International Journal of Scientific Research in Science, Engineering and Technology
Print ISSN: 2395-1990 | Online ISSN : 2394-4099 (www.ijsrset.com)
doi : https://doi.org/10.32628/IJSRSET207624

The Effect of Calcium Chloride on Drought Tolerance of Mulberry

Cha JinMyong[1], Ri CholUk[2], Kim Chol Min[3], Ri Huang Gi[1], Ri Do Hun[1]，Qin Bo[4]

[1] Kyeungsang Sariwon Agricultural University, Pyongyang, 950003, Democratic People's Republic of Korea
[2]Kim Chaek University of Technology, Pyongyang, 950003, Democratic People's Republic of Korea
[3] Branch of Biotechnology, State Academy of Sciences, Pyongyang, 950003, Democratic People's Republic of Korea
[4] Northeast Forestry University Haerbin,150040, China

Article Info
Volume 7 Issue 6
Page Number: 177-186
Publication Issue :
November-December-2020

Article History
Accepted : 01 Dec 2020
Published : 20 Dec 2020

ABSTRACT

Drought stress greatly affects the quality and yield of mulberry leaves, which eventually influences the production of silkworm cocoon. In this study, the effect of calcium chloride on drought tolerance of mulberry was investigated. Different concentrations of $CaCl_2$ solutions were sprayed on the leaves of mulberry under drought condition, and the physiological and biochemical responses were measured. As a result, the spray of $CaCl_2$ on leaves ($CaCl_2$-spray-on-leaves) was proved to have gradual increases in measure parameters as compared to $CaCl_2$-untreated case under the same drought condition; furthermore, 20mM $CaCl_2$-treated group showed a significant increase ($P<0.05$), which indicates the optimal $CaCl_2$ concentration for improving the drought tolerance of mulberry. This study demonstrated that $CaCl_2$-spray-on-leaves can be an effective measure to ameliorate the drought tolerance of mulberry in the severe-drought areas.

Keywords: Mulberry, Calcium Chloride, Drought Toleranceield

I. INTRODUCTION

The quality and yield of mulberry (*Morus spp.*) leaves is considered as an important factor influencing the production of silkworm (Bombyx mori L.) cocoon and greatly affected by drought and disease [1]. Mulberry leaves have a rich protein, antioxidant and mineral content and no any toxic compounds and hence become more popular for livestock forage in recent years [2]. However, the recently-continuous global warming causes a severe drought in the cultivation areas of mulberry, and the loss of mulberry leaves yield due to the drought is 15~30%. Such a drought affects the mulberry leaves quality as well, which results in a considerable reduction of silkworm cocoon yield [3]. Therefore, investigating how to enhance the drought tolerance of mulberry is an important issue to maintain a stable production of mulberry leaves even under a drought condition.

Calcium, as an essential constituent element of plant tissue, plays an important role in the maintenance and regulation of diverse cell functions [4]. Ca^{2+} is not only a main element necessary for the growth of plant[5] but also a secondary signal molecule included in many signaling pathways[6], which enhances the tolerance of plant against biotic or abiotic stresses[7]. In particular, drought stress causes a considerable reduction of Ca^{2+} absorption, which results in some adverse effects.

In a lot of previous studies, calcium chloride has been reported to ameliorate the adverse effects of drought stress on many plants. Literature [8] investigated the influence of calcium chloride on the improvement of drought resistance in maize. Literature [9] reported the effectiveness of calcium chloride on *Zoysia japonica*. In addition, the effects of calcium chloride on maize plant [10]. In this study, the effect of calcium chloride on drought tolerance of mulberry was investigated. Different concentrations of $CaCl_2$ solutions were sprayed on the leaves of mulberry under drought condition, and the physiological and biochemical responses were measured. As a result, $CaCl_2$-treatment significantly increased the leaf relative water content (LRWC) and leaf chlorophyll content (LCC) of mulberry under drought stress and improved SOD, POD and CAT activities decreasing MDA and proline level, which shows that $CaCl_2$ assists mulberry to have drought resistance. In addition, 20mM $CaCl_2$-treatment was proved to be the most effective. This study provides an important guidance and experimental foundation for improving the survival rate and economic value of mulberry under drought condition.

