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Original Article

Effect of salinity stress on physiological aspects of pumpkin (*Cucurbita moschata* Duchesne. 'Laikaotok') under hydroponic condition

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Abstract

Pumpkin (*Cucurbita moschata*) 'Laikaotok' is an important edible vegetable but growth and crop yield are affected by salinity stress. Response of physiological traits to salinity stress was investigated under hydroponic culture using Hoagland's solution at different NaCl concentrations of 0, 15, 30, 45, 60 and 120 mM. All treatments were cultured for four weeks and physiological traits were recorded. Results showed that pumpkin growth decreased after NaCl treatment, especially at 45 mM and higher concentrations. Leaf number, leaf width, leaf length, root number, stem length, stem diameter, SPAD unit, chlorophyll fluorescence in dark condition for 30 min (Fv'/Fm'), chlorophyll fluorescence in light condition (Fv/Fm), total chlorophyll, chlorophyll a and chlorophyll b contents significantly decreased ($p < 0.05$). Results indicated that salt concentration at less than 45 mM NaCl was suitable for *C. moschata* 'Laikaotok' growth. Phenotypic correlation coefficient estimation of 12 physiological traits showed positive correlation at a highly significant level, except for the correlation between plant height - Fv/Fm; plant height - Fv'/Fm'; root number - Fv/Fm and root number - Fv'/Fm'. This is the first report on salt stress response in *C. moschata* 'Laikaotok'. Knowledge gained will be useful for pumpkin culture or rootstock selection under sensitive and moderately sensitive saline conditions.

Keywords: Growth, Hydroponic culture, NaCl, Pumpkin, Phenotypic correlation

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Introduction

Soil salinity is an important issue, which is expected to worsen as a result of climate change. The FAO (2022) estimated that 20-50% of irrigated land was impacted by salinity stress, negatively impacting plant growth and development with lower crop production worldwide (Wang and Huang, 2019). Salinity impairs plant growth by reducing the osmotic potential of the soil, causing plant cells to lose water, thereby diminishing growth and photosynthetic rate with stomatal closure (Ma et al., 2020). Sodium and chloride ions also have indirect toxic effects on plant nutrients and changes in metabolic pathways (Kurum et al., 2013; Martins et al., 2013). Salinity stressors cause an overabundance of reactive oxygen species (ROS) which harm important macromolecules and ultimately cause cell death by inhibiting enzyme activity, lipid peroxidation and loss of membrane integrity (Huang et al., 2019). However, many plant species can tolerate saline conditions through multiple biochemical mechanisms that facilitate metabolic reactions and plant growth (Kurum et al., 2013; Albuquerque et al., 2016). Many reports have addressed plant adaptations under saline conditions including watermelon (Martins et al., 2013), squash (Oliveira et al., 2014), beet (Oliveira et al., 2015), lettuce (Oliveira, 2013) and cucumber (Albuquerque et al., 2016). Different salinity levels exist in saline soil areas. Plants react to stress both individually as cells and collectively as an entire organism (Reddy et al., 2017). Therefore, the effects of various salinity stress levels on plant growth and development require detailed study.

Pumpkin is an economically nutritious crop belonging to the family Cucurbitaceae. Various well-known cultivars include *C. moschata* and *C. pepo*, and the squash *C. maxima*. Cucurbitaceae contains species representing the second highest crop value in the world (Resende et al., 2013; FAO, 2014). Pumpkin is a popular crop in Thailand due to the low cultivation cost and relatively short harvesting time, while the yield is high compared to other crops. Many pumpkin varieties are cultivated in Thailand but information about the effects of salt stress regarding their growth and development is lacking. Bischoff (2018) found that some pumpkin species were moderately sensitive to salinity but no reports exist on local Thai pumpkin cultivars. ‘Laikaotok’ is a local Thai pumpkin variety that has been grown for many years. This variety is considered easy to grow with good yield and good taste and is suitable for cultivation as an economic crop. This study investigated the survival rate, growth and some physiological characteristics of *C. moschata* ‘Laikaotok’ under saline conditions using a hydroponics system. We hypothesized that this pumpkin variety may respond differently under diverse salinity stress levels and compared growth and physiological characteristics under various salinity levels during 4 weeks of treatment. Findings advance the understanding of adaptation and response mechanisms by *C. moschata* ‘Laikaotok’ under salt stress. Our data can be utilized to evaluate the growth of this pumpkin variety under different saline environments.

