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Research Article

Drought Tolerance in Transgenic Potato Cultivar IPB CP1 Expressing *MmCuZn-SOD* Gene

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ABSTRACT

Drought stress induces reactive oxygen species (ROS) accumulation, which is harmful to plant cells. Consequently, it inhibits plant growth and decreases yield. The enzyme superoxide dismutase (SOD), which is encoded by the *SOD* gene, is the first defence enzyme in the cells that detoxify ROS. The study aimed to analyze the tolerance of transgenic potato cultivar IPB CP1 and its yield under drought-stress conditions. The results showed that transgenic plants had superior morphological characteristics, such as plant height, number of leaves, stem diameter, and plant biomass, than non-transgenic plants. However, photosynthetic rate, chlorophyll content, stomatal conductance, and transpiration rate showed similar levels between the transgenic and non-transgenic plants. The transgenic plants expressing the *MmCu/Zn-SOD* gene showed lower lipid peroxidation levels than the non-transgenic plants, indicating that the gene works well to reduce the cell's ROS level. Transgenic plant clone CP1S6 showed 13 times higher gene expression and tuber yield than non-transgenic plants. These research indicated that the transgenic plants expressing the *MmCu/Zn-SOD* gene are more tolerant to drought stress than the non-transgenic plants.

1. Introduction

Global climate change can impact food crop agriculture, affecting food availability. The changes in rainfall patterns alter water availability, thus leading to drought stress that negatively affects plant growth and development, which consequently reduces plant production (Yordanov *et al.* 2000). Plants under drought stress will have limited water supply, reduced stomatal conductance on CO_2, and increased transpiration (Anjum *et al.* 2011).

Drought stress leads to the formation of excessive Reactive Oxygen Species (ROS), such as $^1O2$ (singlet oxygen), H_2O_2 (hydrogen peroxide), O_2•− (superoxide radical), and OH• (hydroxyl radical), that are very harmful for plants (Gill *et al.* 2010). ROS causes cell damage, protein degradation, enzyme inactivation, changes in gene expression, and disrupts metabolic processes in plants (Choudhury *et al.* 2013). Increased ROS in plants affected by drought stress can cause a decrease in root growth, plant height, relative water content, and chlorophyll content (Zhanassova *et al.* 2021). Silencing the lipocalin gene in tomato leads to increased accumulation of reactive oxygen species (ROS), specifically H_2O_2 and O_2•−. This affects various plant phenotypes, including flowers, fruits, calyxes, and mature tomato fruits (Wahyudi *et al.* 2018).

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Plants develop defense mechanisms against stress through ROS detoxification. Superoxide dismutase (SOD) is one of the main antioxidant enzymes in plant cells that can counteract oxidative stress and free radicals. Superoxide dismutase catalyzes the change of harmful superoxide radical (O_2•−) to oxygen and hydrogen peroxide (del Río *et al*. 2018). Transgenic tobacco plants expressing the SOD gene tolerant to drought stress characterized by high water use efficiency and better photosynthesis rates (Faize *et al*. 2011). Transgenic cassava plants have higher SOD and catalase activities than the wild type when exposed to low temperatures and drought stress (Xu *et al*. 2013).

Potatoes are an essential plant in the world that contribute to food security. Public demand for potatoes continues to increase due to the increased social economy and diet. The potato is a relatively sensitive plant to drought. Drought stress on potato plants negatively affects plant growth, e.g., plant height, stem diameter, number of roots, and plant weight (Albiski *et al*. 2012), production (Schafleitner *et al*. 2007), and size and number of tubers (Eiasu *et al*. 2007). Potato cultivation on dry land is prone to water shortage conditions that cause drought stress. Utilizing varieties that are tolerant to drought stress is a measure to solve such a problem.

The potato cv. IPB CP1 potato variety is a suitable raw material for industrial potatoes, such as the potato chip industry. Previous research was carried out on the genetic engineering of potato by introducing the *MmCuZn-SOD* gene into the genome of the potato cv. IPB CP1 (Musawira *et al*. 2022). The transgenic potato cv. IPB CP1 is expected to express the SOD gene and tolerant to drought stress while maintaining high yields. This study aimed to analyze the tolerance of transgenic potato plants expressing the *MmCuZn-SOD* gene to drought stress.

2. Materials and Methods

2.1. Research Location and Plant Materials

The study was conducted from January 2023 to December 2023. The laboratory experiment was conducted at the Plant Cell and Tissue Engineering Laboratory, Biotech Center, and the Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University. The field and greenhouse experiment was carried out at the Bumi Agro Technology Experimental Field Station, Cisarua Village, Kertawangi District, West Bandung Regency.