II. MATERIALS AND METHODS

2.1. Experimental location and plant material

Mulberry cultivar (Ryongchon cv) of 3-month-old was selected and the healthy seedlings were transplanted in 35L plastic pots. The study was undertaken at the greenhouse of Pyong Yang Agricultural University, DPRK, from March 15th to May 15th in 2019. The seedlings were transferred to the greenhouse one month before the experiment. The walls and ceiling of greenhouse were transparent so that provide enough sunlight (900~1600μmol m^{-2} s^{-1} PPFD, 20±1℃(early morning)~30±4℃(early afternoon), 25±5%~45±2% relative humidity, 350 ± 10μmol mol^{-1} CO_2 concentration). During the adaption period, all samples were watered 3-4 times and fertilized one time per week. A total of 150 seedlings were used in this experiment and they were randomized and divided into 5 groups with 3 replicates consisting of 10 individuals. In the beginning of the experiment, the leaves and branches observed were ranged from 5 to 6 and 3 to 4 respectively. Aqueous solutions of $CaCl_2$ (10, 20, 30mM) were sprayed on the leaves of each group until run-off occurred twice a day for 3 days. The control group 1 was maintained at 100% PC (Said and Hugh, 2005), while for the control group 2 distilled water was similarly sprayed to runoff. In test groups except control 1, drought was induced withholding water for 12 days after treatment of $CaCl_2$. The treatment procedures were as follows.

Treatment	Specification
Control1 (100% PC)	No $CaCl_2$ and No drought
Control2 (distilled water)	No $CaCl_2$ and drought
10mM	10mmol/L $CaCl_2$ pretreatment and drought
20mM	20mmol/L $CaCl_2$ pretreatment and drought
30mM	30mmol/L $CaCl_2$ pretreatment and drought

All the measurements and samplings were conducted in period of 0, 4, 8, 12 days after the treatment of $CaCl_2$. Samplings were performed taking fully expanded leaves of third or fourth location of top branch apes. The results were reported in mean value.

III. EXPERIMENTAL METHODS

2.2.1. Leaf relative water content and soil moisture content

Relative leaf water content (RLWC) was calculated in every sampling satge according to the following formula: RLWC = [(FM – DM) / (SM – DM)] × 100 %. The fresh mass (FS), saturated mass(SM) and dry mass (DM) were evaluated respectively using an analytical balance(0.1 mg precision). The saturated leaves were obtained by submerging in distilled water in dark place. The dry mass was measured after drying in the oven at 60℃ for 48h. Soil water content (SWC) was determined according to the following equation: SWC= [(soil wet mass) – (soil dry mass)] / (soil wet mass) × 100%. The dry mass of soil was obtained after desiccating in the oven at 80℃ for 48h.

2.2.2. Chlorophyll contents

Chl content was quantified according to the method of literature [11]. Chl was extracted from 0.5g of fresh leaf using 10 ml of 80% acetone and followed by centrifugation at 10,000g for 5min. The absorbance value of supernatant solution was measured at 663.2nm 646.8nm.

2.2.3. Enzyme Assays

0.5g of fresh leaf was ground in extraction buffer (0.1M phosphate buffer pH 6.8) and this homogenate was further centrifuged at 12,000×g for 15min at 4℃. The obtained solution was stored for the enzyme assay (superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD).)

SOD activity was determined based on the protocol of literature [12], in which measuring the ability of the previously obtained supernatant to suppress the photochemical reduction of nitroblue tetrazoium(NBT) by superoxide radicals induced by photochemical reaction. One unit of SOD was equivalent to the amount of enzyme required to suppress the reduction rate of NBT by 50% at 25℃.

