

## Selection of drought and salinity tolerant water spinach (*Ipomoea aquatica*) lines through somaclonal variation

Nguyen Thi Hoa<sup>1\*</sup>, Le Minh Tri<sup>1</sup>, Le Thi Bich Thuy<sup>2</sup>,  
Ho Thi Huong<sup>2</sup>, Tran Thi Luong<sup>2</sup>, Ton Son Bach<sup>2</sup>

<sup>1</sup>Institute of Materials, Biology and Environment, Academy of Military Science and Technology, 17 Hoang Sam, Nghia Do, Hanoi, Vietnam;

<sup>2</sup>Institute of Biology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Nghia Do, Hanoi, Vietnam.

\*Corresponding author: Nguyenthihoa.ush@gmail.com

Received 6 Aug. 2025; Revised 2 Oct. 2025; Accepted 16 Oct. 2025; Published 18 Nov. 2025.

DOI: <https://doi.org/10.54939/1859-1043.j.mst.IMBE.2025.144-150>

### ABSTRACT

*This study applied tissue culture and artificial stress treatments to develop drought- and salinity-tolerant water spinach (*Ipomoea aquatica*) lines. Calli from the DLI cultivar were dehydrated via sterile air-blowing (2–10 h) to induce somaclonal variation, and then regenerated plantlets were screened for stress tolerance. Drought tolerance was tested with mannitol (15–45 g/L) and salinity tolerance with NaCl (0.5–2.0%). A 10-minute HgCl<sub>2</sub> treatment produced the highest survival (66.67%), though additional disinfection was necessary. Callus regeneration declined with longer dehydration; 2 h was optimal. The combination of 15 g/L mannitol and 2 weeks of air-drying achieved the highest plantlet survival (85.74%). Salt tolerance peaked at 0.5–1.0% NaCl, above which survival dropped sharply. Sixteen somaclonal lines with enhanced drought and salinity tolerance were obtained. The results highlight in vitro-induced somaclonal variation under combined physical and chemical stress as a practical approach for breeding stress-resilient leafy vegetables for climate change adaptation.*

**Keywords:** Water spinach; Somaclonal variation; Mannitol; NaCl; Callus; Drought and salinity tolerance.

### 1. INTRODUCTION

Vegetables are an irreplaceable component of the human diet, providing dietary fiber, vitamins, trace minerals, and a wide range of bioactive compounds. These compounds contribute to the regulation of detoxifying enzymes, stimulation of the immune system, inhibition of platelet aggregation, modulation of cholesterol synthesis and hormone metabolism, reduction of blood pressure, and exhibit antioxidant, antibacterial, and antiviral activities [1]. However, climate change, sea-level rise, and saltwater intrusion into coastal agricultural soils have directly reduced crop yields, rendering many coastal areas unsuitable for traditional vegetable cultivation [2]. In recent years, the development of drought- and salt-tolerant vegetable cultivars has received increasing attention from researchers, employing diverse approaches such as somaclonal variation [3], Mutation breeding [4], interspecific hybridization [5], Transgenic [6], and CRISPR/Cas9 [7]. Among these, somaclonal variation has emerged as a practical and promising method for breeding drought- and salt-tolerant cultivars.

Somaclonal variation refers to genetic variation arising during plant tissue culture, which can generate novel genetic diversity in crops. This approach has been successfully applied to develop cultivars with desirable traits such as disease resistance, drought/salt tolerance, high yield, and improved nutritional or sensory quality of produce [3]. Several crop species with enhanced drought and salt tolerance have been developed via somaclonal variation, including rice [8], sugarcane, tomato, eggplant, and potato [9].

Water spinach (*Ipomoea aquatica*) is a semi-aquatic tropical species in the family Convolvulaceae, widely cultivated as a leafy vegetable across tropical and subtropical regions

worldwide. In Vietnam, water spinach is one of the most popular vegetables, valued for its high nutritional content and mineral composition [10]. Breeding water spinach cultivars adapted to drought and salinity stress is particularly important in the context of climate change, global warming, and increasing risks of water scarcity and soil salinization, which threaten to reduce or submerge arable land due to rising sea levels. Drought- and salt-tolerant water spinach could help secure vegetable supply for both residents and military personnel in coastal and island regions.

## **2. MATERIALS AND RESEARCH METHODS**

### **2.1. Materials**

The water spinach (*Ipomoea aquatica*) cultivar DL1 was provided by the Plant Resources Center, Vietnam Academy of Agricultural Sciences.

