DONG THAP UNIVERSITY JOURNAL OF SCIENCE
Tạp chí Khoa học Đại học Đồng Tháp
Natural Sciences Issue
ISSN 0866-7675 | e-ISSN 2815-567X

DOI: https://doi.org/10.52714/dthu.ns.3285.2006

# ASSESSMENT OF SALT TOLERANCE THRESHOLDS IN PAK CHOI (*Brassica rapa subsp. chinensis* L.)THROUGH GERMINATION AND EARLY SEEDLING GROWTH INDICES

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*Article history*

*Received: 30/3/2026; Received in revised form: 25/5/2026; Accepted: 28/5/2026*

**Abstract**

*Salinity acts as a key abiotic stress that hampers seed germination and early seedling growth. This study examined how salinity affects germination and initial development in Pak Choi (Brassica rapa subsp. chinensis L.) at electrical conductivity (EC) levels from 0 up to 16 dS $m^{-1}$. We measured parameters like germination percentage (GP), germination index (GI), mean germination time (MGT), $T_{50}$, seedling vigor index (SVI), shoot and root lengths, fresh and dry biomass, plus dry weight-to-length ratios. At mild salinity (EC ≤ 4 dS $m^{-1}$), GP stayed high at 91.8%, and seedlings actually developed better - with fresh weight achieving a maximum of 51.30 mg at EC 4 dS $m^{-1}$ (almost twice the control's 22.88 mg) and SVI-shoot topping surpassing 250—suggesting a favorable response to low-level salt stress. Germination time-related indices increased progressively with EC, with $T_{50}$ rising from 12.38 h at EC 0 to 27.02 h at EC 16 dS $m^{-1}$. Seedling growth and vigor declined sharply at EC 10 dS $m^{-1}$, growth and vigor dropped off quickly: shoot length fell from 3.2 cm to just 0.72 cm at EC 16 dS $m^{-1}$. Shoots suffered more than roots, and higher salinity boosted dry weight-to-length ratios (from 0.60 to 1.51 mg $cm^{-1}$), pointing to a change toward denser biomass rather than longer growth. Thus, these findings provide quantitative salinity thresholds for Pak choi at the germination and early seedling stage, offering practical references for agronomic management in saline-affected areas and a basis for selecting salt-tolerant genotypes within the Brassicaceae family.*

**Keywords:** *Germination, Growth and development, Pak choi, Salt stress.*

Cite: Nguyen, T. D. T., Tran, T. K., Trinh, M. N., Pham, L. T. V., Lam, Q. N., Nguyen, N. N., Auguste, A., & Kasim, M. (2026). Effect of Salinity on germination of (Brassica rapa subsp. chinensis L.) through germination and early seedling growth indices. *Tạp chí Khoa học Đại học Đồng Tháp*, *Online First*, 1-15. https://doi.org/10.52714/dthu.ns.3285.2006

# ĐÁNH GIÁ NGƯỠNG CHỊU MẶN CỦA PAK CHOI (*Brassica rapa subsp. chinensis* L.) THÔNG QUA CÁC CHỈ SỐ NẢY MẦM VÀ GIAI ĐOẠN ĐẦU SINH TRƯỞNG CỦA CÂY CON.

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*Lịch sử bài báo*

*Ngày nhận: 30/3/2026; Ngày nhận chỉnh sửa: 25/5/2026; Ngày duyệt đăng: 28/5/2026*

**Tóm tắt**

*Độ mặn đóng vai trò là tác nhân gây stress phi sinh học quan trọng, cản trở sự nảy mầm của hạt và sự phát triển ban đầu của cây con. Chúng tôi đã nghiên cứu ảnh hưởng của độ mặn đến sự nảy mầm và phát triển ban đầu của cải thìa (Brassica rapa subsp. chinensis L.) ở các mức độ dẫn điện (EC) từ 0 đến 16 dS $m^{-1}$. Chúng tôi đã đo các thông số như tỷ lệ nảy mầm (GP), chỉ số nảy mầm (GI), thời gian nảy mầm trung bình (MGT), $T_{50}$, chỉ số sức sống cây con (SVI), chiều dài thân và rễ, sinh khối tươi và khô, cùng với tỷ lệ trọng lượng khô trên chiều dài. Ở độ mặn vừa phải (EC ≤ 4 dS $m^{-1}$), tỷ lệ nảy mầm (GP) vẫn duy trì ở mức cao 91,8%, và cây con phát triển tốt hơn – với trọng lượng tươi đạt tối đa 51,30 mg ở EC 4 dS $m^{-1}$ (gần gấp đôi so với đối chứng là 22,88 mg) và chỉ số SVI-shoot vượt quá 250 – cho thấy phản ứng thuận lợi với stress mặn ở mức thấp. Các chỉ số liên quan đến thời gian nảy mầm tăng dần theo EC, với $T_{50}$ tăng từ 12,38 giờ ở EC 0 đến 27,02 giờ ở EC 16 dS $m^{-1}$. Sức sinh trưởng và sức sống của cây con giảm mạnh ở độ dẫn điện EC 10 dS $m^{-1}$, chiều dài chồi giảm từ 3,2 cm xuống chỉ còn 0,72 cm ở độ dẫn điện EC 16 dS $m^{-1}$. Chồi bị ảnh hưởng nhiều hơn rễ, và độ mặn cao hơn làm tăng tỷ lệ trọng lượng khô trên chiều dài (từ 0,60 đến 1,51 mg $cm^{-1}$), cho thấy sự thay đổi hướng tới sinh khối dày đặc hơn là sự phát triển dài hơn. Những phát hiện này cung cấp ngưỡng độ mặn định lượng cho cải thìa ở giai đoạn nảy mầm và cây con sớm, đưa ra các tài liệu tham khảo thực tiễn cho việc quản lý nông học ở các khu vực bị ảnh hưởng bởi độ mặn và là cơ sở để lựa chọn các giống chịu mặn trong họ Brassicaceae.*

