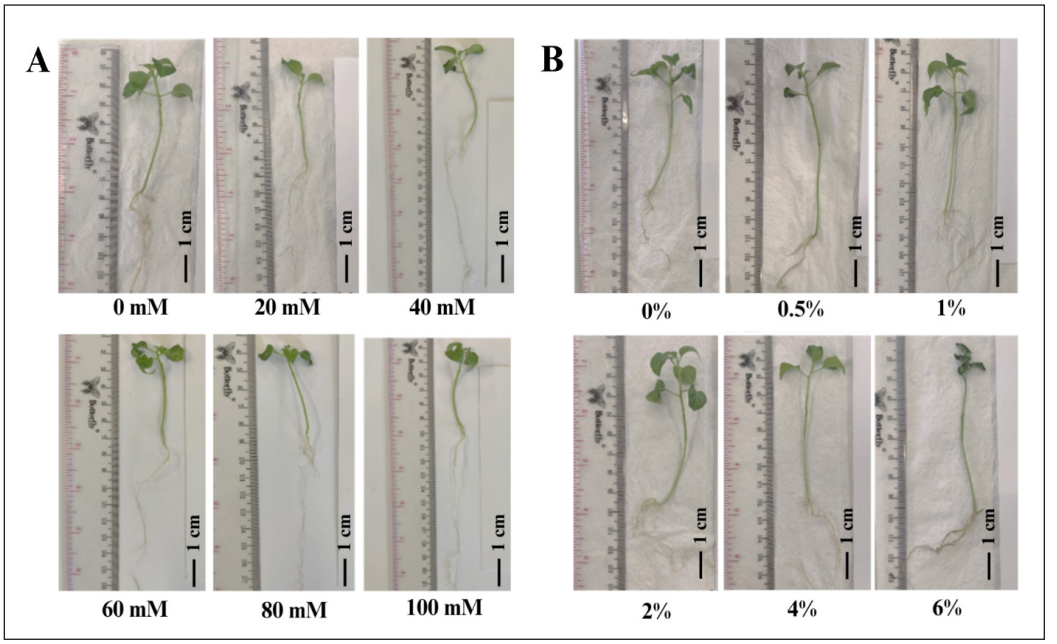


*Figure 2*. Chili pepper plants' morphological documentation under different abiotic stress conditions at 6 weeks after planting: (A) saline stress; (B) drought stress

by a reduction in plant height, number of leaves, stem diameter, and stomata size due to the two treatments given, turned out to be able to help plants in carrying out their physiological activities to remain in normal conditions.

Growth is an irreversible volume, size, or weight increase, including the phases of cell division, cell elongation, and cell differentiation, in which cell division and enlargement are affected by drought stress conditions due to disruption of enzyme activity, turgor loss, and decreased energy supply (Farooq et al., 2009). In conditions of salt stress and drought, there will be a decrease in dry matter accumulation in all plant organs. However, different organs show different levels of decline according to the data obtained in this study. Other studies also add information that drought stress causes a decrease in leaf area and leaf count due to turgor loss (Farooq et al., 2010).

**Dehydrin Gene Expression Analysis**

The tolerant nature of chili pepper plants of the TM999 variety against salt stress and drought in this study cannot be separated from its ability to increase the production of dehydrin compounds. Dehydrin is an intrinsically irregular protein belonging to one of the late embryogenesis abundant (LEA) family of genes. LEA is a protein that plants express as one of the molecular mechanisms in response to abiotic stress. *In vitro* evidence shows that dehydrin is involved in the protection of membranes, proteins, and DNA from abiotic stress. So, dehydrin is considered an abiotic stress-protective protein with multiple roles (Smith & Graether, 2022).

The dehydrin gene expression analysis results in plants from both treatment types showed a significant increase in expression compared to control plants. Figure 3A shows the highest to lowest increase in dehydrin gene expression in NaCl treatment with concentrations of 20, 100, 60, and 40 mM. While in NaCl treatment concentration of 80 mM, there was a decrease in dehydrin gene expression. It is not yet known why a concentration of 80 mM NaCl can decrease the expression of the dehydrin gene of red pepper plants var. TM999 in this study, compared to other concentration levels, tends to increase the expression of the gene. However, this is thought to occur because the increase in dehydrin gene expression is largely determined by the control of interactions between genes and other molecules that are influenced by the concentration of the abiotic stress inducer given. Therefore, no linear pattern was found between increased NaCl concentrations and dehydrin gene expression. A similar result to this study has been found in the research of Alharby et al. (2016), which studied mRNA expression of *superoxide dismutase* (*SOD*) and *glutathione peroxidase* (*GPX*) genes in tomatoes under stress NaCl and/or zinc oxide (ZnO) nanoparticles. Based on Alharby et al. (2016) research, which investigated the effect of nanoparticles of zinc oxide (NPs-ZnO) on the expression mRNA levels of *SOD* and *GPX* genes under salinity stress and confirmed that