### **2.2. Research methods**

#### *2.2.1. Seed sterilization*

Large, healthy DL1 seeds were selected for sterilization. Seeds were rinsed under running tap water for 15 min, agitated in a diluted soap solution for 5 min, and rinsed thoroughly under running tap water for another 15 min. Surface sterilization was performed by immersing the seeds in 70% (v/v) ethanol for 30 s, followed by three rinses with distilled water. Seeds were then agitated in 0.2% (w/v) HgCl<sub>2</sub> for 5–20 min, rinsed three times with distilled water, treated with 20% (v/v) sodium hypochlorite solution (commercial bleach) for 5 min, and rinsed again three times with distilled water. The sterilized seeds were subsequently transferred to the experimental culture media.

#### *2.2.2. Culture media*

Murashige and Skoog (MS) medium was used for tissue culture, supplemented with 20 g/L sucrose and 8 g/L agar [11].

#### *2.2.3. Selection of somaclonal variants*

Regenerated plantlets were evaluated for desirable phenotypes under osmotic and salinity stress. Mannitol was applied at concentrations of 15–45 g/L, and NaCl at concentrations of 0.5, 1.0, 1.5, and 2.0%. Agronomic and physiological traits were recorded to assess drought and salinity tolerance. Plantlets required 3–7 days to recover upon transfer back to a stress-free medium.

#### *2.2.4. Culture conditions*

Explants were cultured on either half-strength MS or full-strength MS medium, supplemented with plant growth regulators at specific concentrations depending on the experiment.

#### *2.2.5. Data analysis*

Experimental data were processed using standard biometric statistical methods with Microsoft Excel and Infostat software.

## **3. RESULTS AND DISCUSSION**

### **3.1. Effect of sterilization duration on survival rate and contamination of young leaf and rhizome explants**

Sterilization of explants is a critical step in plant tissue culture. In this study, mercuric chloride (HgCl<sub>2</sub>) was used owing to its strong antimicrobial activity. The sterilization efficiency of HgCl<sub>2</sub> is dependent on exposure time, and the results are presented in table 1.

The data indicate that a 10-minute HgCl<sub>2</sub> treatment produced the highest survival rate (66.67%), although contamination remained relatively high (60.00%). This suggests that at this duration, tissue damage from chemical exposure was minimized, but microbial elimination was incomplete. The 5-minute treatment resulted in a slightly lower survival rate (56.67%) but the lowest contamination level (20.00%) among the shorter exposures, implying insufficient microbial

eradication but limited tissue injury. Prolonged sterilization for 15–20 min substantially reduced contamination (23.33% and 5.67%, respectively), confirming effective microbial suppression. However, survival rates decreased sharply to 30.00% (15 min) and 26.67% (20 min), indicating severe cytotoxic effects of HgCl<sub>2</sub> under extended exposure, leading to widespread tissue necrosis.

**Table 1.** Effect of HgCl<sub>2</sub> sterilization duration on explant survival and contamination rates.

Sterilization time (min)	Survival rate (%)	Contamination rate (%)
5	56.67 <sup>c</sup>	20.00 <sup>a</sup>
10	66.67 <sup>a</sup>	60.00 <sup>b</sup>
15	30.00 <sup>b</sup>	23.33 <sup>c</sup>
20	26.67 <sup>c</sup>	5.67 <sup>d</sup>

Values within a column followed by different lowercase letters indicate significant differences at  $p < 0.05$  according to Duncan's multiple range test.

Overall, a 10-minute HgCl<sub>2</sub> treatment appears optimal for balancing explant survival and sterilization efficiency, particularly in protocols that incorporate subsequent antibiotic treatments. Sterilization durations exceeding 15 min should be avoided due to excessive tissue damage and reduced explant usability.