**Từ khóa:** *Cải thìa, Sự nảy mầm, Sự sinh trưởng và phát triển, stress mặn.*

## 1. Introduction

Abiotic stress and biotic stress are considered major stressors for crops when they are present below/above optimal levels, individually or in combination (Moulick et al., 2024). Abiotic stress includes low and high temperatures, drought, heat, and salinity. Salinity causes significant damage to crop yields and threatening food security in countries already suffering from sea level rise and saltwater intrusion in the context of climate change (Ludwig et al., 2018). Salinity inhibition of crop growth can be categorized into two main components; in the short term, it creates osmotic stress, which can lead to the plant being unable to maintain turgor pressure, resulting in dehydration and poor cell function (Egamberdieva et al., 2019). While in the long term, salinity stress leads to ion toxicity due to the excessive accumulation of plant-toxic ions, especially sodium ($Na^+$) and chloride ($Cl^-$) (Abou Seeda et al., 2022). Plant tissues disrupted by ion balance caused by too much accumulation of sodium ($Na^+$) and chloride ($Cl^-$) ions and the replacement of potassium ($K^+$) ions, which are crucial for many physiological functions including enzyme activation and osmotic regulation, by sodium ($Na^+$) are severely impaired in the physiological functions of plant metabolic processes (Chakraborty et al., 2018). Besides these osmotic and toxic effects, salt stress also causes oxidative stress, which leads to cell membrane peroxidation, ion leakage, and damage to nucleic acids, cell membranes, and cellular structures (Imran et al., 2021). The combination of these factors together contributes to the adverse effects of salinity on crops, such as metabolism, which can manifest as reduced growth and, ultimately, reduced quality and total yield of crops (Zhang et al., 2022). Under salt stress, plants exhibit a two-phase response to limit damage and maintain growth. In the osmotic phase, rising external salt concentration reduces shoot growth and leaf expansion while prioritizing root development to conserve water. In the subsequent ion-specific phase, toxic accumulation of $Na^+$ in mature leaves accelerates leaf senescence and impairs photosynthesis, thereby further restricting plant growth. While osmotic stress exerts an immediate effect, ionic stress typically emerges later and is generally less severe except under high salinity or in sensitive genotypes. The capacity to tolerate salt stress varies among species and is closely linked to the efficiency of $Na^+$ and $Cl^-$ regulation within plant tissues (Munns et al., 2006; Munns & Tester, 2008)

Several attempts have been made to determine how salinity affects all vegetative stages of crops. During the vegetative stage, it negatively impacts seed germination, causes leaf damage that impairs photosynthesis, and reduces stem biomass, leaf area, and plant height under saline conditions (Petretto et al., 2019; Rajabi Dehnavi et al., 2020). Similarly, salinity stress negatively affects germination rate, root and stem length, fresh and dry weight of seedlings, and germination index in several different plants such as sorghum (*Sorghum bicolor* L.) (Rajabi Dehnavi et al., 2023), *Amorpha fruticosa* L. (Gao et al., 2024), barley (*Hordeum vulgare*) (Gupta et al., 2019), and canola (*Brassica napus* L.) (Shahbazi et al., 2011).

Although the effects of salt stress on the Brassicaceae family have been investigated in numerous studies worldwide, most have focused on limited salinity ranges or isolated growth parameters, leaving tolerance boundaries poorly defined. In Vietnam, research on *Brassica rapa subsp. Chinensis* L. has concentrated primarily on cultivation techniques and nutrient management (Nhi & Quyen, 2025; Trung Thanh & Huynh Anh Tuyet, 2025), with studies specifically addressing salinity stress responses remaining absent. With saltwater creeping into vital veggie-growing areas like the Mekong Delta, it's more pressing than ever to figure out how much salt our local Brassicaceae crops can take. We all know germination and those tender early seedlings are the most vulnerable stages - if they falter, the whole crop suffers. That's why this study gently tested Pak choi's germination and early growth in a lab, from EC levels (0 dS $m^{-1}$) up to salty (16 dS $m^{-1}$) under controlled laboratory conditions, using multiple parameters including GP, MGT, $T_{50}$, SVI, shoot and root length, and biomass traits, to

characterize salinity response patterns and establish tolerance thresholds as a basis for future breeding and agronomic management in saline-affected environments.

## 2. Materials and Research Methods

### 2.1. Seed Preparation and Treatments

Pak Choi (*Brassica rapa subsp. chinensis L*) seeds were obtained from a certified supplier of Tay Nam Co., Ltd (MESSI 10) with germination percent > 80%. Germination was observed daily according to the method of the Association of Official Seed Analysis. The seeds were tested by the top-of-paper method, using Newstart 102 Ø 7 filter paper. The test seeds were placed on sterilized 90 mm Petri dishes. Salinity treatments (0–16 dS $m^{-1}$) were prepared using analytical-grade NaCl (AR ≥99.5% purity; Ghtech, CAS No. 76-14-5). A stock solution with an electrical conductivity (EC) of approximately 20 dS $m^{-1}$ was prepared by dissolving about 12.8 g of analytical-grade NaCl in 1 L of distilled water. The stock solution was then diluted with distilled water to obtain the desired salinity levels (0–16 dS $m^{-1}$). The EC of each solution was verified and adjusted using a conductivity meter.