The modified method of literature [13] was used to evaluate POD and CAT activities. The major composition of the POD reaction solution were phosphate buffer (50 mM, pH 7.8), guaiacol (25mM), H_2O_2 (200mM) and the enzyme extract respectively. All the changing absorbance values of the reaction solution were measured at 470nm. Meanwhile, for CAT activity, potassium phosphate buffer (50mM, pH 7.0, containing 0.1 mM EDTA), H_2O_2 (200 mM) and the enzyme extract was added in a reaction solution. After adding the enzyme extract, decomposition rate of H_2O_2 was measured at 240 nm.

2.2.4. Lipid Peroxidation

Lipid peroxidation in leaf tissue was assayed measuring the amount of MDA. 0.5g of tissue sample was homogenized in 5 mL of 0.1 % TCA (trichloroacetic acid) and liquid nitrogen. The homogenate was then centrifuged at 5000 g for 10min at 4℃ and 500μL of supernatant was used to measure MDA content according to literature [14].

2.2.5. Proline content

Proline content was investigated using the method supposed by literature [15]. 0.5g of fresh leaf was homogenized in 10ml of 3 % sulphosalicyclic acid and followed by centrifugation at 9000g for 15min at room temperature. The reaction solution consisting of 1 ml of leaf extract, 2 ml of acid ninhydrin, 2ml of glacial acetic acid was boiled in water bath for 1h. After incubation, 4 ml of toluene was added to the solution and mixed vortexing for 15-20s. After separating toluene part with pink color from the upper solution, the absorbance value was measured at 520nm. Authentic proline (Sigma) was used to prepare standard calibration curve and proline content was expressed in mg g^{-1} fw.

2.2.6. Estimation of leaf calcium concentration

Leaf samples were harvested in the final stage of experiment (12day) to determine calcium concentration. The leaves were then dried in the oven for 30min at 105 and further dried at 65℃ until the mass keeps constant. Dried leaves were pulverized using plant grinding tool. Wet digestion process was

occurred in the dried plant material and $Ca2^+$ concentration of leat tissue was calculated using Flame-photometer based on the method described by literature [16].

2.2.7. Determination of Growth Parameters

The plants were harvested in the final stage of experiment (12day) and the final growth and the leaf number per plant were recorded. The total length of buds were calculated measuring the length of all primary bud from each plant (literature [17]). After collecting the leaves from each plant, number and area of leaves were measured. The leaf area was estimated by plotting the margins of the leaf on graph paper.

2.2.8. Statistical analysis

All data were obtained from three replicates and were expressed as mean ± SE. Statistical analysis and comparisons was done using *SPSS 17.0* software. When the main effect was a significant, *Duncan's* multiple range test was performed at the 0.05 level of significance.

IV. RESULTS

3.1. Soil water content

Table1 shows the time dependent variation of soil water content (SWC) under drought condition for 12 days. The SWC of control group 1 has been kept as 100%PC, while that of control group 2 and all test groups (treated with $CaCl_2$) has been significantly($P<0.05$) decreased along the continuation of drought condition. The SWC of test group 3 at 12 day of the drought was decreased to even 12.7%. There was no significant difference among all the test groups and control group 2, meaning that all groups have been under the same level of drought.

Table1. Time dependent soil water content under drought condition (%)

Time (d) / $CaCl_2$ concentration	0	4	8	12
0mM, no drought (Control 1)	100%PC	100%PC	100%PC	100%PC
0mM, drought (Control 2)	38.1±3.2 Aa	29.8±1.9 Ba	23.3±1.9 Ca	13.4±2.1 Da
10mM	37.7±3.3 Aa	30.2±2.4 Ba	22.8±1.5 Ca	13.2±1.1 Da
20mM	37.9±2.9 Aa	30.1±2.2 Ba	22.9±1.2 Ca	12.9±2.4 Da
30mM	38.0±2.5 Aa	29.9±1.8 Ba	23.0±1.7 Ca	12.7±1.6 Da

The data in the figure represent mean ± SE (n = 3). Different capital letters indicate statistical differences between drought treatment stages, and lowercase letters indicate significant difference between Ca treatments at the same drought treatment stage, at $P \leq 0.05$ according to Duncan's multiple range test (DMRT).