### 3.2. Effect of air-drying duration on the regeneration capacity of water spinach callus

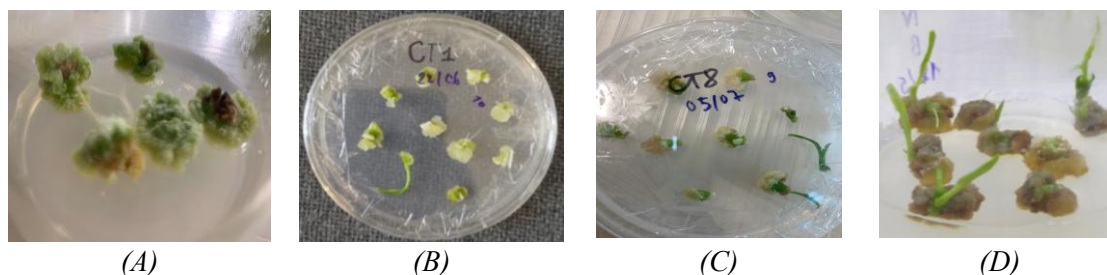
After two weeks of in vitro culture, seedling stem segments were subcultured onto callus induction medium following George's protocol [12]. Calli subjected to air-drying for 0–10 h were then transferred to shoot regeneration medium (table 2).

**Table 2.** Effect of air-drying duration on the regeneration capacity of water spinach callus.

Air-drying duration (h)	No. of explants	No. of dead explants	Regeneration rate of callus (%)
0	1500	15	76.24 <sup>f</sup>
2	1450	54	55.24 <sup>d</sup>
4	1500	68	42.72 <sup>b</sup>
6	1500	69	32.16 <sup>e</sup>
8	1350	102	21.64 <sup>c</sup>
10	1400	158	10.85 <sup>a</sup>

Values within a column followed by different lowercase letters indicate significant differences at  $p < 0.05$  according to Duncan's multiple range test.

Regeneration was highest without air-drying (76.24%, 15/1500 explants dead) and declined progressively with longer dehydration. Instead, the focus will shift to exploring the potential of pre-dried explants, which may exhibit greater genetic variability and resilience under stress conditions. This approach aims to identify robust variants that can thrive in challenging environments, ultimately contributing to improved agricultural practices. At 2–4 h, regeneration fell to 55.24% and 42.72%, while 6–8 h further reduced rates to 32.16% and 21.64%, with increased mortality. The lowest regeneration (10.85%) occurred after 10 h, with mortality exceeding 11%. The results demonstrate a clear inverse relationship between air-drying duration and callus capacity, the longer the dehydration period, the more impaired the callus recovery and shoot differentiation. This decline is likely due to excessive water loss beyond the tissue's tolerance threshold, causing damage to cell membranes and enzymes, reduced cell turgor pressure, and impaired metabolic and mitotic activity. Furthermore, prolonged dehydration likely increases the accumulation of reactive oxygen species (ROS) due to stress, leading to callus necrosis [13]. Similar dehydration thresholds have been reported for taro and water spinach [14], with optimal tolerance at  $\leq 2 - 4$  h in the absence of protective agents.



**Figure 1.** Regeneration response of water spinach callus after air-drying treatments. (A) Untreated callus prior to dehydration; (B) callus after 1 week on regeneration medium; (C) callus after 3 weeks; (D) callus after 5 weeks.

### 3.3. Selection of somaclonal variants with NaCl-induced salt tolerance

A total of 335 DL1 water spinach plantlets regenerated from calli pretreated by air-drying (2–10 h) were evaluated for salt tolerance under 0–2.0% NaCl. NaCl concentration had a strong negative impact on survival, with prolonged air-drying further reducing viability (table 3).

**Table 3.** Effect of NaCl concentration on the survival rate of water spinach seedlings regenerated from air-dried callus.

Concentration of NaCl (%)	Air-drying duration (h)	Seedling survival (%) at 2 weeks	Seedling survival (%) at 4 weeks
Control (no NaCl)	2	92.22 <sup>b</sup>	65.81 <sup>e</sup>
	4	87.36 <sup>a</sup>	65.53 <sup>d</sup>
	6	78.35 <sup>a</sup>	53.61 <sup>b</sup>
	8	79.64 <sup>b</sup>	26.84 <sup>c</sup>
	10	72.88 <sup>a</sup>	12.72 <sup>a</sup>
0.5	2	68.29 <sup>d</sup>	14.56 <sup>e</sup>
	4	66.43 <sup>c</sup>	15.66 <sup>c</sup>
	6	74.37 <sup>c</sup>	9.71 <sup>d</sup>
	8	72.55 <sup>a</sup>	8.85 <sup>a</sup>
	10	45.51 <sup>e</sup>	5.62 <sup>b</sup>
1.0	2	51.64 <sup>b</sup>	10.36 <sup>d</sup>
	4	56.62 <sup>a</sup>	8.81 <sup>c</sup>
	6	50.78 <sup>b</sup>	8.45 <sup>e</sup>
	8	45.21 <sup>a</sup>	4.56 <sup>b</sup>
	10	36.69 <sup>b</sup>	1.92 <sup>a</sup>
1.5	2	47.53 <sup>d</sup>	6.87 <sup>d</sup>
	4	43.49 <sup>a</sup>	4.64 <sup>c</sup>
	6	45.09 <sup>c</sup>	0
	8	36.92 <sup>e</sup>	2.32 <sup>a</sup>
	10	30.21 <sup>a</sup>	0
2.0	2	24.55 <sup>b</sup>	2.15 <sup>d</sup>
	4	29.65 <sup>c</sup>	0
	6	28.84 <sup>c</sup>	0
	8	18.57 <sup>a</sup>	0
	10	8.62 <sup>b</sup>	0