a decrease in mRNA expression of *SOD* and *GPX* genes occurred during exposure to NaCl. In contradictive, Alharby et al. (2016) research has also found that a low and/or appropriate dose of NPs-ZnO has a positive response on plant metabolism to increase the expression of mRNA levels of the *SOD* and *GPX* genes in tomatoes under salinity. Another assumption was that the increases in the mRNA levels of *SOD* and *GPX* genes could be a result of increased stability of transcribed mRNAs. Further research was needed in this study to reveal the other molecules that play an important role in affecting the decreasing and increasing of DHN1 mRNA expression levels under salinity. These other molecules could be predicted from the MS medium content used in this study.

In contrast to the results obtained in the PEG treatment (Figure 3B), a linear pattern was found between an increase in the concentration of PEG used and an increase in the expression of the dehydrin gene. The highest to lowest increases in dehydrin gene expression in PEG treatment were 6, 2, 1, and 4%, respectively. At a PEG concentration of 0.5%, there was a decrease in dehydrin gene expression. The lowest increase in dehydrin gene expression at a PEG concentration level of 4% further strengthens previous predictions that increased dehydrin gene expression is largely determined by control of interactions between genes and other molecules. Although higher eukaryotes still respond to environmental signals by regulating their genes, there is an additional layer of regulation resulting from cell-to-cell interactions that regulate the development of an organism. In the end, although there was a variation in the increase and decrease in dehydrin gene expression between concentration treatment levels of both NaCl

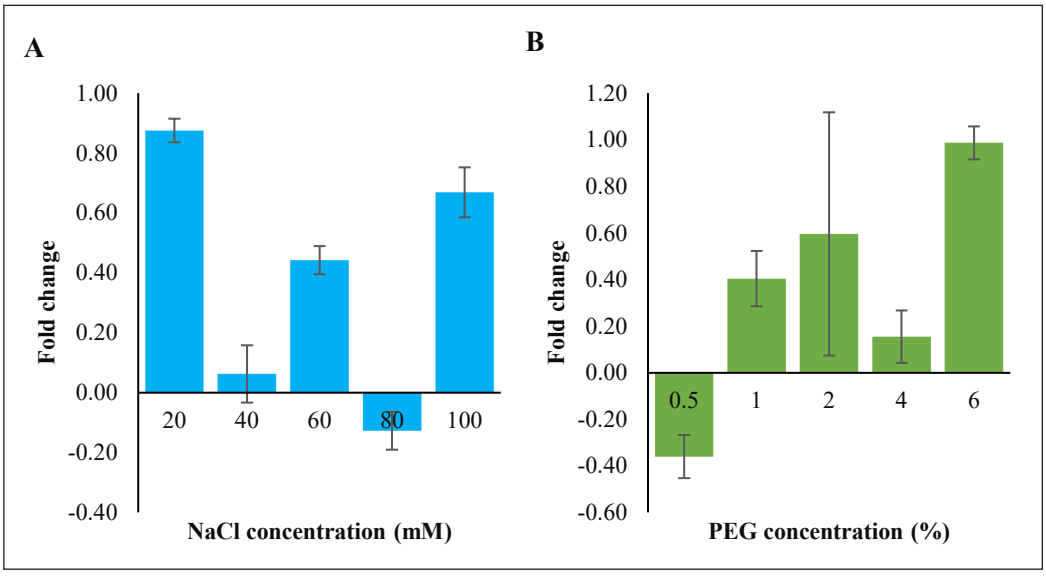


*Figure 3*. Dehydrin gene expression analysis under different abiotic stress conditions at 6 weeks after planting: (A) sodium chloride (NaCl) treatment; (B) polyethylene glycol (PEG) treatment

and PEG, it did not change the fact that salt and drought stress applied were able to significantly increase the expression of the dehydrin gene in the treatment plants compared to the respective control plants. The high expression of the dehydrin gene in chili pepper plants of the TM999 variety in both treatments (NaCl and PEG) proved the role of dehydrin compounds in helping to increase plant resistance to salt stress and drought when associated with morphological and physiological growth responses. The average morphological and physiological growth response of treatment plants in this study is classified as able to catch up or be parallel to the growth of control plants, especially in certain observation variables that show no real influence between treatment plants and control plants.

## CONCLUSION

The chili pepper plants of the TM999 variety are tolerant to both types of stress treatments tried, especially drought stress, and have the potential to increase their resistance through molecular approaches or over-expression of dehydrin genes with a gene editing method.

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