TOLERANCE OF CHINESE CABBAGE (*BRASSICA RAPA CHINENSIS*) TO INDUCED DROUGHT AND SALINITY STRESSES

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ABSTRACT

Chinese cabbage (*Brassica rapa chinensis*) is a common vegetable consumed in the Asian region. The cultivation of this popular vegetable is however limited by unfavourable conditions such as drought and salinity stress. In this study, we analyzed the physiological response of cabbage seedlings to various concentrations of polyethylene glycol 6000 (PEG 6000) and sodium chloride (NaCl) used to induce drought and salinity stresses, respectively. The *in vitro* screening showed that after a 14-day period of stress challenge, drought and salinity had no effect on the plant survival rate, but plant height, root growth, fresh weight and numbers of leaves were reduced. Their tolerance to stress was attributed to the accumulation of proline and total soluble proteins. Proline accumulation increased significantly in seedlings challenged with drought and salinity stress. The seedlings also reduced its total chlorophyll content under drought and salt environment. In NaCl-amended medium, increased total soluble protein content was also observed as a tolerance response towards stress. To conclude, the results from the study suggested that *Brassica rapa* seedlings had developed its own tolerance mechanisms in order to overcome or avoid damages brought by the drought and salinity stresses. As such, the cultivation of Chinese cabbage could be manipulated in marginal lands to levels which will not affect their growth.

Keywords: *Brassica ra pa,* drought stress, salinity stress, tolerance mechanisms, proline

INTRODUCTION

Chinese cabbage (*Brassica rapa chinen sis*) is a common vegetable found in many dietary practices in the Asian region. As a leafy vegetable, they prefer full-sun and well drained fertile soils, with pH 5.5 to 7 (Huxley 1992). Their widespread cultivation is limited by the diminishing fertile lands. It is estimated that 90% of the arable lands in the world is affected by the environmental stresses with drought and salinity being the most widespread (Ashraf 1994). An estimated 2 million ha of world agricultural land turns saline every year, leading to reduced or no crop productivity (Syverstein et al. 1989). As a result, marginal lands are increasing while arable lands are decreasing, thus, there is a need to understand the potential of

cultivating crops on soils affected with drought and salinity factors. To date, there is no proper documentation on the tolerance level of common crops like Chinese cabbage to drought and salinity stress although investigations on other important crops such as bean (*Phaseolus vulgaris*), corn (*Zea mays*) and cotton (*Gossypium hirsutum*) have been documented (Maas 1990). Thus, this study was conducted to determine the impact and physiological response of Chinese cabbage to drought and salinity stress.

MATERIALS AND METHODS

Sixty seeds of *Brassica rapa chinensis* (Serbajadi®) were first surface sterilized in 2 % Clorox® solution with two drops of Tween-20, rinsed in sterile distilled water and germinated on Murashige-Skoog (MS) agar media in the culture room (16 h light, 8 h darkness). After 7 days, seedlings with two to three leaves and of uniform size were subcultured onto fresh MS media incorporated with different concentrations of PEG 6000 and NaCl. For drought stress challenge, MS media was incorporated with 0.5 g, 1.0 g and 1.5 g of PEG 6000 (Duchefa Biochemie) to achieve 5 g/L, 10 g/L and 15 g/L, respectively. For salinity stress challenge, 0.29 g, 0.58 g and 0.88 g of NaCl (Duchefa Biochemie) were added to achieve 50 mM, 100 mM and 150 mM, respectively.

Seedlings were assessed for their percentage of survival and vegetative growth parameters prior to and after 14 days of sub-culturing into the stress medium. The vegetative parameters assessed included plant height, root growth, fresh weight and number of leaves. For physiological response, proline assay, total chlorophyll assay and total soluble protein assay were conducted to determine the tolerance of Chinese cabbage seedlings to the stresses.

Proline accumulation was determined as described by Bates et al. (1973). Briefly, seedlings were grinded and the slurry was added with 1.2 mL of 3 % sulphosalicyclic acid (Acros Organics) to precipitate protein. The samples were centrifuged (10000 rpm) for 10 min and the supernatant collected and made up to 1 mL with distilled water. Glacial acetic acid (1 mL) (Fisher Scientific) and ninhydrin reagent (1 mL) [3 % (w/v) ninhydrin (Fisher Scientific) in 60 % (v/v) 6M phosphoric acid (R&M chemical)] were then added and the mixture incubated for 1 h in boiling water bath (Memmert). The tube was cooled on ice to terminate the reaction. The products were extracted with 2 mL of toluene with the upper (toluene) phase transferred into a glass cuvette and the absorbance values read at 520 nm using a spectrophotometer (GENESYS 20). Proline concentration was calculated from a standard curve constructed separately using L-proline standards (Acros Organics) in a series of concentration (0, 1, 2, 3, 4 and 5 g/10 mL) and using the following equation:

$$\mu g \text{ proline / g dry weight} = \frac{(\mu g \text{ proline / ml} * \text{ml toluene})}{(115.5\ \mu g \text{ / mole}) / (\text{g sample} / 3)}$$