Material and Methods

Plant material

C. moschata ‘Laikaotok’ seeds were individually germinated in 3x5 cm soil pots. Fourteen-day seedlings with 1-2 leaves were used as explants for hydroponic culture. All seeds were germinated in the greenhouse at the Department of Biology, Faculty of Science, Khon Kaen University during June 2020 (rainy season in Thailand) with 29.4±0.8°C of daily dry-bulb temperature and approximately 40 µmol.$m^2.s^{-1}$ of light intensity.

Hydroponic culture by Deep Flow Technique (DFT) and salinity treatment

Rectangular tubes size 10x25 cm with a foam sheet were used as planting materials for hydroponic culture. Each tube was filled with one liter of Hoagland’s solution (pH 5.5) and aerated using an air pump (model ACQ-007, 75 watts, 100 l/s). Selected healthy pumpkin seedlings were cultured in Hoagland’s solution containing various concentrations of NaCl (0, 15, 30, 45, 60 and 120 mM) for four weeks. Leaf number (more than 2 cm long leaf), leaf size, stem length and root number (healthy and more than 2 cm long root) were counted and measured. At least three replicates (10 samples per replicate) were tested in every treatment.

Data collection

Green leaf intensity was measured using a SPAD-502 Plus Chlorophyll Meter in three areas (leaf base, middle and leaf apex) of mature leaf blades. Average SPAD units were calculated and total chlorophyll, chlorophyll a and chlorophyll b were analyzed. Mature leaves (0.1 g) were ground in a mortar before dissolution in 5 ml of 80% acetone. This step was repeated until all the green material was dissolved. The solution was filtered using filter paper. If any green color remained, then grinding was continued. When all the green material had dissolved, a further 20 ml of 80% acetone was added. The supernatant was detected by absorbance measurements at 645 and 663 nm using a spectrophotometer (Spectronic 20) and 80% acetone was set as the blank. Chlorophyll contents were calculated following the method of Arnon (1949) using the formulae shown below.

$$\text{Total chlorophyll (mg/g tissue)} = [20.2\ (A645) + 8.02\ (A663)] \times \frac{V}{(1000 \times W)}$$

$$\text{Chlorophyll a (mg/g tissue)} = [12.7\ (A663) - 2.69\ (A645)] \times \frac{V}{(1000 \times W)}$$

$$\text{Chlorophyll b (mg/g tissue)} = [22.9\ (A645) - 4.68\ (A663)] \times \frac{V}{(1000 \times W)}$$

where V = total volume of solution (ml)
and W = weight of leaves (g)

For chlorophyll fluorescence, mature leaves were measured using a Chlorophyll Fluorometer Handy PEA in dark-adapted leaf (30 min dark) and light condition.

Statistical analysis

Completely randomized design (CRD) was used and at least three replicates were tested in every treatment. Statistical analysis was checked using a one-way analysis of variance (One-way ANOVA), while comparative analysis of the mean values was examined using the post hoc test (Duncan's test) at 95% confidence level. Data analysis was conducted using SPSS Program. Relationships between physiological traits were considered using correlation coefficients among interesting pairs of physiological characteristics at phenotypic levels. Phenotypic correlation coefficients were calculated following the methods of Searle (1961) and Singh et al. (2018) as follows:

$$\text{Phenotypic correlation coefficients (rp)} = \frac{\text{cov.XP (p)}}{\sqrt{var.Xp.var.Yp}}$$

where cov.XY (p) indicates phenotypic covariance between characteristics X and Y and var.X (p), and var.Y (p) indicate variance for phenotypic levels of characteristics X and Y, respectively. Data analysis was conducted using the SPSS program.

Results and Discussion

After culture under various concentrations of NaCl using a hydroponic system for four weeks, all pumpkin seedlings increased in growth at all treatments. Results showed that growth performance decreased as NaCl concentration increased, while leaf number, leaf width and length, stem length, root number and stem diameter of treated plants also dramatically decreased (Fig. 1). Salt concentration adversely affected the growth of *C. moschata* 'Laikaotok'. At 45 mM NaCl concentration leaf growth, stem diameter and root number were significantly different when compared with the control and treatment at less than 45 mM NaCl.