The experiment was conducted in November 2025 and arranged in a completely randomized design with one factor, salinity (EC dS $m^{-1}$) , comprising 17 EC levels (0–16 dS $m^{-1}$) with three replicates. Each replicate consisted of four Petri dishes containing 50 seeds each (a total of 204 dishes). Salinity was maintained by adding 1 mL of NaCl solution corresponding to each EC level daily to keep the filter paper moist. To minimize evaporation and prevent changes in salt concentration, Petri dishes were tightly sealed during the first 3 days of incubation. Petri dishes were incubated under laboratory conditions at an average temperature of 28°C. Germination was recorded daily at the same time for 3 days. After initial measurements, the lids were removed, and seedlings were allowed to grow under the same conditions until day 6, when shoot length, root length, fresh weight (FW), and dry weight (DW) were measured.

### 2.2. Seed Germination Parameters

Seeds were considered germinated when the radicle emerged at least 1 mm. The following parameters were calculated:

$$\text{Germination Percentage (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100 \quad (1)$$

$$\text{Germination index} = \sum\left(\frac{\text{Gt}}{\text{Tt}}\right) \quad (2)$$

Gt is the number of seeds germinated on day t; Tt is the number of days.

$$\text{MGT (mean germination time)} = \frac{\sum \text{D} \times \text{n}}{\sum \text{nD}} \quad (3)$$

*nD: where n is the number of seeds germinated on day D;*

*D is the number of days counted from the beginning of the test.*

$$T_{50} = t_i + \left[\frac{(0.5N - G_i)}{(G_j - G_i)}\right](t_j - t_i) \text{ (hour)}; \quad (4)$$

*N = total number of seeds germinated at the end of the test (final germination);*

*0.5N = half of the final germination (50%);* $t_i$ *= time (day/hour) just before cumulative germination first exceeds 50%;* $t_j$ *= time just after cumulative germination exceeds 50%;* $G_i$ *= cumulative germination count at time* $t_i$*;* $G_j$ *= cumulative germination count at time* $t_j$*.*

Ten seedlings were randomly selected from each petri dish at the end of the germination period. After that, shoot length and root length were measured in cm.

Fresh weight (FW) and dry weight (DW) of shoots and roots were determined by weighing all seedlings within each replicate and dividing by the total number of seedlings to obtain the mean fresh weight per plant. Dry weight was measured after drying the shoot and root samples at 70°C until a constant weight was reached. A four-digit analytical balance was used, and all samples were collected at the same time, after 7 days.

$$\text{Shoot Vigor Index} = \text{Germination Percentage} \times \text{Mean Shoot Length}$$

$$\text{Root Vigor Index} = \text{Germination Percentage} \times \text{Mean Root Length}$$

Shoot and root dry matter per unit length (DW/L, mg $cm^{-1}$) were calculated as the ratio of dry weight to the corresponding organ length.

### 2.3. Statistical Analysis

Data were analyzed using ANOVA to detect significant differences among treatments. Means were compared using Duncan's test at $p < 0.05$. The response curve was generated using the mean values of three replicates with their corresponding standard errors and plotted in OriginPro 2006 Student Edition.

## 3. Results and Discussion

### 3.1. Results

#### *3.1.1. Germination percent (GP) and Germination index (GI)*

As can be seen from Table 1, the low EC group (0-6 dS $m^{-1}$) got germination percentages reported at 84.7% - 98.7%, significantly more than the high EC group (7-12 dS $m^{-1}$) at 67.6% -78.3%. What stands out in the table is that the germination percent of Pak Choi (*Brassica rapa subsp. chinensis* L.*)* in EC ≥ 13 dS $m^{-1}$ was the lowest and differed significantly from the other treatments.

#### *3.1.2. Mean germination time (MGT) and $T_{50}$*

An overview of the concentration-dependent increase in mean germination time (MGT) of Pak choi seeds under salt stress is provided in Table 1. At low salinity levels (EC ≤ 2 dS $m^{-1}$), MGT values ranged from 1.02 to 1.04 days and did not diverge significantly among treatments (group g). Moderate salinity led to a gradual but significant increase in MGT (1.07 - 1.20 days; groups fg–cdef), causing a marked delay, with the highest MGT recorded at EC 15 - 16 dS $m^{-1}$ (1.59 - 1.70 days; group a).

**Table 1. Effect of different EC levels on seed germination of Pak choi**

| EC (dS $m^{-1}$) | Germination percent (%) (GP) | % Change | Germination index (GI) | % Change | Mean germination time (day) | % Change | $T_{50}$ (hour) | % Change |
|---|---|---|---|---|---|---|---|---|
| 0 | $98.7^{a}$ | 0.0 | $48.5^{a}$ | 0.0 | $1.04^{g}$ | 0.00 | $12.38^{e}$ | 0.00 |
| 1 | $86.7^{ab}$ | -12.2 | $42.8^{ab}$ | -11.7 | $1.02^{g}$ | -1.50 | $12.28^{e}$ | -0.74 |
| 2 | $84.7^{abc}$ | -14.2 | $41.6^{b}$ | -14.3 | $1.03^{g}$ | -0.47 | $12.44^{de}$ | +0.55 |