The plant materials used in this experiment were G0 tubers of the transgenic potato cv. IPB CP1 namely CP1S2, CP1S3, CP1S4, CP1S5, CP1S6, and non-transgenic potato cv. IPB CP1 plants (Musawira *et al*. 2022).

2.2. Experimental Design and Drought Stress Treatment

The factorial experiment employed a Randomized Block Design with three replications. The first factor was the potato genotype comprising six genotypes, i.e., CP1, CP1S2, CP1S3, CP1S4, CP1S5, and CP1S6. The second factor was drought stress treatment, consisting of drought stress and without drought stress (control) treatments.

Potato tubers (bulbs) were planted in polybags containing a mixture of cocopeat and manure in a ratio of 4:1 (w/w). In each polybag, 3 bulbs were planted, and then one plant per polybag was maintained. The water content of the planting media was maintained at field capacity until 30 days after planting. The drought stress treatment was administered by intermittently stopping the plant watering for 30 days with watering intervals every 10 days. Regular watering of potato plants was carried out again after 60 days after planting. For the control treatment (normal watering), the plant was watered every two days during the planting period. Soil moisture of the plant growing media was measured every day during stress treatment using Handheld VT-05 Soil Moisture Meter (Guangzhou Juanjuan Electronic Technology, China).

2.3. Chlorophyll Content Analysis

Chlorophyll was extracted from leaves using 80% acetone, following a method by Quinet *et al*. (2012). The absorbance of the chlorophyll extract was measured using a Vis spectrophotometer JenwayTM 7315 (Keison, UK) at 470, 663, and 646 nm wavelengths. The chlorophyll content was calculated following Lichtenthaler (1987) as follows:

$$[\mathrm{Chl}]\ (\mathrm{mg/g\ FW}) = (7.15\times A663) + (18.71\times A646)$$

2.4. Photosynthesis Rate Measurement

Photosynthesis rate, stomatal conductance, and transpiration rate were measured using Portable Photosynthesis System LICOR type LI-6400XT (LI-COR, USA) on young leaves that have reached their maximum size on seven-week-old potato plants after planting. The measurement followed manual instruction under radiation intensity 1,000 µmol/m^2/s.

2.5. Lipid Peroxidation Analysis

Lipid peroxidation was measured based on malondialdehyde (MDA) content. The MDA content in leaf samples was analyzed, following Du *et al*. (2010) with slight modifications. The MDA absorbance value was measured using a Vis spectrophotometer at 450, 532, and 600 nm wavelengths with 0.5% TBA in 5% TCA as a blank. MDA concentration was measured based on the method of Wang *et al*. (2013) as follows:

$$\begin{array}{c}\text{CMDA}\\ (\mu\text{mol/ FW})\end{array} = [6.45\times(\text{A532-A600})] - [0.56\times\text{A450}]$$

2.6. Gene Expression Analysis

The total RNA of transgenic and non-transgenic plants was isolated using buffer TRIzol® Reagent (Invitrogen, USA). The quality of the RNA was then observed using a 1% agarose electrophoresis gel in 1× MOPS buffer, and the quantity of the RNA was measured using a MaestroNano Pro Spectrophotometer (MaestroGen, Taiwan). cDNA synthesis was done using ReverTra Ace™ qPCR RT Master Mix with gDNA Remover (TOYOBO, Japan). Gene expression analysis was done using the qRT-PCR technique using ThunderBirdTM Next SYBR® qPCR Mix (TOYOBO, Japan). Amplification was carried out using actin primers as an internal control (Forward, Tact-qF: 5'-ACATCGTCCTTAGTGGTGGA-3'; reverse, Tact-qR: 5'-GTGGACAATGGAAGGACCAG-3') and specific primers to amplify the MmCu/Zn-SOD gene (Forward, SOD-qF: 5'-CCATCACAGACAAGCAGATTCC-3'; reverse, SOD-qR: 5'-GCCACCCTTTCCGAGATCATC-3'). The target sequence amplification process was carried out by Applied Biosystems™ QuantStudio™ 5 Pro Real-Time PCR System machine (Thermo Fisher Scientific, US) using an amplification program consisting of a denaturation stage of 95°C for 10 seconds, annealing 57°C for 30 seconds, and extension 72°C for 40 seconds for 40 cycles. The relative expression of the *MmCu/Zn-SOD* gene was calculated based on the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

2.7. Data Analysis

The data obtained were analyzed using a two-way ANOVA test using SPSS software. If the ANOVA test results provide a significant difference, then a further DMRT test is carried out at a confidence level of 5%.