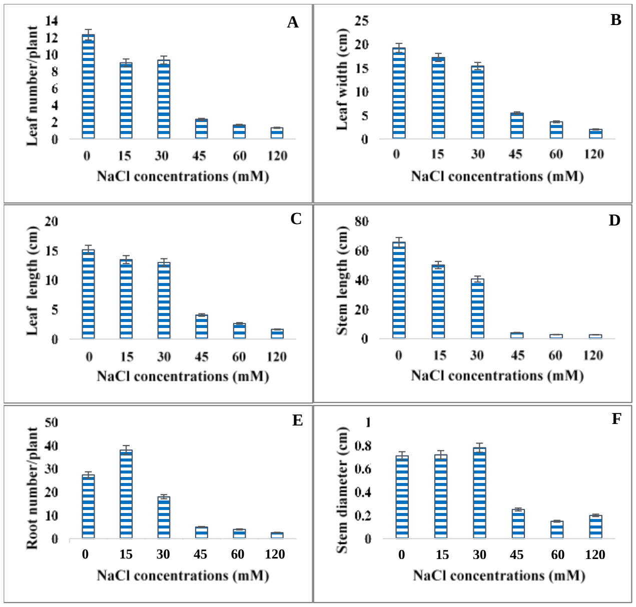

Figure-1. Leaf measurement, stem length, root number and stem diameter of *C. moschata* 'Laikaotok' pumpkin under a hydroponic system with various concentrations of NaCl after culture for four weeks.

Pumpkin growth was adversely affected by high NaCl concentrations. Kurum et al. (2013) reported that salt stress impacted the growth of pumpkin rootstock, while stem length, root length, leaf length, fresh weight and dry weight decreased when treated with high NaCl concentrations. *C. moschata* 'Laikaotok' growth was inhibited when cultured on higher concentrations of NaCl. This result was consistent with our previous reports on *C. moschata* 'Butternut' cultivation. Under different NaCl concentrations, number of leaves, their width and length, number of roots, height and diameter of the stems, amount of green pigment measured in SPAD units, chlorophyll fluorescence (Fv'/Fm', Fv/Fm), total chlorophyll and chlorophyll a and b content all decreased significantly ($p<0.05$) (Taratima et al., 2022). Root and shoot length were determined as an early indicator for salinity tolerance in *Brassica napus* (Long et al., 2013). High salinity stress affects plant growth characteristics such as seedling growth, leaf size, shoot and root length and total biomass (Hakim et al., 2010; Gupta and Huang, 2014). Plant growth under salt stress was impacted at different levels based on many factors such as plant species, growth and developmental stage and cultivation period (Roy et al., 2014).

Chlorophyll fluorescence in 30 min dark condition (dark-adapted leaves) and light condition slightly decreased in high concentration NaCl treatments (Fig. 2E, 2F), while total chlorophyll, chlorophyll a and chlorophyll b contents significantly decreased at higher NaCl concentrations (Fig. 2A, 2B, 2C). However, green intensity (SPAD value) of the 30 mM NaCl treatment showed the highest value and was significantly different compared to the other treatments, except for the 15 mM NaCl concentration (Fig. 2D). SPAD value, total chlorophyll, chlorophyll a and chlorophyll b decreased depending on NaCl concentrations, especially at concentrations higher than 45 mM NaCl (Fig. 2D-3).

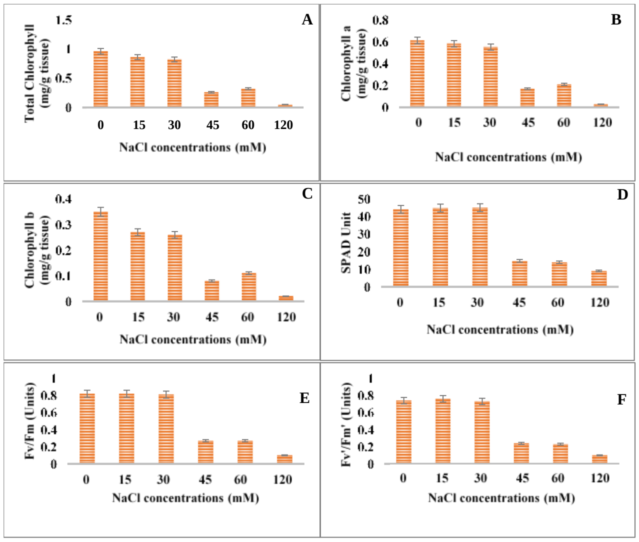

Figure-2. Chlorophyll fluorescence in dark condition for 30 min (Fv'/Fm') and light condition (Fv/Fm), green intensity (SPAD unit), total chlorophyll, chlorophyll a and chlorophyll b of *C. moschata* 'Laikaotok' leaf under a hydroponic system with various concentrations of NaCl after culture for four weeks.