| EC (dS $m^{-1}$) | Germination percent (%) (GP) | % Change | Germination index (GI) | % Change | Mean germination time (day) | % Change | $T_{50}$ (hour) | % Change |
|---|---|---|---|---|---|---|---|---|
| 3 | $96.8^{a}$ | -1.9 | $47.1^{ab}$ | -3.0 | $1.07^{fg}$ | +2.39 | $12.66^{de}$ | +2.25 |
| 4 | $91.8^{ab}$ | -6.9 | $44.4^{ab}$ | -8.5 | $1.07^{fg}$ | +2.90 | $12.84^{de}$ | +3.73 |
| 5 | $91.3^{ab}$ | -7.4 | $43.4^{ab}$ | -10.5 | $1.1^{efg}$ | +5.73 | $13.29^{de}$ | +7.36 |
| 6 | $89.7^{ab}$ | -9.1 | $42.3^{b}$ | -12.7 | $1.12^{efg}$ | +7.21 | $12.84^{de}$ | +3.72 |
| 7 | $78.3^{bcd}$ | -20.6 | $35.5^{c}$ | -26.8 | $1.2^{cdef}$ | +13.65 | $13.29^{de}$ | +7.39 |
| 8 | $78^{bcd}$ | -20.9 | $32.8^{cd}$ | -32.6 | $1.33^{b}$ | +27.82 | $16.59^{bc}$ | +34.05 |
| 9 | $72.2^{cd}$ | -26.9 | $31.3^{cd}$ | -35.5 | $1.28^{bcd}$ | +22.94 | $16.69^{bc}$ | +34.85 |
| 10 | $70.7^{cd}$ | -28.4 | $32.1^{cd}$ | -33.8 | $1.19^{cdef}$ | +14.54 | $14.04^{cde}$ | +13.44 |
| 11 | $71.8^{cd}$ | -27.2 | $31.0^{cd}$ | -36.1 | $1.27^{bcd}$ | +21.75 | $16.82^{bc}$ | +35.95 |
| 12 | $67.6^{d}$ | -31.4 | $28.5^{d}$ | -41.3 | $1.33^{bc}$ | +27.65 | $17.67^{b}$ | +42.84 |
| 13 | $48.0^{e}$ | -51.4 | $21.7^{e}$ | -55.2 | $1.18^{def}$ | +13.36 | $14.61^{bcde}$ | +18.04 |
| 14 | $45.8^{e}$ | -53.5 | $20.2^{e}$ | -58.4 | $1.23^{bcde}$ | +18.64 | $15.75^{bcd}$ | +27.24 |
| 15 | $45^{e}$ | -54.4 | $16.4^{ef}$ | -66.3 | $1.59^{a}$ | +52.33 | $25.37^{a}$ | +105.00 |
| 16 | $38.3^{e}$ | -61.1 | $13.3^{f}$ | -72.5 | $1.7^{a}$ | +63.42 | $27.02^{a}$ | +118.28 |
| CV(%) | 10.6 | | 9.8 | | 6.3 | | 11.1 | |

*Note: Means followed by different letters within the same column are significantly different at $P < 0.05$ (ANOVA, followed by Duncan's test)*

As shown in Table 1, the time to 50% germination ($T_{50}$) of Pak Choi seeds was significantly increased by salinity ($p \leq 0.05$). Treatments reached the rapid germination period on the first day, whereas the EC $\geq 15$ dS $m^{-1}$ treatment reached the rapid germination stage on the second day. Under low EC 0 - 4 dS $m^{-1}$, no significant increase in $T_{50}$ was observed (12.28 - 12.84 h), indicating that early germination was not adversely affected by mild salinity. A medium postponement was observed at EC 8 - 14 dS $m^{-1}$ (14.04 - 16.82 h). At EC $\geq 15$ dS $m^{-1}$, $T_{50}$ increased sharply, reaching 25.37 - 27.02 h; this result reflects severe osmotic and ionic stress that disturbs ion homeostasis and enzyme activity (Table 1).

*3.1.3. Shoot length and SVI - shoot (Seedling Vigor Index- shoot)*

There were statistically significant differences in shoot length at the 5% level between treatments. At EC 1 - 4 dS $m^{-1}$, shoot length was approximately 3.2 cm. Shoot length decreased gradually, ranging from 2.07 to 2.66 cm at medium salinity (EC 5 - 8 dS $m^{-1}$). This number

continued to decrease sharply at EC ≥10 dS $m^{-1}$, and the lowest value was measured at EC 16 dS $m^{-1}$ (0.72 cm) (Table 2). The trend of shoot length response to salinity was illustrated in Figure 1.