For chlorophyll assay, the pigments were extracted from 1 g of leaf tissues by grinding in 100 mL of 85 % acetone (MERCK). The homogenate was filtered and the filtrate made up to 100 mL with 85 % acetone. The absorbance values of the extracts were read at both 663 nm and 644 nm (Arnon 1949). The concentrations of chlorophyll *a* and *b*, in mg per g of tissue, were calculated by the following formula:

mg chlorophyll *a* /g tissue = [1.07 ($O.D._{663}$) – 0.094 ($O.D._{664}$)] x 3
mg chlorophyll *b* /g tissue = [1.77 ($O.D._{644}$) – 0.280 ($O.D._{663}$)] x 3
Total chlorophyll content = chlorophyll *a* + chlorophyll *b*

Total soluble protein was determined using the method by Lowry et al. (1951). The seedlings were grinded with 10 mL of phosphate buffer (pH 6.5) (Fisher Scientific). The slurry was strained through cheese cloth, filtered through absorbent cotton and the filtrate collected for centrifuge for 20 min at 4°C (10,000 rpm). The supernatant was discarded, the pellet was resuspended in 5 mL of phosphate buffer (pH 6.5) and filtered again through absorbent cotton. One mL of the mixture (0.01 ml filtrate + 0.99 ml distilled water) was then added into 0.9 mL solution A [2 g potassium sodium tartrate (Fisher Scientific), 100 g Na_2CO_3 (SYSTERM) dissolved in 500 ml 1M NaOH (MERCK) and top up to 1 L]. The mixture was mixed thoroughly and incubated at 50°C in a water bath (Memmert) for 20 min, then cooled to room temperature. To the mixture, 0.1 mL Solution B [2 g potassium sodium tartrate and 1 g $CuS_4.5H_2O$ (Bendosen) dissolved in 90 mL distilled water and 10 mL 1M NaOH] was added and incubated at room temperature for 10 min. After that, 3 mL of dissolved Folin-Ciocalteu (MERCK) was added and the mixture incubated at 50°C water bath for 10 min. Finally, the tubes were cooled down to room temperature and the absorbance values were measured at 750 nm. Total soluble content was calculated using known concentrations of bovine serum albumin (BSA) (Sigma) (0, 0.05, 0.10, 0.15, 0.20 and 0.25g/L).

The experiment was conducted in triplicates throughout the sampling period. The analysis of variance (ANOVA) and means comparisons (Tukey's Studentized Range Test) ($HSD_{(0.05)}$) were performed using SAS (Statistical Analysis System) version 6.12.

RESULTS AND DISCUSSION

Our results showed that Chinese cabbage seedlings achieved 100 % of survival rate when challenged with drought and salinity stresses. The high concentrations of PEG 6000 and NaCl had no severe adverse effect on plant survival rate, although the leaves suffered some wilting effect, especially where the older leaves developed chlorosis. Symptoms were more prevalent in seedlings-stressed with 150 mM NaCl and 15 g/L PEG 6000. This showed that Chinese cabbage seedlings developed tolerance to the PEG 6000 and NaCl mediated drought and salinity stress, respectively. The concentrations used here are also commonly used in other plant-

stress studies (Murillo et al. 2002), hence eliminating the possibility that tolerance was attributed to low levels of concentrations.

The vegetative growth of the seedlings was less affected by the salinity stress compared to drought stress. Increase in height, root growth, fresh weight and number of leaves for seedlings cultivated on NaCl amended media (50 mM, 100 mM, 150 mM NaCl), were not significantly different from the growth values of seedlings in control (0 mM NaCl) although the values were lower (Figure 1). The slight inhibition of growth observed may be attributed to the ion accumulation by the changing membrane permeability which affected the metabolism (Cramer et al. 1985). In contrast, drought stress caused a significant reduction in the root growth, fresh weight, and number of leaves compared to growth values for seedlings in control (0 g/L PEG 6000). The loss of water content inhibited photosynthesis, respiration, translocation, ion uptake and nutrient metabolism, resulting in loss of turgour, wilting and decrease in cell growth (Morgan 1984; Coca et al. 1996). In both stresses, the general trend is that the growth values decrease with the increase in concentrations of NaCl and PEG 6000. Seedlings in control (T1) which was free from any stress treatments produced the highest increment for all values assessed with 0.91 cm, 0.57 cm, 0.15 g, 3.33 leaves for plant height, root growth, fresh weight, and number of leaves, respectively (Figure 1).