Some physiological traits such as SPAD value, total chlorophyll, chlorophyll a and chlorophyll b were impacted by high NaCl concentrations. This result concurred with Sevengor et al. (2011) but differed from Gong et al. (2018) who reported that salt stress reduced total chlorophyll and chlorophyll a of *Kalidium foliatum*, while chlorophyll b content remained unaffected. Reduction in chlorophyll content is caused by loss of photosynthetic ability and accumulation of ions, resulting in inhibition of the chlorophyll biosynthesis pathway (Hakim et al., 2014).

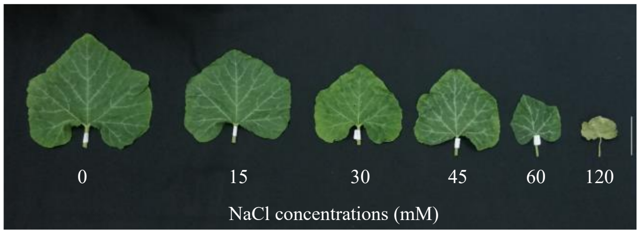

Figure-3. Development of *C. moschata* 'Laikaotok' leaves after treatment with various concentrations of NaCl for four weeks under a hydroponic system (scale = 5 cm).

Our results found that salt stress affected total chlorophyll content but no clear difference was shown in leaf greenness under 0, 15, 30, 45 and 60 mM NaCl treatments. However, some leaves showed the first indication of chlorosis. Mature proteins in thylakoid membranes are damaged or degraded under salinity stress, resulting in a significant reduction in photochemical efficiency and salt stress tolerance level (Shu et al., 2012). Salt stress induces thylakoid membrane disruption by free radicals. Chlorophyll is damaged, with reduction of properties to become a colorless form called bleaching chlorophyll (Theerakulpisut, 2016). Our results showed that pumpkin leaves exposed to saline conditions turned pale green. The dark green chlorophyll a was more disrupted by free radicals, while light green chlorophyll b was more stable under salt stress (Zhao et al., 2019). However, chlorophyll a can be modified to chlorophyll b by oxidation of the methyl group to a formyl group (Ito et al., 1996).

Chlorophyll fluorescence in dark (Fv/Fm) and light condition (Fv'/Fm' indicated photosystem II (PSII) performance. Our results proved that salt stress affected PSII because both Fv/Fm and Fv'/Fm' dramatically decreased after treatment at higher NaCl concentrations.

Table-1. Estimates of phenotypic correlation coefficients between 12 traits in pumpkin 'Laikaotok' treated with various concentrations of NaCl for four weeks under a hydroponic system.

Trait	LW	LL	SL	RN	SD	Fv/Fm	Fv'/Fm'	SU	TC	Cha	Chb
LN	0.949**	0.945**	0.929**	0.866**	0.902**	0.694*	0.706*	0.920**	0.929**	0.919**	0.926**
LW		0.997**	0.866**	0.883**	0.942**	0.729*	0.722*	0.945**	0.940**	0.945**	0.909**
LL			0.86**	0.867**	0.949**	0.726*	0.724*	0.944**	0.928**	0.936**	0.891**
SL				0.885**	0.745**	0.571	0.608	0.762**	0.810**	0.783**	0.839**
RN					0.800**	0.624	0.642	0.822**	0.840**	0.835**	0.828**
SD						0.737**	0.727*	0.965**	0.878**	0.894**	0.828**
Fv/Fm							0.990**	0.758**	0.725*	0.735*	0.688*
Fv'/Fm'								0.731*	0.700*	0.708*	0.667*
SU	Low	Medium	High						0.948**	0.962**	0.899**
TC										0.995**	0.984**
Cha											0.962**

*Significant correlation at $p < 0.05$, ** Significant correlation at $p < 0.001$. (LN = leaf number; LW = leaf width; LL = leaf length; SL = stem length; RN = root number; SD = stem diameter; Fv/Fm = chlorophyll fluorescence in light condition; Fv'/Fm' = chlorophyll fluorescence in dark condition for min; SU = SPAD unit; TC = total chlorophyll; Cha = chlorophyll a; Chb = chlorophyll b.