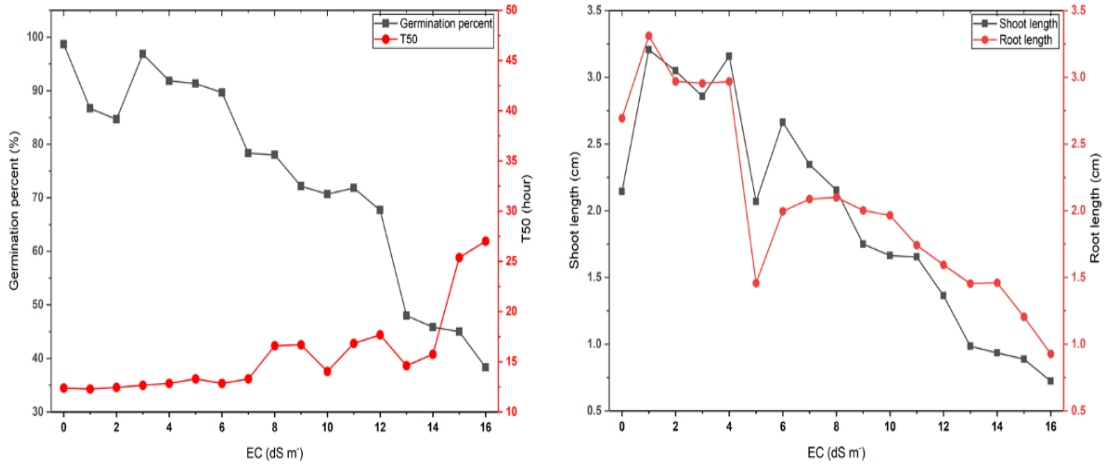


**Figure 1. Germination percent, $T_{50}$, shoot length, and root length of seedling at different EC levels.**

When analysis of variance (ANOVA) SVI showed that the value of shoots at EC levels from 1 to 4 dS $m^{-1}$ was highest (>250) and higher than at EC level 0 dS $m^{-1}$ (212), while this value gradually decreased at medium salinity levels (EC 5 - 8 dS $m^{-1}$). At EC ≥10 dS $m^{-1}$, there was a significant decrease in the SVI of shoots (< 120), and this number reached the lowest value at EC 16 dS $m^{-1}$ (29) (Table 2).

*3.1.4. Root length and SVI - root (Seedling Vitality Index- root)*

As shown in Table 2, root length data for Pak Choi showed significant differences under varying salinity levels, specifically at low salinity (EC 0 - 4 dS $m^{-1}$). The longest recorded roots ranged from 2.69 to 3.31 cm, but they were relatively stable at EC 6 - 10 dS $m^{-1}$ (1.96 - 2.10 cm). Notably, at high salinity (EC ≥ 11 dS $m^{-1}$), root length decreased to below 1.8 cm and reached a minimum value at EC 16 dS $m^{-1}$ (0.93 cm).

Statistical analysis of seedling root vitality index (SVI-root) showed significant differences ($p < 0.05$) between different EC salinity levels. At low salinity (EC 0 - 4 dS $m^{-1}$), the SVI has values from 251 to 287, indicating strong root vigor under mild stress conditions. However, at EC 5 - 10 dS $m^{-1}$ and medium salinity, the SVI-root tends to decrease rapidly to values of 133 - 179. This index continues to decrease sharply at EC ≥ 11 dS $m^{-1}$, with the lowest value recorded at EC 16 dS $m^{-1}$ (35).

**Table 2. Effect of different EC levels on Pak Choi seedling**

| EC (dS.$m^{-1}$) | Shoot length (cm) | % Change | SVI shoot | % Change | Root length (cm) | % Change | SVI root | % Change |
|---|---|---|---|---|---|---|---|---|
| 0 | $2.14^{de}$ | 0 | $212^{d}$ | 0 | $2.69^{b}$ | 0 | $266^{ab}$ | 0 |
| 1 | $3.21^{a}$ | +49.84 | $278^{ab}$ | +31 | $3.31^{a}$ | +22.91 | $287^{a}$ | +8 |
| 2 | $3.05^{ab}$ | +42.42 | $258^{bc}$ | +22 | $2.97^{b}$ | +10.27 | $251^{b}$ | -5 |
| 3 | $2.86^{b}$ | +33.50 | $277^{ab}$ | +31 | 2.95b | +9.70 | $286^{a}$ | +8 |

| EC ($dS.m^{-1}$) | Shoot length (cm) | % Change | SVI shoot | % Change | Root length (cm) | % Change | SVI root | % Change |
|---|---|---|---|---|---|---|---|---|
| 4 | $3.16^{a}$ | +47.57 | $290^{a}$ | +37 | $2.97^{b}$ | +10.20 | $273^{ab}$ | +3 |
| 5 | $2.07^{e}$ | -3.35 | $189^{de}$ | -11 | $1.46^{ef}$ | -45.90 | $133^{def}$ | -50 |
| 6 | $2.66^{c}$ | +24.41 | $238^{c}$ | +13 | $2.00^{cd}$ | -25.90 | $179^{c}$ | -33 |
| 7 | $2.35^{d}$ | +9.60 | $184^{e}$ | -13 | $2.09^{c}$ | -22.49 | $163^{cd}$ | -38 |
| 8 | $2.15^{de}$ | +0.69 | $168^{e}$ | -21 | $2.10^{c}$ | -22.02 | $164^{cd}$ | -38 |
| 9 | $1.75^{f}$ | -18.21 | $126^{f}$ | -40 | $2.00^{cd}$ | -25.65 | $144^{de}$ | -46 |
| 10 | $1.66^{f}$ | -22.28 | $118^{fg}$ | -44 | $1.96^{cd}$ | -27.05 | $138^{def}$ | -48 |
| 11 | $1.65^{f}$ | -22.73 | $118^{fg}$ | -44 | $1.74^{de}$ | -35.32 | $124^{ef}$ | -53 |
| 12 | $1.36^{g}$ | -36.31 | $94^{g}$ | -55 | $1.59^{e}$ | -40.80 | $109^{f}$ | -59 |
| 13 | $0.98^{h}$ | -54.00 | $47^{h}$ | -78 | $1.45^{ef}$ | -46.02 | $69^{g}$ | -74 |
| 14 | $0.93^{hi}$ | -56.33 | $43^{h}$ | -80 | $1.46^{ef}$ | -45.79 | $67^{g}$ | -75 |
| 15 | $0.89^{hi}$ | -58.51 | $39^{h}$ | -82 | $1.20^{fg}$ | -55.28 | $54^{g}$ | -80 |
| 16 | $0.72^{i}$ | -66.26 | $29^{h}$ | -86 | $0.93^{g}$ | -65.59 | $35^{g}$ | -87 |
| CV (%) | 6.6 | | 9.1 | | 8.4 | | 11.8 | |