3.2. Leaf relative water content

Table 2 shows the time dependent variation of leaf relative water content (LRWC) under drought condition for 12 days. The LRWC of all test groups were significantly ($P<0.05$) higher than that of control group 2. The LRWC of the 20mM $CaCl_2$ treated test group at 12 day of drought was highest (69.9%).

Table 2. Effect of the $CaCl_2$ with different concentration on the leaf relative water content

Time (d) / $CaCl_2$ concentration	0	4	8	12
	LRWC (%)	LRWC (%)	LRWC (%)	LRWC (%)

0mM, no drought (Control 1)	82.3±2.1 Aa	82.5±2.4 Aa	81.9±2.3Aa	82.3±2.0Aa
0mM, drought (Control 2)	82.6±2.3 Aa	75.5±1.6 Bb	64.8±2.2Cd	49.5±2.5De
10mM	81.8±1.8 Aa	76.5±2.1 Bb	66.5±2.1Ccd	56.2±2.4Dd
20mM	83.1±2.2 Aa	80.5±1.3 Aa	78.6±1.8Bb	69.9±2.4Cb
30mM	82.3±2.1 Aa	76.9±1.7 Bb	70.1±2.3Cc	63.8±2.7Dc

The data in the figure represent mean ± SE (n = 3). Different capital letters indicate statistical differences between drought treatment stages, and lowercase letters indicate significant difference between Ca treatments at the same drought treatment stage, at P≤0.05 according to Duncan's multiple range test (DMRT).

1.3. Leaf Chlorophyll contents

Following tables (Table 3, Table 4, and Table 5) show the effect of the $CaCl_2$ on the chlorophyll content during drought for 12 days. $CaCl_2$ treatment significantly slowed the chlorophyll content decrease caused by the drought condition. 20mM $CaCl_2$ treatment produced the best effect.

Table 3. Effect of the $CaCl_2$ on the leaf chlorophyll$_a$ content (mg g^{-1} fw)

Time (d) / $CaCl_2$ concentration	0	4	8	12
0mM, no drought (Control	2.80±0.05 Aa	2.71±0.21 Aba	2.82±0.02 Aca	2.82±0.03 Ada
1)	2.79±0.04 Aa	2.04±0.22 Bc	1.93±0.05 Cd	1.42±0.17De
0mM, drought (Control 2)	2.75±0.25 Aa	2.42±0.09 Bb	2.21±0.09 Cc	1.63±0.08Dde
10mM	2.80±0.02 Aa	2.63±0.07 Bab	2.53±0.05 BCb	1.91±0.11CDb
20mM	2.73±0.14 Aa	2.54±0.14 Bb	2.32±0.03 Cc	1.68±0.07Dc
30mM				

Table 4. Effect of the $CaCl_2$ on the leaf chlorophyll$_b$ content (mg g^{-1} fw)

Time (d) / $CaCl_2$ concentration	0	4	8	12
0mM, no drought (Control	0.78±0.07Aa	0.80±0.06Aba	0.82±0.02Aca	0.81±0.03Ada
1)	0.80±0.02Aa	0.62±0.14Bc	0.50±0.06Ce	0.46±0.06De
0mM, drought (Control 2)	0.83±0.03Aa	0.69±0.03Bb	0.53±0.09Cd	0.49±0.05Dd
10mM	0.84±0.05Aa	0.75±0.02Ba	0.62±0.05BCb	0.58±0.07CDb
20mM	0.82±0.02Aa	0.70±0.02Bb	0.56±0.02Cc	0.52±0.04CDc
30mM				

Table 5. Effect of the $CaCl_2$ on the leaf chlorophyll$_c$ content (mg g^{-1} fw)

Time (d) / $CaCl_2$ concentration	0	4	8	12
0mM, no drought (Control	3.64±0.03Aa	3.60±0.06ABa	3.62±0.03ACa	3.61±0.03ADa
1)	3.61±0.06Aa	2.66±0.06Bd	2.43±0.06Cd	1.88±0.05Dd
0mM, drought (Control 2)	3.62±0.02Aa	3.11±0.07Bc	2.74±0.08BCc	2.12±0.06Dc
10mM	3.60±0.06Aa	3.38±0.04Bb	3.15±0.05BCb	2.49±0.08CDb
20mM	3.64±0.04Aa	3.24±0.08Bc	2.88±0.03BCc	2.20±0.04Dc
30mM				

The data in the figure represent mean ± SE (n = 3). Different capital letters indicate statistical differences between drought treatment stages, and lowercase letters indicate significant difference between Ca treatments at the same drought treatment stage, at P≤0.05 according to Duncan's multiple range test (DMRT).