The results for proline, total chlorophyll and total soluble phenol assay showed that the Chinese cabbage seedlings tolerated drought and salinity stresses by producing significantly higher amounts of proline, significantly lesser chlorophyll pigments, and non-significant difference in levels of total soluble proteins. Proline is produced as a response to stress, with higher proline amounts assayed from higher concentrations of NaCl and PEG 6000. The lowest proline level was assayed from controlled seedlings at 5.59 mg/L (Figure 2). Between the two stresses, proline levels were higher in seedlings cultured on PEG 6000 media, with values almost twice the amount in NaCl-treated seedlings (Figure 2). Proline act as a protector from dessication and from harmful effects derived from solute accumulation (Yoshiba et al. 1997). They are also associated with the "recovery resistance" serving as a source of respiratory energy for the recovering plant (Blum & Ebercon 1976).

The reduction of total chlorophyll content was significant as concentrations of NaCl and PEG 6000 increased (Figure 3). Lowest total chlorophyll content was recorded from seedlings challenged with 150 mM NaCl with 0.34 mg total chlorophyll/g fresh weight tissue, and from 15 g/L PEG 6000 with 0.29 mg total chlorophyll / g fresh weight tissue. Controlled seedlings recorded the highest amount of total chlorophyll content with 0.55 mg total chlorophyll/g fresh weight tissue (Figure 3). The reduction in chlorophyll content may be an adaptation mechanism to stress conditions to inhibit photosynthesis and decrease CO_2 assimilation (Kicheva et al. 1994).