*Note: Means followed by different letters within the same column are significantly different at $P < 0.05$ (ANOVA, followed by Duncan's test).*

*3.1.5. Seedling biomass (fresh and dry weight)*

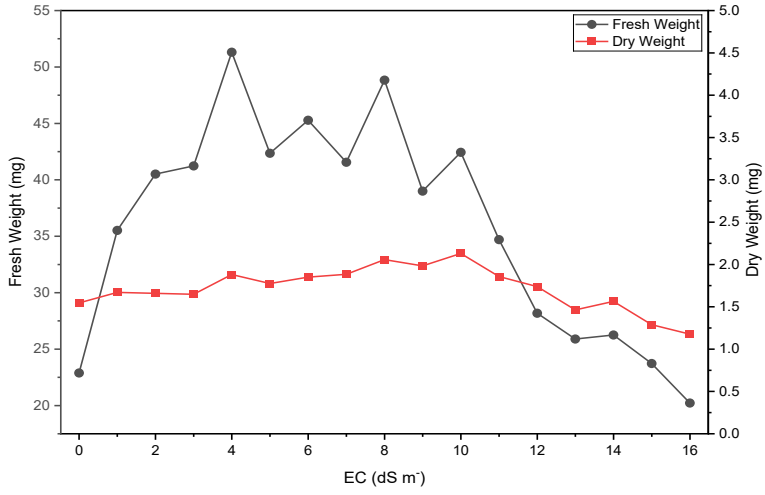


**Figure 2. Fresh weight and dry weight of seedling at different EC levels.**

Statistical analysis of fresh weight (FW) and dry weight (DW) of Pak choi seedlings showed that seedling weight was significantly affected at different salinity levels. Fresh weight increased significantly at low to medium salinity levels, reaching a maximum at EC 4 dS $m^{-1}$ (51.30 mg), more than double that of EC 0 dS $m^{-1}$ (22.88 mg). Fresh weight remained significantly higher than the control at EC 9 dS $m^{-1}$ before decreasing sharply at EC 11 dS $m^{-}$

[1] and falling down to reach its lowest value at EC ≥14 dS $m^{-1}$ (20.22 mg). Besides, seedling dry weight (DW) showed a similar but less pronounced response, with dry weight peaking at EC 10 dS $m^{-1}$ (2.13 mg), significantly higher than the control EC 0 dS $m^{-1}$ (1.55 mg), and then decreasing at higher salinity (EC ≥ 13), reaching its low value of 1.18 - 1.46 mg.

*3.1.6. Shoot and root dry matter per unit length (DW/L, mg $cm^{-1}$)*

There was an analysis of the statistical difference in the dry weight per unit length in shoots and roots of Pak choi at EC levels. ($p < 0.05$). Compared with the control (EC 0 dS $m^{-1}$), where DW shoot/shoot length and DW root/root length were 0.60 and 0.10 mg $cm^{-1}$, respectively, both ratios remained low and statistically unchanged at EC ≤4 dS $m^{-1}$, indicating balanced elongation and biomass accumulation. At moderate salinity levels (EC 5–9 dS $m^{-1}$), DW shoot/shoot length increased gradually to 0.72 - 0.96 mg $cm^{-1}$, while DW root/root length showed a smaller rise (0.14 - 0.17 mg $cm^{-1}$). At high salinity (EC ≥10 dS $m^{-1}$), the DW shoot/shoot length ratio increased sharply and peaked at EC 16 dS $m^{-1}$ (1.51 mg $cm^{-1}$), whereas DW root/root length remained significantly higher than the control but exhibited a more moderate response (Figure 3 ).

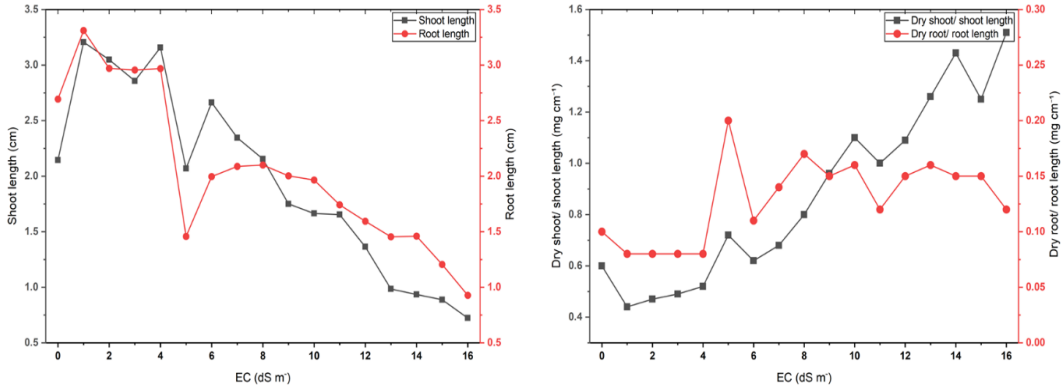


**Figure 3. Shoot length, root length, dry weight per unit shoot length, and dry weight per unit root length at different EC levels.**

### 3.2. Discussion

The present study determined the effect of salinity was clearly influenced by the seed germination of Pak Choi (*Brassica rapa subsp. chinensis* L.*)* by increasing levels as reflected by changes in germination percentage (GP), germination index (GI), mean germination time (MGT), and time to 50% germination ($T_{50}$). Under low salinity conditions (EC ≤6 dS $m^{-1}$), seeds maintained a high germination capacity, and all of the seeds at EC levels germinated relatively rapidly on day 1. However, the postponement of seed germination as salinity increased to moderate and high levels meant that both the final germination percentage and the rate of germination were markedly reduced. When analyzing GP, which was decreased, the estimated $EC_{50}$ value of approximately 13 dS $m^{-1}$ suggests that Pak Choi exhibits only moderate tolerance to salinity during the germination stage. The first stage of seed germination involved water absorption in the seed; at low EC levels, mild salinity did not substantially restrict water uptake or metabolic activation in the seeds. Under such conditions, water always moves by osmosis from an area of higher water potential to an area of lower water potential; this potential gradient is what drives the rapid initial influx of water into the seed so that osmotic adjustment mechanisms may be sufficient to sustain the physiological processes required for germination.