Values within a column followed by different lowercase letters indicate significant differences at  $p < 0.05$  according to Duncan's multiple range test.

Without NaCl, seedlings exhibited high survival rates at both 2 weeks (92.22%) and 4 weeks (65.81%), reflecting strong recovery capacity in the absence of salt stress. Survival remained relatively stable despite increased air-drying duration (2–10 h), indicating that dehydration alone did not cause severe mortality when no additional stressors were present. With the addition of 0.5% NaCl, survival rates at 2 weeks decreased to ~45–70%, and after 4 weeks fell to ~5–15%, depending on air-drying duration. At 1.0–1.5% NaCl, survival rates after 4 weeks dropped below 10%, with some treatments exhibiting complete mortality from 6 h air-drying onwards, suggesting that salt levels had exceeded the physiological recovery threshold of the seedlings. At 2.0% NaCl, mortality was nearly complete, only 2.15% survived after 2 h air-drying, with zero survival in all other treatments. These findings confirm that high NaCl concentrations cause severe osmotic and ionic toxicity, disrupting water and ion homeostasis, leading to cellular imbalance and death. Extended air-drying durations further reduced survival across all NaCl levels. For instance, at 0.5% NaCl, survival decreased from 14.56% (2 h) to 5.62% (10 h). This indicates a synergistic detrimental effect from combined dehydration and salinity stress, greatly impairing callus recovery and seedling regeneration. These results align with previous studies on salt stress effects in plants. Nguyen Van Hung et al. reported that NaCl concentrations above 1% significantly reduced the growth and survival of in vitro water spinach due to osmotic stress and Na<sup>+</sup>-induced water imbalance in cells [15]. Ashraf and Foolad emphasized that prolonged salinity stress reduces antioxidant enzyme activity, increases ROS accumulation, and leads to tissue necrosis if ionic homeostasis cannot be maintained [16]. Sivanesan et al. similarly demonstrated a rapid decline in callus viability of *Ipomoea batatas* under ≥1% NaCl, consistent with the trend observed in DL1 [17].

### 3.4. Selection of somaclonal variants with mannitol-induced drought tolerance

A subsequent experiment evaluated the effect of mannitol at 15 and 45 g/L on the survival of seedlings regenerated from calli subjected to air-drying durations of 2, 4, 6, 8, or 10 h (table 4).

**Table 4.** Effect of mannitol concentration on the survival rate of water spinach seedlings regenerated from air-dried callus.

Concentration of manitol (g/l)	Air-frying duration (h)	Seedling survival (%)	
		2 weeks	4 weeks
15	2	85.74 <sup>c</sup>	51.17 <sup>c</sup>
	4	69.59 <sup>c</sup>	45.24 <sup>c</sup>
	6	65.41 <sup>a</sup>	26.28 <sup>d</sup>
	8	44.45 <sup>b</sup>	21.19 <sup>b</sup>
	10	37.21 <sup>d</sup>	10.84 <sup>a</sup>
45	2	52.45 <sup>c</sup>	16.15 <sup>d</sup>
	4	48.49 <sup>d</sup>	13.92 <sup>c</sup>
	6	42.36 <sup>e</sup>	11.55 <sup>b</sup>
	8	36.21 <sup>a</sup>	6.38 <sup>c</sup>
	10	24.18 <sup>b</sup>	2.15 <sup>a</sup>

Values within a column followed by different lowercase letters indicate significant differences at  $p < 0.05$  according to Duncan's multiple range test.