3. Results

3.1. Morphological Response of Transgenic IPB CP1 Potato Plants under Drought Stress Conditions

The observations of the morphological response of transgenic and non-transgenic IPB CP1 potato plants were carried out after the plants aged 6 to 11 weeks after planting. Soil moisture of the plant growing media was measured from the first day of the treatment applied until the 10th day after drought stress treatment. The soil moisture of drought-stressed soil media significantly decreased to 43% on the last day of treatment in each period of intermittent drought stress (Figure 1), which indicates that the drought treatment could reduce the supply of water to the plant.

The research showed that there was no significant difference in morphological characteristics between transgenic and non-transgenic plants when grown in the control treatment. Meanwhile, drought stress treatment significantly affected the growth of transgenic and non-transgenic plants, with a more significant reduction occurring in non-transgenic plants. Transgenic plants had higher plant height, number of leaves, number of nodes, and stem diameter than non-transgenic plants under drought stress conditions. Clones CP1S5 and CP1S6 had the highest values in those four morphological characters observed (Table 1).

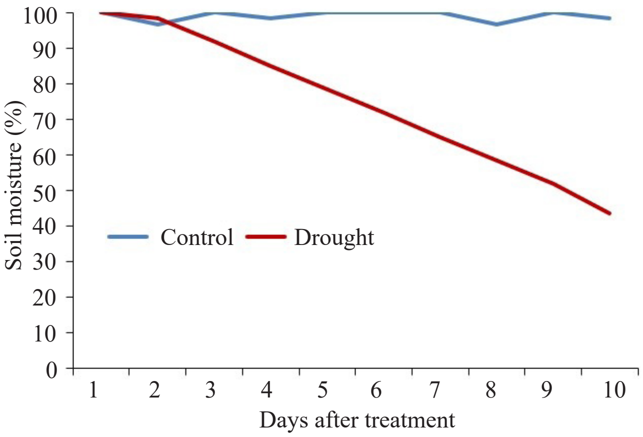


Figure 1. The soil moisture of the plant growing media under control and drought stress treatment at ten days of the treatment

Despite the reduction in plant morphological characters, drought stress also affected the plant biomass weight of transgenic and non-transgenic plants. However, transgenic plants showed less reduction in plant biomass weight than non-transgenic plants. There was no significant difference in fresh and dry biomass weight between transgenic dan non-transgenic plants under control conditions. However, some transgenic plants produced significantly higher fresh and dry biomass than non-transgenic plants under drought stress. The CP1S6 clone had higher shoot and root weight than non-transgenic and other transgenic plants (Table 2). The results are in accordance with the research on tomato and spinach plants which have high SOD enzyme activity under drought conditions and have a higher total biomass compared to plants which have low SOD enzyme activity. The transgenic plants appear healthy under normal watering and drought stress conditions. In contrast, the non-transgenic plants showed more wilting compared to the transgenic plants under drought stress (Figure 2).

3.2. Physiological Response of Transgenic Potato cv. CP1 IPB to Drought Stress

Analysis of chlorophyll content showed that drought stress decreased leaf chlorophyll content in transgenic and non-transgenic plants. However, the decrease in leaf chlorophyll content of transgenic plants was less than that of non-transgenic plants, and the chlorophyll content in all transgenic plants was much higher compared to the non-transgenic plants. The result also showed that there was a significant difference in the chlorophyll content between transgenic and non-transgenic plants under drought stress conditions. The average chlorophyll content of all transgenic plants under drought stress condition was 12.4 mg/g, while the chlorophyll content of non-transgenic plant was 9.0 mg/g.

The *MmCuZn-SOD* gene caused the transgenic plants to adapt better to drought stress through the stabilization of the photosynthesis rate, stomatal conductance, and transpiration rate. Among the five clones of transgenic plants, only clone CP1S2 showed a significant reduction in photosynthetic rate, stomatal conductance, and transpiration rate under drought stress treatment. In contrast, other clones did not experience significant changes in both variables, even though they showed higher photosynthetic rate and stomatal conductance than the non-transgenic plant under control conditions. The result showed that the CP1S6 clone had the highest photosynthesis rate and stomatal conductance compared to the other transgenic and non-transgenic plants (Table 3).