Past studies reported the relevance between seed germination in Brassica species and salinity, where similar responses under low salt stress were observed, indicating that slight salinity did not immediately impair germination performance (Bakirov et al., 2022; Jia et al., 2020). However, once salinity exceeded 7 dS m-¹, both GP and GI declined significantly, suggesting that osmotic stress and ion toxicity began to interfere with normal germination processes. In addition to reducing germination capacity, the most obvious finding emerges from the analysis is that salinity stress also delayed germination, as indicated by the increases in MGT and T50. The results at EC ≤ 4 dS $m^{-1}$ showed that the absence of significant changes in these parameters and early germination events were relatively unaffected by mild salinity. However, at moderate salinity levels, germination was noticeably delayed, while at EC ≥13 dS $m^{-1}$, both MGT and T50 increased sharply; the concurrent decline in GP and GI together with the increase in MGT and T50 indicates that salinity primarily affects the kinetics of germination rather than only final seed viability. In other words, salt stress first slows down water uptake and metabolic activation, which delays radicle emergence, and only at higher salinity levels does it substantially reduce the total number of seeds able to germinate (Vujaković et al., 2017). This coordinated response suggests that germination speed is a more sensitive indicator of salt stress than final germination percentage. The negative effects of salinity on seed germination can be explained by the combined influence of osmotic stress, excessive ion accumulation, and oxidative damage. The gradient pressure between the seed and its environment is high when external salinity reduces the water absorption, thereby slowing imbibition and delaying the reactivation of metabolic pathways (Manono, 2025). At the same time, the accumulation of $Na^+$ and $Cl^-$ ions disrupts cellular ion balance and may impair membrane stability and enzyme function (Yang & Guo, 2018).

Furthermore, the overproduction of reactive oxygen species can damage cellular macromolecules, including proteins, carbohydrates, nucleic acids, and lipids, or cellular structures like membranes, resulting in the inhibition of seed germination (Li et al., 2018; Nadarajah, 2020). Besides, salinity stress is also known to alter hormonal regulation during germination; increased abscisic acid (ABA) levels and reduced gibberellin (GA) consequently downregulate the GA/ABA ratio so that seeds under saline conditions tend to maintain dormancy and delay radicle emergence (Shuai et al., 2017). This hormonal imbalance provides a plausible explanation for the prolonged MGT and T50 observed at higher EC levels in the present study, and similar mechanisms have been reported in other Brassica species and other species exposed to NaCl stress (Hmissi et al., 2024; Khan et al., 2022).

The results of this study showed that early seedling growth of Pak Choi was strongly influenced by increasing EC, as evidenced by reductions in shoot length, root length, and the corresponding vitality indices (SVI-shoot and SVI-root). Both shoot and root growth remained relatively stable, and SVI values were high under low salinity conditions (EC ≤ 4 dS $m^{-1}$), indicating that mild osmotic stress did not substantially impair early development. This experiment detected evidence that seedlings can maintain cell expansion and biomass accumulation within a limited salinity range, likely through effective osmotic adjustment and temporarily stimulating metabolic activity and enhancing seedling development (Nakaune et al., 2012). Under moderate salinity conditions, shoot and root elongation decreased progressively, concomitant with substantial declines in both shoot seedling vigor index and root seedling vigor index. The parallel reduction in organ length and SVI values suggests that limitations in cell expansion are closely associated with reduced seedling vigor under salinity stress (Fatema et al., 2024). Since SVI integrates both germination performance and seedling growth, its stronger decline compared with individual traits indicates that early seedling establishment is governed by the combined effects of delayed germination and restricted post-germination growth. Notably, a non-linear response in seedling growth was observed across

salinity levels. Growth was slightly stimulated at EC 1–4 dS $m^{-1}$ compared to the control, consistent with a hormetic response in which mild ionic stress promotes osmotic adjustment and metabolic activity (Fu & Yang, 2023). However, at EC 5 dS $m^{-1}$, both shoot and root length declined sharply, suggesting that this level represents a physiological threshold beyond which osmotic stress suddenly restricts water uptake before adaptive mechanisms are fully activated. At moderate salinity levels (EC 6–9 dS $m^{-1}$), growth partially stabilized rather than continuing to decline linearly, which may reflect the early activation of ion homeostasis and osmotic adjustment responses under sustained stress.