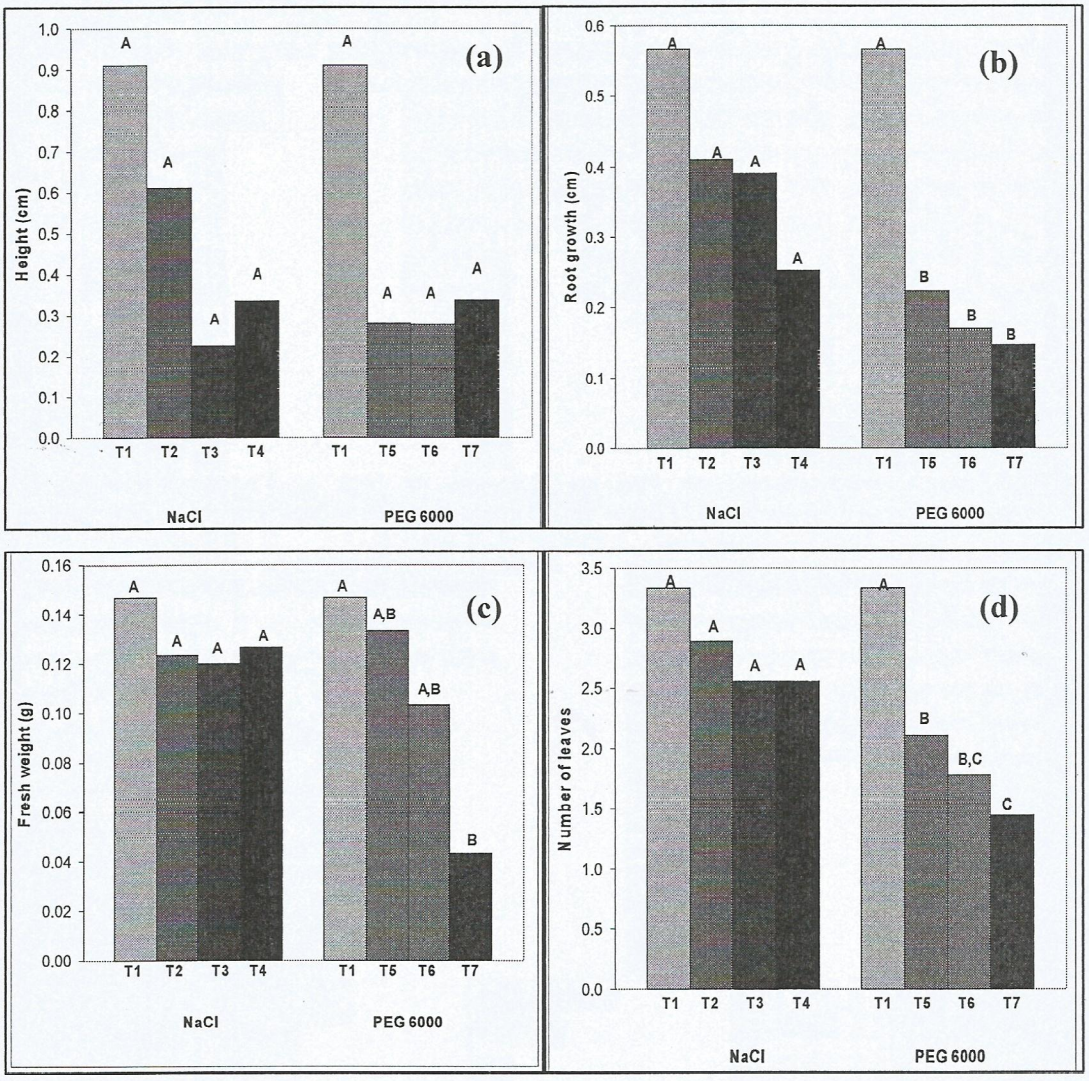

Figure 1. Increase in height (a), root growth (b), fresh weight (c) and number of leaves (d) of Chinese cabbage seedlings cultivated in a series of different concentrations of PEG 6000 and NaCl. Means with the same letters are not significantly different ($HSD_{(0.05)}$). (Note: T1- 0 mM NaCl, T2-50 mM NaCl, T3-100 mM NaCl, T4-150 mM NaCl, T5-5 g/L PEG 6000, T6-10 g/L PEG 6000, T7-15 g/L PEG 6000).

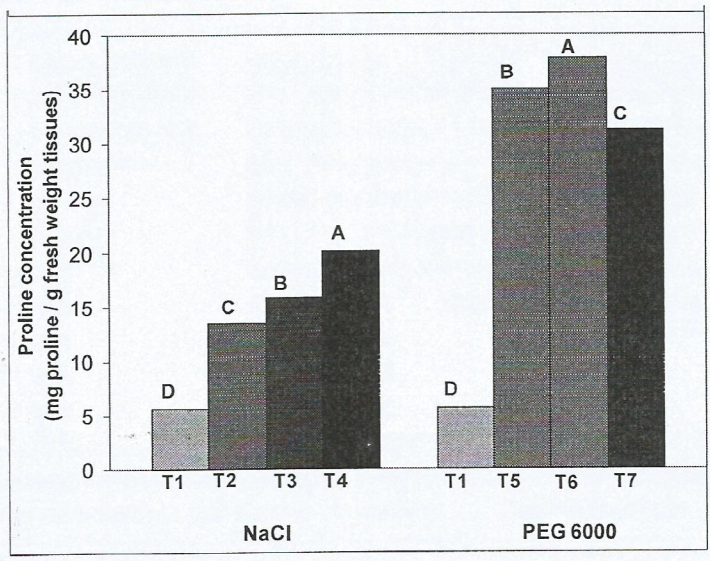

Figure 2: Proline concentrations in Chinese cabbage seedlings challenged with different concentrations of PEG 6000 and NaCl. Means with the same letters are not significantly different (HSD $_{(0.05)}$). (Note: T1- 0 mM NaCl, T2-50 mM NaCl, T3-100 mM NaCl, T4-150 mM NaCl, T5-5 g/L PEG 6000, T6-10 g/L PEG 6000, T7-15 g/L PEG 6000).