1.4. Changes in antioxidant enzyme activities

Following tables (Tables 6 to 8) show the effect of the $CaCl_2$ on leaf antioxidant enzyme activities. The SOD activity of control group 1 has been kept without significant difference during drought for 12 days. All the rest groups show great increase in SOD activity. SOD activity of all $CaCl_2$ treated groups were significantly higher than that of control group 2. 20mM $CaCl_2$ treatment induced the highest SOD activity.

Table 6. Effect of the $CaCl_2$ on SOD activity (Ug^{-1} Dw min^{-1})

Time (d) / $CaCl_2$ concentration	0	4	8	12
0mM, no drought (Control 1)	56.8±5.8Da	58.0±4.8CDb	57.0±4.2BDe	55.9±8.3ADe
0mM, drought (Control 2)	52.5±7.4Da	94.8±5.2Ca	153.3±5.6Bd	182.4±5.8Ad
10mM	54.2±6.3Da	92.9±7.4Ca	196.6±7.3ABc	225.2±8.6Ac
20mM	53.7±8.2Da	97.4±8.2Ca	324.5±7.3Aba	344.7±8.5Aa
30mM	55.7±5.2Da	98.3±7.2Ca	232.1±9.5ABb	251.3±7.4Ab

The data in the figure represent mean ± SE (n = 3). Different capital letters indicate statistical differences between drought treatment stages, and lowercase letters indicate significant difference between Ca treatments at the same drought treatment stage, at $P \leq 0.05$ according to Duncan's multiple range test (DMRT).

In addition, the POD activity of control group 1 has been not significantly changed during drought for 12 days. For the rest groups, POD activity has been increased until 8 day of drought and then slightly decreased. POD activity of $CaCl_2$ treated groups were significantly higher than that of control group 2. 20mM $CaCl_2$ treatment showed the highest POD activity.

Table 7. Effect of the $CaCl_2$ on POD activity (Ug^{-1} Dw min^{-1})

Time (d) / $CaCl_2$ concentration	0	4	8	12
0mM, no drought (Control 1)	24.3±1.8Da	24.4±1.6CDc	23.6±2.2BDd	23.8±2.3ADd
0mM, drought (Control 2)	22.8±1.4Da	24.4±1.2CDc	42.6±1.6Ac	37.2±3.8Bc
10mM	24.1±1.3Da	28.5±1.4Cb	45.4±2.3Ab	42.8±3.6ABb
20mM	23.5±1.2Da	32.4±2.2Ca	58.4±4.3Aa	55.2±5.5Aba
30mM	25.6±1.2Da	28.6±1.2Cb	47.6±2.5Ab	44.3±4.4ABb

Changing pattern of CAT activity was similar to that of POD activity. $CaCl_2$ treatment induced the increase in CAT activity, the highest at 20mM $CaCl_2$ treatment.

Table 8. Effect of the $CaCl_2$ on CAT activity (Ug^{-1} Dw min^{-1})

Time (d) / $CaCl_2$ concentration	0	4	8	12
0mM, no drought (Control 1)	3.4±0.4Ca	3.4±0.2Ca	3.3±0.2ACd	3.3±0.3ABCd
0mM, drought (Control 2)	3.2±0.4Ca	3.3±0.2Ca	5.4±0.6Ac	5.1±0.2ABc
10mM	3.4±0.3Ca	3.4±0.4Ca	6.8±0.5Abc	5.6±0.4ABbc
20mM	3.3±0.2Ca	3.5±0.5Ca	8.4±0.3Aa	6.9±0.3Aba
30mM	3.5±0.2Ca	3.4±0.2Ca	7.3±0.5Ab	6.2±0.2ABb

The data in the figure represent mean ± SE (n = 3). Different capital letters indicate statistical differences between drought treatment stages, and lowercase letters indicate significant difference between Ca treatments at the same drought treatment stage, at $P \leq 0.05$ according to Duncan's multiple range test (DMRT).