Drought stress induces the lipid peroxidation of the cell membranes, which is represented by an increased level of MDA content. Analysis of leaf MDA contents showed an increase in MDA content in drought-stressed plants (Table 4). The increase in MDA content in non-transgenic plants was greater than the increase in transgenic plants,

Table 1. Effect of drought stress on plant height, number of leaves, number of nodes, and shoot diameter of transgenic and non-transgenic potato cv. IPB CP1 at 6 weeks after planting

Clones	Plant height (cm)		Number of leaves		Number of nudes		Stem diameter (mm)	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought
CP1	40.8^{b}	28.0^{d}	13.5^{a}	6.0^{c}	14.5^{bc}	7.8^{e}	5.75^{ab}	4.1^{c}
CP1S2	45.0^{ab}	31.0^{cd}	15.0^{a}	10.2^{b}	16.2^{ab}	10.2^{d}	5.9^{ab}	5.0^{bc}
CP1S3	43.3^{ab}	42.3^{ab}	15.8^{a}	10.3^{b}	16.8^{a}	11.5^{d}	6.0^{ab}	5.5^{ab}
CP1S4	43.5^{ab}	35.2^{c}	16.7^{a}	10.0^{b}	17.2^{a}	11.2^{d}	6.0^{ab}	5.3^{ab}
CP1S5	43.5^{ab}	47.7^{a}	15.0^{a}	11.3^{b}	16^{ab}	11.8^{d}	6.2^{a}	5.9^{ab}
CP1S6	42.5^{ab}	45.0^{ab}	14.7^{a}	10.7^{b}	15.8^{ab}	13.7^{d}	6.2^{a}	6.1^{ab}

The values followed by the same letter in the same variable are not significantly different according to the DMRT's test at $\alpha = 0.05$

Table 2. Effect of drought stress on plant biomass weight of transgenic and non-transgenic potato cv. IPB CP1 at 6 weeks after planting

Clones	Fresh shoot weight (g)		Dry shoot weight (g)		Fresh Root Weight (g)		Dry Root Weight (g)	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought
CP1	32.9^{a}	15.4^{c}	5.9^{ab}	2.7^{e}	2.3^{bc}	1.0^{d}	1.5^{ab}	0.6^{e}
CP1S2	33.7^{a}	21.0^{bc}	5.8^{ab}	3.1^{de}	2.8^{abc}	1.1^{d}	1.2^{abcd}	0.8^{de}
CP1S3	33.2^{a}	23.5^{b}	5.7^{ab}	4.3^{bcd}	2.8^{abc}	2.1^{c}	1.3^{abc}	0.9^{cde}
CP1S4	33.7^{a}	21.7^{b}	6.0^{a}	4.0^{cde}	2.5^{abc}	1.3^{d}	1.4^{abc}	0.8^{de}
CP1S5	34.9^{a}	28.8^{b}	6.1^{a}	5.2^{abc}	2.8^{abc}	2.2^{bc}	1.5^{ab}	1.1^{bcd}
CP1S6	36.0^{a}	30.3^{b}	6.0^{a}	6.7^{a}	3.1^{a}	2.8^{ab}	1.7^{a}	1.4^{ab}

The values followed by the same letter in the same variable are not significantly different according to the DMRT's test at $\alpha = 0.05$

CP1 CP1S5 CP1 CP1S5

(A) (B)

Figure 2. Transgenic plant performance under (A) control and (B) drought stress conditions at 6 weeks after planting. CP1 = non-transgenic, CP1S5 = transgenic clone. Bar = 10 cm

Table 3. Effect of drought stress on chlorophyll total, photosynthesis rate, stomatal conductance, transpiration rate of transgenic and non-transgenic potato cv. IPB CP1 at 6 weeks after planting

Clones	Chlorophyll total (mg/g)		Photosynthesis Rate (µmol $CO_2/m^2/s$)		Stomatal Conductance (mol $H_2O/m^2/s$)		Transpiration Rate (mol $H_2O/m^2/s$)	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought
CP1	15.3^{abc}	9.0^{e}	18.0^{bc}	15.9^{d}	1.3^{a}	0.7^{b}	1.9^{ab}	1.1^{d}
CP1S2	15.6^{ab}	12.1^{d}	20.3^{ab}	16.1^{cd}	1.4^{a}	0.8^{b}	2.0^{a}	1.2^{d}
CP1S3	15.3^{abc}	12.6^{cd}	18.8^{ab}	19.6^{ab}	1.7^{a}	1.3^{a}	1.9^{ab}	1.6^{abc}
CP1S4	15.4^{abc}	12.4^{cd}	20.4^{a}	18.5^{ab}	1.6^{a}	1.4^{a}	1.8^{ab}	1.4^{bcd}
CP1S5	15.6^{ab}	12.7^{bcd}	20.2^{a}	19.8^{ab}	1.5^{a}	1.5^{a}	1.8^{ab}	1.6^{abc}
CP1S6	16.0^{a}	12.6^{bcd}	20.4^{a}	20.3^{a}	1.8^{a}	1.7^{a}	1.8^{ab}	1.8^{abc}