The concurrent sharp increase in DW/length at EC 5 dS $m^{-1}$ onward further supports a shift from elongation-driven growth to biomass consolidation as the dominant growth strategy under salt stress. Another important point is that the decrease in vitality indices, combined with limitations on organ elongation and dry matter accumulation, indicates that moderate salinity stress restricts water uptake, disrupts nutrient balance, and partially inhibits enzymatic processes involved in cell wall expansion and growth (Basnet et al., 2015).The most effective severe inhibition of seedling growth occurred at high salinity (EC ≥10 dS $m^{-1}$), where both organ length and vitality indices declined sharply. These responses indicate that osmotic stress and ion toxicity exceeded the adaptive capacity of the seedlings, with excessive $Na^+$ and $Cl^-$ accumulation impairing membrane stability and enzyme function, leading to reduced metabolic activity and biomass production (Cheng et al., 2022). The salinity-induced growth inhibition was positively correlated with a higher accumulation of $Na^+$ ions and an elevated $Na^+/K^+$ ratio in radicles and plumules of salt-stressed seedlings. In explanation, adverse salt stress decreases the permeability of the root plasma membrane that induces accumulation of intracellular $Na^+$ content by substituting $K^+$ and other nutrients from plant tissues, increasing the $Na^+/K^+$ ratio and $Na^+$ ion toxicity. The ion toxicity, in turn, persuades osmotic stress and limits the uptake of water, thereby restricting seedlings' growth and development, which is supported by the previous study (Basnet et al., 2015). The following results of the study portrayed a dramatic decline in radicle and plumule length, for example, in choysum seedlings under NaCl stress (Kamran et al., 2021) and *Lactuca sativa* L. (Breś et al., 2022), *Pisum sativum* L. (Khan et al., 2022), and *Lupinus albus* L. (Beyaz & Uslu, 2025). The stronger reduction in SVI compared with organ length alone highlights that seedling vigor is more sensitive to salinity stress than morphological traits. It was underscoring the value of vitality indices as integrative indicators of early seedling performance under saline conditions (Zhang et al., 2022).

This study also found that salinity significantly affected seedling biomass accumulation in Pak choi, as reflected by changes in fresh weight (FW) and dry weight (DW). FW increased markedly compared with the control at low-to- moderate salinity levels and reached a maximum at EC 4 dS $m^{-1}$. The increase in FW without a proportional increase in DW suggests that early biomass stimulation under mild salinity is primarily associated with enhanced water retention rather than true dry matter accumulation. This indicates that tissue hydration plays a dominant role in early seedling growth under low salt stress, while metabolic biomass production becomes more constrained as salinity increases. This discrepancy between FW and DW responses suggests that biomass stimulation under mild salinity was partly driven by increased tissue hydration, which promoted early seedling growth (Ghaffar et al., 2025). However, in this study, both FW and DW at higher salinity levels were found to cause it to decline sharply; these results indicated that osmotic stress and ion toxicity constrained biomass production. Comparison of the findings with those of other studies confirms were similar in *Brassica rapa L.* (Jia et al., 2020), *Brassica napus* L. (Zhang. G et al., 2022).

The most obvious finding to emerge from the analysis is that changes in dry matter per unit length (DW/L) further revealed salinity-induced alterations in growth patterns. DW/L for

both shoots and roots values at EC ≤ 4 dS $m^{-1}$ remained low and comparable to the control, indicating coordinated elongation and biomass accumulation. As salinity increased to moderate levels (EC 5 - 9 dS $m^{-1}$), DW/L growth gradually decreased, particularly in shoots, reflecting reduced elongation relative to dry matter deposition. Under high salinity (EC ≥ 10 dS $m^{-1}$), DW shoot/shoot length increased sharply; this evidences severe inhibition of shoot elongation accompanied by increased tissue density (Wu et al., 2022). The inverse relationship between decreasing organ length and increasing DW/L highlights a shift in growth strategy from elongation to biomass consolidation under salt stress. This implies that cell division and expansion are more strongly inhibited than dry matter accumulation, leading to shorter but denser tissues, which is a typical adaptive response to osmotic stress conditions. The stronger increase in DW/L observed in shoots compared with roots indicates that shoot growth is more sensitive to salinity stress than root growth during early seedling development. This differential sensitivity may be attributed to the greater dependence of shoot elongation on turgor-driven cell expansion, which is highly susceptible to osmotic stress, whereas roots are able to maintain limited elongation to sustain water and nutrient uptake under adverse conditions. Comparatively less suppression of root growth than shoot growth may be credited to lower retention of sodium ions in roots (Xue et al., 2011), so that roots have a more moderate response with the objective of the need to maintain minimal elongation for water and nutrient uptake, while shoot tissues exhibit pronounced growth restraint under high EC levels. Overall, these results demonstrate that Pak choi responds to salinity through biomass response and organ-specific sensitivity, with shoots representing the primary target of salinity-induced growth inhibition at the seedling stage.

## 4. Conclusion

In conclusion, Pak Choi (*Brassica rapa subsp. chinensis* L.) exhibited a clear salinity-dependent response during germination and early seedling growth. Low salinity levels (≤ 4 dS $m^{-1}$) had minimal adverse effects, allowing seeds and seedlings to maintain high germination performance, growth, and vitality. Moderate salinity (5–9 dS $m^{-1}$) caused gradual declines in seedling vigor and biomass accumulation, while high salinity (≥ 10 dS $m^{-1}$) markedly inhibited germination progression, shoot and root elongation, and dry matter production. EC 5 dS $m^{-1}$ appeared to represent a critical threshold, beyond which salinity stress substantially restricted seedling growth. Increased dry weight per unit length under high salinity was associated with reduced elongation and altered biomass distribution. Overall, these findings indicated that seedling vigor indices and biomass-related parameters are sensitive indicators of salinity stress beyond germination rate alone. The study provides useful insights for future research on salt tolerance and for the selection of salt-tolerant crops in saline-affected agricultural systems.

**Acknowledgements:** The author acknowledges Tra Vinh University and Andalas University for institutional support. This research was funded by the Southeast Asian Regional Center for Graduate Study and Research in Agriculture (SEARCA).

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