1.5. Changes in MDA level

Table 9 shows the effect of the $CaCl_2$ on leaf MDA level. Compared to the MDA level of control group 1, that of the rest groups has been increased. $CaCl_2$ treated groups showed lower MDA level than control group 2. MDA level of 20mM $CaCl_2$ treated group was the lowest, 20.5nmol g^{-1} fw.

Table 9. Effect of the $CaCl_2$ on MDA level (nmol g^{-1} fw)

Time (d) / $CaCl_2$ concentration	0	4	8	12
0mM, no drought (Control 1)	12.2±0.9Da	12.3±1.3CDb	12.0±1.2CDc	12.2±1.4ABd
0mM, drought (Control 2)	11.9±1.3Da	15.5±2.5Ca	28.9±2.3Ba	36.6±2.6Aa
10mM	12.1±1.5Da	15.3±2.7Ca	24.6±1.8Bab	29.8±2.3ABb
20mM	12.2±0.7Da	14.7±1.3Ca	15.2±2.7BCbc	20.5±2.5ABc
30mM	11.8±1.7Da	14.9±2.1Ca	21.9±2.5Bb	25.3±1.8ABbc

The data in the figure represent mean ± SE (n = 3). Different capital letters indicate statistical differences between drought treatment stages, and lowercase letters indicate significant difference between Ca treatments at the same drought treatment stage, at P≤0.05 according to Duncan's multiple range test (DMRT).

1.6. Changes in proline content

As shown in Table 10, leaf proline content has been increased proportionally to the drought time. $CaCl_2$ treated groups showed lower proline content than control group 2. The proline content of 20mM $CaCl_2$ treated group was the lowest, 2.4mg g^{-1} fw.

Table 10. Effect of the $CaCl_2$ on proline content (mg g^{-1} fw)

Time (d) / $CaCl_2$ concentration	0	4	8	12
0mM, no drought (Control 1)	1.4±0.06Da	1.5±0.03CDd	1.4±0.02BCe	1.4±0.02ABe
0mM, drought (Control 2)	1.5±0.08Da	1.9±0.07Ca	3.9±0.12Ba	4.4±0.11Aa
10mM	1.4±0.13Da	1.7±0.04Cb	3.6±0.03Bb	4.1±0.04ABb
20mM	1.4±0.12Da	1.5±0.03CDc	2.2±0.06Bd	2.4±0.14Abd
30mM	1.4±0.06Da	1.7±0.08Cb	3.1±0.07Bc	3.7±0.06ABc

The data in the figure represent mean ± SE (n = 3). Different capital letters indicate statistical differences between drought treatment stages, and lowercase letters indicate significant difference between Ca treatments at the same drought treatment stage, at P≤0.05 according to Duncan's multiple range test (DMRT).

1.7. Leaf calcium concentration

Table 11 shows the leaf calcium concentration. The calcium concentration of control group 2 was 2.1mg g^{-1} DW, great lower than 3.2 mg g^{-1} DW of control group 1. $CaCl_2$ treatment increased the leaf Ca concentration during the drought. Ca concentrations of 10mM, 20mM, and 30mM $CaCl_2$ treated groups were 3.4, 4.3, 3.8mg g^{-1} DW, respectively, higher than control group 2, even the control group 1.