The values followed by the same letter in the same variable are not significantly different according to the DMRT's test at α =0.05

Table 4. Effect of drought stress on leaf MDA content of transgenic and non-transgenic potato cv. IPB CP1 at 6 weeks after planting

Clones	MDA content (µmol/g)	
	Control	Drought
CP1	0.14^{c}	0.46^{a}
CP1S2	0.14^{c}	0.46^{a}
CP1S3	0.12^{c}	0.31^{abc}
CP1S4	0.18^{c}	0.40^{ab}
CP1S5	0.15^{c}	0.20^{bc}
CP1S6	0.16^{c}	0.21^{a}

indicating that transgenic plants have better drought stress tolerance than non-transgenic plants. Clones CP1S5 and CP1S6 had lower MDA contents than other clones, and it was not significantly different with MDA content in control conditions, indicating less lipid peroxidation and fewer ROS formed during the onset of drought stress.

3.3. Tuber Yield of Transgenic Potato cv. CP1 IPB under Drought Stress

IPB CP1 potato tubers were harvested 13 weeks after planting. Under control conditions without drought stress treatment, the number and weight of tubers did not differ significantly among genotypes. Drought stress significantly reduced the number and weight of tubers in transgenic plants and non-transgenic plants. Still, the reduction was higher in non-trangenic plants compared to the transgenic plants. The number of tuber yield of transgenic plant clones CP1S5 and CP1S6 was almost 2 times higher than that of non-transgenic CP1 plants under drought stress (Table 5, Figure 3). Clones CP1S5 and CP1S6 produced a similar number of tubers with the non-transgenic plant under control conditions without drought stress.

3.4. Expression of the *MmCu/Zn-SOD* Gene in Transgenic Potato cv. IPB CP1

Expression analysis of SOD gene showed that transgenic plants expressed the gene higher than non-transgenic plant in both control and drought stress conditions. The CP1S6 clone showed the highest gene expression, about 24 and 13 times higher than non-transgenic plant under control and drought stress condition, respectively (Figure 4). All transgenic clones expressed the gene in a similar pattern between control and drought conditions.

The activity of SOD and catalase enzymes was also analyzed in transgenic and non-transgenic plants. Statistical data analysis indicated that the transgenic plants exhibited higher activity levels of SOD and catalase enzymes compared to non-transgenic plants (Figure 5). The CP1S6 clone demonstrated the highest activity levels of SOD and catalase enzymes compared to the other clones and non-transgenic plant.

Table 5. Effect of drought stress on tuber yield of transgenic and non-transgenic potato cv. IPB CP1 at 11 weeks after planting

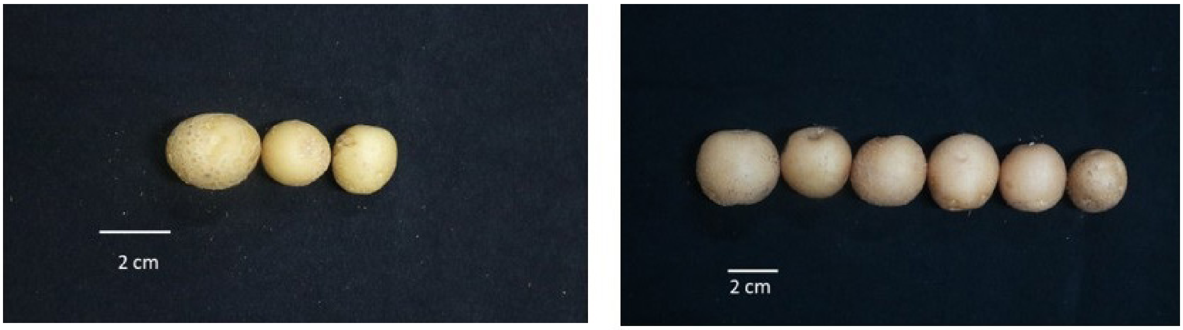
Clones	Number of tuber		Tuber weight (g)	
	Control	Drought	Control	Drought
CP1	7.0^{ab}	3.3^{d}	27.7^{a}	16.6^{c}
CP1S2	7.7^{ab}	4.3^{cd}	28.0^{a}	19.9^{bc}
CP1S3	8.0^{a}	5.3^{bc}	27.7^{a}	22.5^{b}
CP1S4	7.3^{a}	4.3^{cd}	28.5^{a}	20.4^{b}
CP1S5	8.3^{a}	6.3^{abc}	27.9^{a}	22.9^{b}
CP1S6	8.3^{a}	6.3^{abc}	29.5^{a}	21.9^{b}