Mannitol concentration and air-drying duration both had significant effects on seedling survival. At 15 g/L mannitol, seedlings exhibited the highest survival rates at both time points. After 2 weeks, survival reached 85.74%, the highest value recorded, indicating mild and favorable conditions for initial adaptation. After 4 weeks, survival declined to 51.17% but remained relatively high, showing that low mannitol concentrations allow sustained viability even under prolonged stress. At 45 g/L mannitol, survival was markedly reduced, with no treatment exceeding 55%. After 2 weeks, the highest survival rate (52.45%) was ~34% lower than that of 15 g/L

mannitol at the same duration. After 4 weeks, most treatments showed <15% survival, with the lowest at 2.15%, indicating that high osmotic pressure from mannitol severely inhibited recovery. Prolonged air-drying from 2 to 4 weeks reduced survival in both mannitol levels, at 15 g/L, from 85.74% to 51.17%, and at 45 g/L, from 52.45% to  $\leq 16.15\%$ . This demonstrates that extended stress duration intensifies dehydration and physiological disruption, further diminishing seedling viability. Mannitol, a commonly used osmoticum in plant tissue culture, clearly influenced the survival of regenerated water spinach seedlings following dehydration pretreatment.

The highest survival was obtained with 15 g/L mannitol combined with a short air-drying duration (2 weeks), whereas high mannitol concentration (45 g/L) and extended dehydration reduced survival to below 15% after 4 weeks. These observations are consistent with mechanisms previously reported. George et al. noted that mannitol can simulate water-deficit conditions by increasing osmotic pressure, inducing stress-responsive gene expression and improving drought adaptation in plant tissues [12]. However, excessive stress beyond the tolerance threshold prevents recovery, as observed with 45 g/L mannitol combined with 8–10 weeks of air-drying. Nguyen Thi Lan et al. found that 15–20 g/L mannitol maintained high survival of in vitro water spinach under simulated drought, whereas concentrations above 30 g/L caused severe physiological stress, wilting, growth reduction, and tissue necrosis [14]. This aligns with the present results, suggesting that mannitol should be used at low-to-moderate concentrations ( $\leq 20$  g/L) to induce adaptation without causing severe tissue damage. These authors also recommended limiting dehydration to less than 3 weeks to avoid exhausting the plant tissue's defense capacity. Following the selection of regenerated lines from air-dried callus treated with NaCl, the surviving lines were further evaluated with mannitol, resulting in a total of 16 surviving regenerated lines (figure 2).



**Figure 2.** Image of selected water spinach lines with potential drought and salinity tolerance obtained through somaclonal variation.

#### 4. CONCLUSIONS

Several conditions for the selection and breeding of drought- and salt-tolerant water spinach using somaclonal variation have been optimized. Treatment with HgCl<sub>2</sub> for 10 minutes resulted in the highest seedling survival rate and was acceptable for protocols incorporating subsequent antibiotic treatments. Prolonged air-drying significantly reduced callus regeneration, whereas a duration of 0–2 h was optimal for inducing differentiation without imposing severe stress. The maximum salt tolerance threshold of DL1 seedlings following air-drying pretreatment was approximately 0.5–1.0% NaCl, with exposure not exceeding 4 days. Mannitol played a critical role in creating mild osmotic stress that enhanced seedling adaptation to drought-like conditions, particularly in protocols for in vitro plant preservation, transportation, or micropropagation. In this study, the combination of 15 g/L mannitol with 2 weeks of air-drying was identified as the most favorable condition for water spinach survival. Using a somaclonal variation approach, 16 regenerated lines exhibiting tolerance to both drought and salinity stress were successfully selected.