However, chlorophyll fluorescence of dark condition for 30 min was higher than chlorophyll fluorescence of light condition. This result concurred with Omovbude and Hamadina (2018) and Zhong et al. (2019) who found that chlorophyll fluorescence of light condition decreased after salt stress treatment. However, some growth and photosynthetic characters in our study differed from previous reports (Kurum et al., 2013; Tang et al., 2018) that noted root number, stem diameter, Fv'/Fm' and SPAD unit of some salinity treatments as higher than the control. Root number of 15 mM NaCl treatment was significantly higher than control. This result was similar to previous studies in rice (*Oryza sativa* L.) 'KDML 105' and 'IR29' that found increased root numbers and root length under 14-day salt stress (Ninmanont and Chaidee, 2016). Moreover, root surface area of two rapeseed varieties increased under 100 mM treatment (Arif et al., 2019). More conditions may affect root length, root surface area and volume such as aeration condition (Li et al., 2019), soil enzyme activity and soil microorganisms (Yang et al., 2016) and also plant species, developmental stage and environmental conditions.

Phenotypic correlation coefficient estimation of 12 physiological traits was investigated (Table 1). Almost all showed positive correlation at a highly significant level, except for the correlation between stem length (SL) - chlorophyll fluorescence in light condition (Fv/Fm) (0.571); stem length (SL) - chlorophyll fluorescence in dark condition for 30 min (Fv'/Fm') (0.608); root number (RN) - chlorophyll fluorescence in light condition (Fv/Fm) (0.624) and root number (RN) - chlorophyll fluorescence in dark condition for 30 min (Fv'/Fm') (0.642). However, some traits showed significant correlation only at $p < 0.05$ level, especially Fv'/Fm', when considered for leaf width (LW) (0.722*), leaf length (LL) (0.724*), SPAD unit (SU) (0.731*), total chlorophyll (TC) (0.700*), chlorophyll a (Cha) (0.708*) and chlorophyll b (Chb) (0.667*).

This result showed a positive correlation that was not significantly different for all physiological traits, implying that increasing some physiological traits increased growth. Phenotypic correlation between stem length and chlorophyll fluorescence in light condition (Fv/Fm) was also low (0.571), indicating that increasing stem length did not increase photosynthetic rate. The value of chlorophyll fluorescence in light condition (Fv/Fm) in this study was higher than chlorophyll fluorescence in dark condition for 30 min (Fv'/Fm') This result concurred with Zhong et al. (2019) who reported that overexpression of transglutaminase from cucumber in tobacco increased salt tolerance through regulation of photosynthesis. Transglutaminase (TGase) is indissolubly related to plant growth and development (Shu et al., 2020). In higher plants, TGase can catalyze the covalent binding of polyamines (PAs) to membrane protein complexes, and this helps to maintain the stability of photosynthetic membrane proteins (Ioannidis et al., 2009).

Pumpkin growth under salt stress conditions has been extensively studied but physiological correlation investigations are scant. Factors including varying the cultivar, planting techniques or environmental management and climate change also impact correlation investigation. Pumpkins are classified as glycophytes and do not grow well under salt stress situations; however, the plants can adapt when cultured under mild salt stress conditions (Zhu et al., 2008). Our findings confirmed that pumpkin 'Laikaotok' can grow in low to moderate saline conditions of between 30 and 45 mM NaCl. In Thailand, large areas of land are impacted by salinity. Therefore, our results will be useful for farmers who are interested in growing pumpkin 'Laikaotok" in saline areas that cannot be used to cultivate other crops. Planting in various soil salinities requires further investigation to assess the interaction between plants and soil microorganisms under aeration and environmental stress.

Conclusion

Results indicated that concentrations at less than 30 mM NaCl were appropriate for *C. moschata* 'Laikaotok' growth. Survival rate, growth and some physiological characteristics under this condition were not significantly different compared to the control. Phenotypic correlation coefficients of all physiological traits were positive. Our results can be applied to pumpkin cultivation and production in saline areas that may be associated with climate change in Thailand or other global regions. These results will provide important data to improve the adaptation mechanisms of tolerant pumpkin varieties under salinity stress.

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Contribution of Authors

Taratima W: Conceived and planned the experiments, collected and analysed data and wrote the manuscript
Kunpratum N: Supervised the project and edited manuscript
Maneerattanarungroj P: Helped in data collection and analysis.