4. Discussion

Drought stress affects morphological and physiological characteristics as well as tuber yield of potato plants. Drought can particularly affect plant growth by reducing plant height, stem diameter, and the number of shoots in potato plants (Chang *et al*. 2018; Pourasadollahi *et al*. 2019). The limited water supply to plants could disrupt cell elongation, leading to a decrease in internode length (Litvin *et al*. 2016). In drought stress conditions, plants tend to reduce shoot biomass to maintain optimal root absorption capacity and water use efficiency (Tátrai *et al*. 2016). Furthermore, a decrease in plant biomass weight is observed as one of the plant responses to drought stress. Non-transgenic plants experience a significant decrease in plant biomass weight under drought stress conditions. The drought stress can also reduce potato

CP1 CP1S5

(A)



CP1 CP1S5

(B)

Figure 3. Tuber yield of transgenic and non-transgenic potato produced under (A) control and (B) drought stress conditions. CP1 = non-transgenic, CP1S5 = transgenic clone. Bar = 2 cm

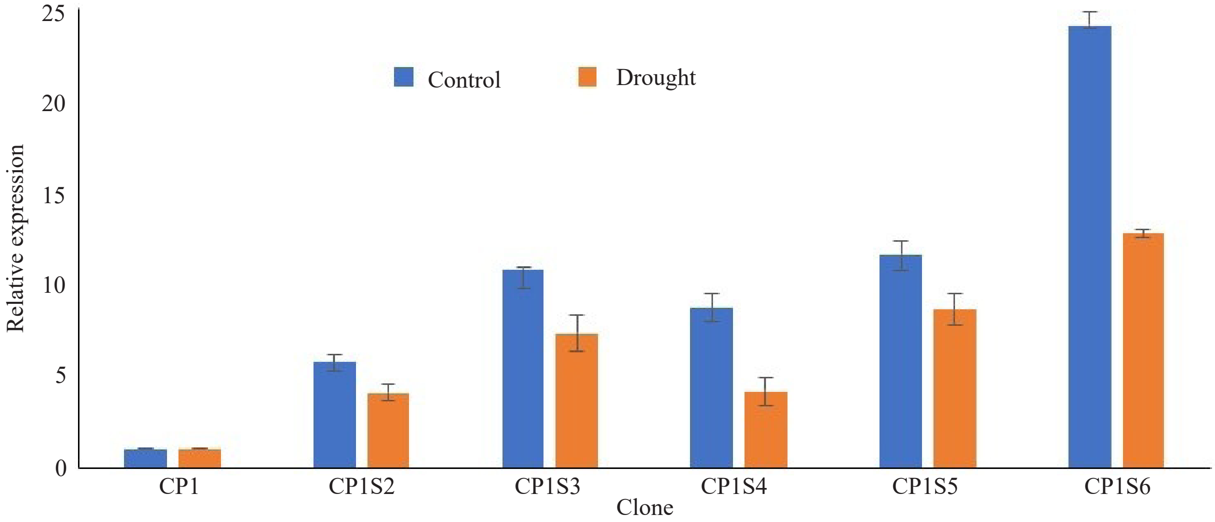


Figure 4. Relative expression of *MmCuZn-SOD* gene in transgenic and non-transgenic lines under control and drought stress using $2^{-\Delta\Delta CT}$ method. Data are presented as mean ± standard deviation (n =3)