Table 11. Leaf calcium concentration (mg g^{-1} fw)

Group	Leaf Calcium concentration(mg g-1 DW)

0mM $CaCl_2$, no drought (Control 1)	3.2±0.13c
0mM $CaCl_2$, drought (Control 2)	2.1±0.10d
10mM $CaCl_2$	3.4±0.08c
20mM $CaCl_2$	4.3±0.05a
30mM $CaCl_2$	3.8±0.07b

The data in the figure represent mean ± SE (n = 3). Lowercase letters indicate significant difference between Ca treatments at the same drought treatment stage, at P≤0.05 according to Duncan's multiple range test (DMRT).

1.8. Changes in plant growth characteristics

Table 12 shows the plant growth characteristics under drought condition. Control group 1 represents the highest value in all the growth parameters, whereas the control group 2 shows the lowest value. Among the $CaCl_2$ treated groups, 20mM $CaCl_2$ treated group was the highest.

Table 12. Plant growth characteristics

Growth characteristics / $CaCl_2$ concentration	Plant height (cm)	Number of leaves per plant	Leaf area (cm^2)
0mM, no drought (Control 1)	6.7 ±1.2a	10.6±0.6a	270.6±5.4a
0mM, drought (Control 2)	3.5±0.5d	6.8±0.6d	158.8±4.2d
10mM	3.9±0.3cd	7.2±0.5cd	166.2±3.8cd
20mM	5.8±0.4b	8.9±0.4b	210.9±6.4b
30mM	4.5±0.6c	7.8±0.4c	181.8±4.6c

The data in the figure represent mean ± SE (n = 3). Lowercase letters indicate significant difference between Ca treatments at the same drought treatment stage, at P≤0.05 according to Duncan's multiple range test (DMRT).

V. DISCUSSION

In this study, drought stress decreased the leaf relative water content (LRWC) of Morus spp. by 40%. Leaf application of $CaCl_2$ solution significantly (P<0.05) increased LRWC under drought condition, where 20mM $CaCl_2$ treatment increased LRWC to 69.9% (24.9% higher than untreatment, as shown in Table 2). Leaf application of $CaCl_2$ solution significantly (P<0.05) increased Morus spp. chlorophyll content during drought, 20mM $CaCl_2$ treatment showed highest chlorophyll content (2.49 mg g-1 fw) among the test groups (Table 3, Table 4 and Table 5).

Therefore, typical leaf antioxidant enzymes such as SOD, POD and CAT were investigated their activity in this study (Table 6, Table 7 and Table 8). The activity of antioxidant enzymes in Morus spp. leaf increased as the drought became more severe. The control group 1 showed no significant change in the leaf antioxidant enzyme activity. Enzyme activities of $CaCl_2$ treated groups represented much greater change than untreated group (P<0.05). 20mM $CaCl_2$ treated group was highest in 3 enzyme activities among test groups.

In this study, drought stress was proved to increase leaf proline level $CaCl_2$ treated groups showed significantly (P<0.05) lower proline level than untreated groups (Table 10). Proline level was the lowest in 20mM $CaCl_2$ treated group.

Leaf Ca^{2+} concentration was measured in this study. Drought dramatically decreased the leaf Ca^{2+} concentration. Ca^{2+} concentration of 20mM $CaCl_2$ treated group was highest (4.3 mg g^{-1} DW) among stressed group, even higher than unstressed control group 1 (3.2 mg g^{-1} DW).

The plant growth characteristics under drought condition and $CaCl_2$ treatment were investigated in this study (Table 12). Drought stress severely inhibited Morus spp. growth and $CaCl_2$ treatment alleviated inhibitory effect of drought stress. The plant height, leaf number per plant and leaf area per plant of 20mM $CaCl_2$ treated group were 122%, 114% and 132% higher than the control group 2.

VI. CONCLUSION

To sum up, drought stress caused the decreases of leaf relative water content (LRWC) and leaf chlorophyll content in mulberry and induced the lipid peroxidation affecting membrane stability and permeability, and consequently inhibited the mulberry growth. Under drought condition, $CaCl_2$ treatment significantly increased LRWC and chlorophyll content and improved SOD, POD and CAT activity decreasing MDA and proline level. This demonstrated that $CaCl_2$ assists mulberry to have drought resistance. 20mM $CaCl_2$ was proved to be the most effective. This study provides an important guidance and experimental foundation for improving the survival rate and economic value of mulberry under drought condition.