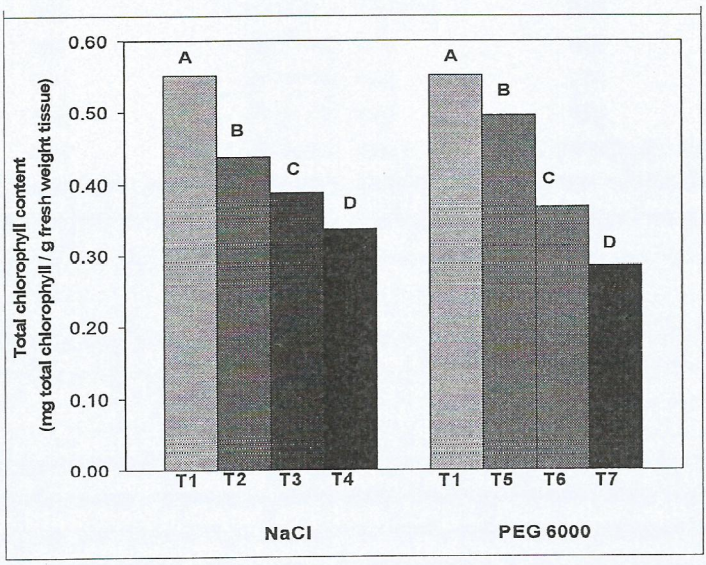

Figure 3: Total chlorophyll content in Chinese cabbage seedlings challenged with different concentrations of PEG 6000 and NaCl. Means with the same letters are not significantly different (HSD $_{(0.05)}$). (Note: T1- 0 mM NaCl, T2-50 mM NaCl, T3-100 mM NaCl, T4-150 mM NaCl, T5-5 g/L PEG 6000, T6-10 g/L PEG 6000, T7-15 g/L PEG 6000).

Total soluble protein content in Chinese cabbage (*Brassica rapa chinensis*) seedlings differed under drought and salt stressed conditions. In salt-stress media, total soluble protein increased gradually as NaCl concentrations increased although the levels were not significantly different among the concentrations (Figure 4). At 150 mM NaCl, Chinese cabbage seedlings recorded the highest total soluble protein content of 0.59 g total soluble protein/g fresh weight tissues (Figure 4). The higher amounts of total soluble proteins with the increase in higher concentrations of NaCl may be due to the fact that salinity enhances protein synthesis and that more nitrogen is converted to proteins under salt-stressed conditions (Langdale et al. 1973; Pareek et al. 1997). These proteins can then serve as a storage form for nitrogen, which is reutilized when the stress is lifted (Singh et al. 1987).

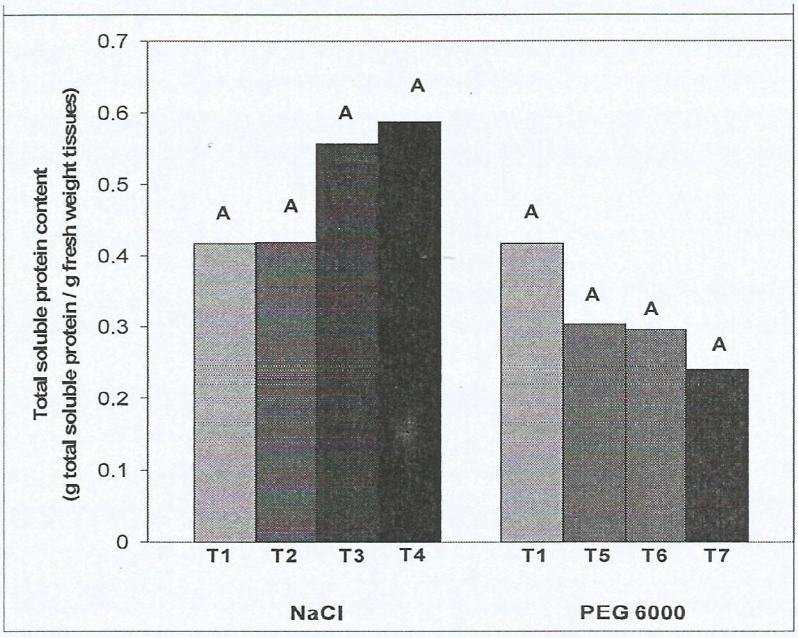

Figure 4: Total soluble protein content in Chinese cabbage seedlings challenged with different concentrations of PEG 6000 and NaCl. Means with the same letters are not significantly different (HSD $_{(0.05)}$). (Note: T1- 0 mM NaCl, T2-50 mM NaCl, T3-100 mM NaCl, T4-150 mM NaCl, T5-5 g/L PEG 6000, T6-10 g/L PEG 6000, T7-15 g/L PEG 6000).

In contrast, seedlings challenged with drought-stress declined with the incline in concentrations of PEG used. The decrease in protein level is due to the lack of protein synthesis and the increase in protein degradation (Lawlor 2002). The decline in total soluble protein levels were, however, not significantly different among all the PEG concentrations.

CONCLUSION

Tithe overall results indicated that Chinese cabbage showed greater mechanism of tolerance to salt stress (up to 150 mM NaCl) compared to drought stress. Although seedlings of Chinese cabbage (*Brassica rapa chi nensis*) maintained 100 % of survival rate in both stress conditions, reduced vegetative growth were observed under drought- and salinity-stressed conditions. Significant reduction of vegetative growth in seedlings was reported in drought stress except for the plant height, but no significant decrease of vegetative parameters was recorded for seedlings under salinity stress conditions. Chinese cabbage tolerated salinity stress by increasing the production of proline and total soluble protein, which balance the osmotic potential to ensure survivability in stress conditions.

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