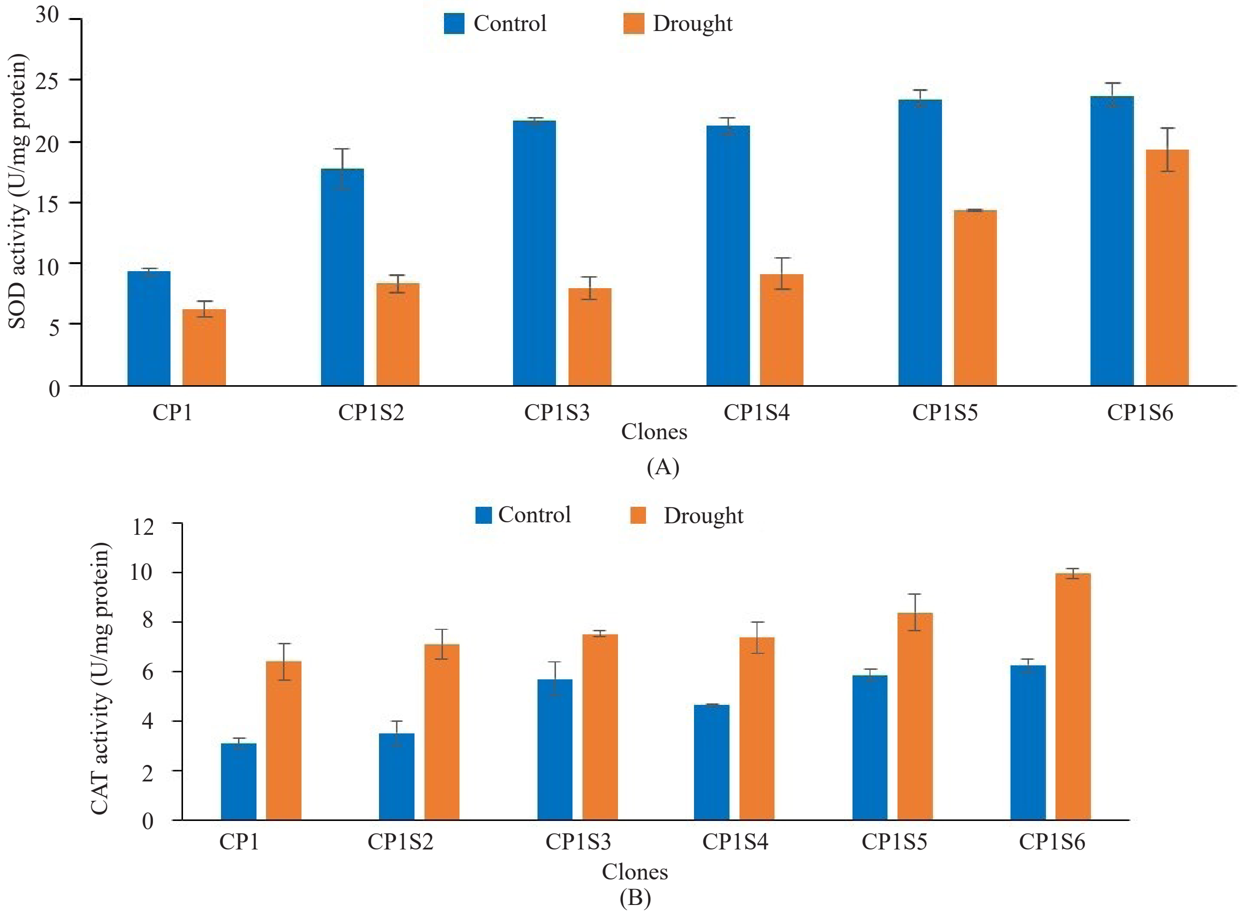


Figure 5. Effect of drought stress on (A) SOD and (B) Catalase activity in transgenic and non-transgenic clones. Data are presented as mean ± standard deviation (n = 3)

tuber weight and number, which is correlated with a decrease in root mass (Boguszewska-Mańkowska *et al*. 2020) and a decrease in the canopy (Aliche *et al*. 2018). Oil palm accessions tolerant to drought stress have higher biomass and metabolite profiles compared to other intolerant accessions during drought stress treatment (Andesmora *et al*. 2025). In this research, we found that transgenic plants overexpressing the *MmCuZn-SOD* gene showed better growth, higher biomass, and tuber yield than non-transgenic plants. The findings align with research on tomato and spinach plants with high SOD enzyme activity under drought conditions, which have a higher total biomass compared to plants that have low SOD enzyme activity (Rahman *et al*. 2004; Sarker and Oba 2018).

The decrease in the number of tubers is an impact of drought stress. Transgenic plants that overexpress the *MmCuZn-SOD* gene can maintain high tuber yields under stress conditions. Several research studies reported that drought stress can reduce the number and weight of tubers by 17% and 6% in potato plants, respectively (Deblonde and Ledent 2001). Reduced plant growth and tuber yield were found when drought stress occurs at the early planting stadium (Chang *et al*. 2018). In drought-tolerant potato genotypes with high SOD and catalase enzyme activities, tuber yield are better than in non-tolerant potato genotypes (Nasir and Toth 2022). The finding of this research is in accordance with those reports, which is the transgenic plant showed higher SOD and catalase enzyme activities and produced higher tuber yield under drought stress compared to the non-transgenic plant.