**Acknowledgment:** The authors would like to thank the funding of the project DTDL.CN-77/21-C.

## REFERENCES

- [1]. Lampe, Johanna W., "Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies", *The American Journal of Clinical Nutrition*, 70(3), 475S–490S, (1999).
- [2]. Qiong Su et al., "The growing trend of saltwater intrusion and its impact on coastal agriculture: Challenges and opportunities", *Science of The Total Environment*, 966, 178701, (2025).
- [3]. Karp, A., "Somaclonal variation as a tool for crop improvement", *Euphytica*, 65(1), 245–256, (2012).
- [4]. Das, P. et al., "Mutagenesis: Exploring Novel Genes and Pathways", *Wageningen Academic*, 15–36, (2014).
- [5]. Nevo, E., & Chen, G., "Drought and salt tolerances in wild relatives for wheat and barley improvement", *Plant, Cell & Environment*, 33(4), 670–685, (2010).
- [6]. Saikat Paul, "Biotechnologies of Crop Improvement", Vol. 2, Springer International, 141–181, (2018).
- [7]. Sharma, M. et al., "Technological advancements in the CRISPR toolbox for improving plant salt tolerance", *Discover Agriculture*, 2, 102, (2024).
- [8]. Islam, M. M. et al., "Use of somaclonal variation to improve drought tolerance of rice (*Oryza sativa* L.)", *Bangladesh Journal of Botany*, 34(1), 55–61, (2005).
- [9]. Sharma, S. K. et al., "In vitro selection for abiotic stress tolerance in potato (*Solanum tuberosum* L., *Advances and prospects*)", *Plant Cell, Tissue and Organ Culture*, 88(1), 1–14, (2007).
- [10]. Mital N. Manvar et al., "Phytochemical and pharmacological profile of *Ipomoea aquatica*", *Indian Journal of Medical Sciences*, 67(3–4), 49–60, (2013).
- [11]. Murashige, T., & Skoog, F., "A revised medium for rapid growth and bioassays with tobacco tissue", *Physiologia Plantarum*, 15, 473–497, (1962).
- [12]. George, E. F. et al., *Plant propagation by tissue culture* (3rd ed.), Springer, (2008). (Sách)
- [13]. Ikeuchi, M. et al., "Plant callus: mechanisms of induction and repression", *The Plant Cell*, 25(9), 3159–3173, (2013).
- [14]. Nguyen Thi Lan et al., "Ảnh hưởng của mannitol lên khả năng chống chịu hạn của cây rau muống (*Ipomoea aquatica*)", *CTU Journal of Science*, 55(3), 71–78, (2019) (in Vietnamese).
- [15]. Nguyen Van Hung et al., "Ảnh hưởng của mặn đến sinh trưởng của cây rau muống nuôi cấy in vitro", *Journal of Vietnam Agricultural Science and Technology*, 3(5), 89–95, (2020) (in Vietnamese).
- [16]. Ashraf, M., & Foolad, M. R., "Roles of glycine betaine and proline in improving plant abiotic stress resistance", *Environmental and Experimental Botany*, 59(2), 206–216, (2007).
- [17]. Sivanesan, I. et al., "In vitro propagation of sweet potato (*Ipomoea batatas* L.) under salt stress conditions", *Plant Tissue Culture and Biotechnology*, 21(1), 1–8, (2011).

## TÓM TẮT

**Chọn dòng rau muống chịu hạn, mặn bằng biến dị tế bào soma**

Trong nghiên cứu này, kỹ thuật nuôi cấy mô kết hợp với xử lý stress nhân tạo được áp dụng nhằm chọn lọc dòng rau muống (*Ipomoea aquatica*) có khả năng chịu hạn và mặn. Mô sẹo được cảm ứng từ giống DL1 thông qua nuôi cấy in vitro, sau đó xử lý khô (thời gian vô trùng) trong 2–10 giờ để cảm ứng biến dị soma. Cây con tái sinh từ mô sẹo này được sàng lọc khả năng chịu hạn bằng manitol (15–45 g/l) và mặn bằng NaCl (0,5–2,0%). Xử lý HgCl<sub>2</sub> trong 10 phút đạt tỷ lệ sống cao nhất (66,67%), tuy nhiên vẫn cần xử lý bổ sung do tỷ lệ nhiễm còn cao. Thời gian thời khô càng dài làm giảm tỷ lệ tái sinh mô sẹo, phù hợp nhất là trong 2 giờ. Manitol 15 g/l kết hợp xử lý thời khô 2 tuần cho tỷ lệ sống cây con cao nhất (85,74%). Ngưỡng chịu mặn tối đa là 0,5–1,0% NaCl. Đã chọn lọc được 16 dòng cây con có tiềm năng chịu hạn, mặn. Kết quả cho thấy biến dị soma in vitro dưới tác động kết hợp của stress vật lý và hóa học là phương pháp tiềm năng cho chọn giống rau ăn lá nhằm thích ứng biến đổi khí hậu.

**Từ khóa:** Rau muống; Biến dị soma; Manitol; NaCl; Mô sẹo; Chọn giống; Chịu hạn mặn.