VII.REFERENCES

[1]. Ihsan M.Z, El-Nakhlawy F.S, Ismail S.M, Fahad S, daur I, Wheat phenological development and growth studies as affected by drought and late season high temperature stress under arid environment. Frontiers in Plant Science, 2016, 7(795).

[2]. Anirban Guha,DebashreeSengupta, Attipalli RamachandraReddy. Physiological optimality, allocation trade-offs and antioxidant protection linked to better leaf yield performance in drought exposed Mulberry. J Sci Food Agric, 2010, 90: 2649–2659.

[3]. Manimegalai S, Chandramohan N.Leaf quality of mildew affected leaves and their effect on mortality and economic characters of silkworm, Bombyx mori L. Sericologia, 2007, 47:87–92

[4]. Elad Y, Kirshner B. Calcium reduces Botrytis cinerea damage to plants of Ruscus hypoglossum. Phytoparasitica, 1992, 20(4): 285–291.

[5]. Yang S, Wang F, Guo F, et al. Calcium contributes to photoprotection and repair of photosystem II in peanut leaves during heat and high irradiance J]. Journal of Integrative Plant Biology, 2015, 57(5): 486-495.

[6]. Lecourieux D, Ranjeva R, Pugin A. Calcium in plant defense-signalling pathways J]. New Phytologist, 2006, 171: 249-269.

[7]. Johnson, G. I. Postharvest handling of tropical fruits. Proceedings of an International Conference held at chiang Mai, Thailand, 1993, 7: 19–23.

[8]. Khan M B, Hussain M, Raza A, et al. Seed priming with CaCl2 and ridge planting for improved drought resistance in maize. Turkish Journal of Agriculture and Forestry, 2015, 39: 193-203.

[9]. Xu C, Li X, Zhang L. The effect of calcium chloride on growth, photosynthesis, and antioxidant responses of Zoysia japonica under drought conditions, PLOS ONE, 2013, 8(7): e68214.

[10]. Nae em M, Naeem MS, Ahmad R, Ahmad R. Foliar-applied calcium induces drought stress tolerance in maize by manipulating osmolyte accumulation and antioxidative responses. Pak J Bot, 2017, 49(2):427-434.

[11]. Agrawal SB, Rathore D. Changes in oxidative stress defense in wheat (Triticum aestivum L.) and mung bean (Vigna radiata L.) cultivars grown with and without mineral nutrients and irradiated by supplemental ultraviolet-B. Environmental and Experimental Botany, 2007, 59(1): 21-33.

[12]. Beyer Jr WF, Fridovich I. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions, 1987, 161(2): 559-566.
[13]. Chance B, Maehly AC. Assay of catalases and peroxidases. Methods in Enzymology, 1955, 2(136):764-775.
[14]. Fu J, Huang B. Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. Environmental and Experiment Botany, 2001, 45(2):105–114.
[15]. Bates, LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant and Soil, 1973, 39: 205-207.
[16]. Wolf, Benjamin. A comprehensive system of leaf analyses and its use for diagnosing crop nutrient status. Communication in Soil Science and Plant Analysis, 1982, 13: 1035-1059.
[17]. Chowfary NB, Rajan MV, Dandin SB. Effect of Poultry Feed Supplemented with Mulberry Leaf Powder on Growth and Development of Broilers. The IUP Journal of Life Sciences, 2009, 3(3): 51-54.

Cite this article as :

Cha JinMyong, Ri CholUk, Kim Chol Min, Ri Huang Gi, Ri Do Hun，Qin Bo " The Effect of Calcium Chloride on Drought Tolerance of Mulberry, International Journal of Scientific Research in Science, Engineering and Technology(IJSRSET), Print ISSN : 2395-1990, Online ISSN : 2394-4099, Volume 7, Issue 6, pp.177-186, November-December-2020. Available at doi : https://doi.org/10.32628/IJSRSET207624
Journal URL : http://ijsrset.com/IJSRSET207624