Drought stress is associated with decreased chlorophyll content, photosynthesis rate, stomatal conductance, and transpiration rate of plants. Drought stress impacts the closure of stomata, which leads to decreased photosynthesis and NADPH demand in the Calvin cycle, causing limited electron transport activity (Liang *et al*. 2020). In this study, transgenic plants exhibited higher photosynthesis rates and stomatal conductance than non-transgenic plants. In wheat plants, tolerant genotypes with high SOD expression can maintain chlorophyll content or even increase it under drought conditions (Khayatnezhad and Gholamin 2021). In sugar cane plants experiencing drought stress, the photosynthesis rate in plants with high SOD activity has a better value compared to sugar cane plants sensitive to drought (Sales *et al*. 2013). Chrysanthemum cultivars tolerant to drought stress conditions have higher SOD enzyme activity, photosynthesis rate, chlorophyll content, and transpiration rate than chrysanthemum plants sensitive to drought stress (Sun *et al*. 2013). In Mulberry, tolerant varieties to drought stress have the highest relative water content, photosynthesis rate, and water use efficiency and the lowest MDA and H_2O_2 compared to intolerant varieties (Nutthapornnitchakul *et al*. 2024).

The excessive production of ROS in plants can lead to higher levels of lipid peroxidation that cause cell damage. MDA, a byproduct of oxidative stress, can be used as a parameter of lipid peroxidation level in cell membranes. Transgenic plants have lower MDA levels compared to non-transgenic plants when they are exposed to drought stress. Furthermore, there is an observed increase in MDA alongside the decreased activity of antioxidant enzymes, osmoprotectants, and chlorophyll content in plants that are subjected to a combination of drought and high-temperature stress (Hanif *et al*. 2021). In tolerant alfalfa plants undergoing drought stress, have low levels of lipid peroxidation with high photosynthetic rates, osmoregulatory capacity, antioxidant activity, and gene expression, especially those involved in the balance between ROS production and detoxification (Zhang *et al*. 2018).

Overexpression of SOD gene becomes a defense system to enhance plant tolerance to stress. Transgenic potato plants that overexpress the SOD gene more tolerant to drought stress than non-transgenic plants. SOD is an enzyme that plays a crucial role in eliminating ROS (Saed-moucheshi *et al*. 2021) and contributes in the dismutation of superoxide radicals (Sarker and Oba 2018). Drought stress leads to a decrease in root weight, sugar content, chlorophyll content, relative water content, and leaf area index in plants. However, increasing the activity of superoxide dismutase, peroxidase, and catalase enzymes, as well as proline content, enhances plant tolerance to these stress conditions (Khodadadi *et al*. 2020). In tomato plants exposed to heat stress, the expression of the SOD gene was stimulated by lipocalin, a protein that respond to environmental stress. The overexpression of the lipocalin gene was able to eliminate ROS formed in tomatoes (Wahyudi *et al*. 2020). In eggplant, applying α-tocopherol exogenously through foliar spraying and pre-sowing seed treatment effectively reduces drought stress as shown by increased levels of chlorophyll, proline, peroxidase, and SOD activity, as well as reduced H_2O_2 content (Akram *et al*. 2023).

In conclusion, transgenic potato CP1 IPB plants overexpressing the *MmCuZn-SOD* gene are more tolerant against drought stress than non-transgenic plants, marked by better morphological and physiological characteristics. Lipid peroxidation in transgenic potato plants is lower than in non-transgenic plants. *MmCu/Zn-SOD* gene expression in transgenic potato CP1 IPB plants is higher than in non-transgenic plants. The transgenic potato IPB CP1 exhibit higher activity of SOD and catalase enzymes compared to non-transgenic plants. Transgenic clones CP1S5 and CP1S6 are more tolerant of drought stress than other transgenic clones, which suggests that both clones can be utilized as plant materials to develop potato lines or cultivars that are tolerant to drought stress.

This research was conducted under controlled conditions on a limited scale, highlighting the need for long-term research and multi-location planting to assess the stability and response of transgenic potato plants accurately. Further research will undoubtedly strengthen these findings and lead to concrete recommendations for transgenic potato clones that exhibit drought stress tolerance.

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