Original Research Article

Impact of Salinity Stress on Germination of Water Spinach (*Ipomoea aquatica*)

ABSTRACT

Aims: Salinity is one of the major abiotic stress that negatively affects plant growth in germination and early seedling stages. Salinity has becoming a serious problem as most of the parts of worldwide lands were affected by high salt concentration. Therefore, the effects of salinity ranging from 0 mM, 25 mM, 50 mM and 75 mM Sodium chloride (NaCl) concentrations on germination and early seedling growth of water spinach and their salt tolerance mechanism.
Study design: Completely Randomized Design (CRD).
Place and Duration of Study: This study was conducted at Tissue Culture Laboratory, Department of Biology, Faculty of Science in Universiti Putra Malaysia (UPM) from June 2018 to August 2018.
Methodology: In order to study the effects of salinity on water spinach, several parameters have been taken into account for measurement which include water uptake percentage, germination percentage, germination index, mean germination time, relative injury rate, seed vigor, seedling height reduction, hypocotyl and radicle length, seedling biomass, salt tolerance, total phenolic content and total flavonoids contents.
Results: The results obtained showed that salinity adversely reduced water uptake efficiency, seed vigor, hypocotyl and radicle length, total phenolic content and total flavonoids content of water spinach. The seedling height reduction of water spinach increased significantly in relative to increasing salinity. However, seeds treated in mild salt concentration at 25 mM of NaCl showed an increment of germination percentage and germination index.
Conclusion: Salt tolerance of water spinach increased as the response towards increasing salinity.

Keywords: salinity, germination, growth, completely randomized design (CRD), salt tolerance

1. INTRODUCTION

Ipomoea aquatica or its common name called water spinach belongs to the family Convolvulaceae. It is an aquatic vascular plant that grows wild and commonly cultivated in Southeast Asia, India and Southern China [1]. Water spinach generally found on moist soil along the margins of fresh water, marshes, ditches and wet rice fields [2]. *Ipomoea aquatica* is a trailing or floating herbaceous plant with long and hollow stem occupied with large number of air passages and rooting at the nodes [3]. Water spinach is restricted to the tropics and subtropics thus does not grow well if the mean temperature is below 24ºC.

Water spinach which also known as kangkong is commonly used to treat fever, bronchitis, constipation and liver complaints in Unani. While in Ayurveda, this plant is effective to treat jaundice and nervous debility. Furthermore, the leaf extract of water spinach is useful to reduce blood sugar levels [5]. *Ipomoea aquatica* consists of high nutritional values with substantial amount of amino acids, non-essential amino acids, macro salts, micro salts, crude fibers, carbohydrates, fatty acids, organic acids and polyphenols [6]. These components are a WHO-recommended pattern for an ideal dietary protein and potentially as food supplement as it comparable to conventional foodstuffs like soybean or egg.

Abiotic stress such as drought and salinity greatly influence plant growth and crop productivity worldwide [7]. A significant part of the world's land area is considered salt-affected by salinity or sodicity. The presence of salinity is mostly natural.

Unfortunately, cultivation in agriculture has made a huge amount of land to become saline due to land reclamation or irrigation [8]. Munns (2005) stated that irrigated land composed of 15% of total cultivated land. Tanji and Wallender estimated that 23% of cultivated lands could be affected by salinity [9]. According to Rengasamy (2002), salts arise from weathering of rocks or aerial deposition of ocean aerosols through wind or rain contributes to salinity [10]. The main salt in the saline soils generally NaCl however there are also other significant concentrations of Ca^{2+}, Mg^{2+}, SO_4^{2-} and CO_3^{2-} [8]. Salinity may bring adverse effects by limiting the plant growth when salts accumulated in the root zone [11]. Salt stress causes detrimental effects in most major processes including germination, growth, photosynthetic pigments and yield [12]. Salt stress also being a factor of delayed seed germination and final germination percentage. It affects germination and seedling growth by creating osmotic pressure which prevents water uptake or by toxic effects of sodium (Na^+) and chloride (Cl^-) ions [13]. Seed germination is the fundamental and vital phase of a plant's growth cycle as it determines the yield. Researcher stated that salinity adversely affects germination process in various plants such as *Posidonia*, *Oryza sativa*, *Triticum aestivum*, *Brassica* sp. and *Zea mays* [14]. According to Gul and co-worker's salinity changes the imbibition of water by seeds due to lower osmotic potential of germination media [15]. Furthermore, salinity induces toxicity which alters the enzymes activities of nucleic acid metabolism, alters protein metabolism and disturbs the hormonal balance in the seeds [16].

2. MATERIAL AND METHODS

2.1 Seed Sterilization

Seeds of water spinach (*Ipomoea aquatica*) were obtained from Green World Sri Serdang, Selangor. Seed sterilization was conducted according to a method reported by Hassen [17]. Seeds of water spinach were surface sterilized for 20 minutes in 5% of Sodium hypochlorite (NaOCl). Then, the seeds were rinsed 3 times with distilled water for 2 minutes.

2.2 Preparation of Saline Solution

Three different concentration of Sodium chloride (NaCl) were prepared separately from 25mM, 50mM and 75mM. Deionized distilled water was used as a control.

Table 1: Preparation of Saline Solution

Treatment (mM)	Amount of NaCl (g)	Amount of Distilled Water (mL)
0	**0**	**1000**
25	**1.46**	**1000**
50	**2.92**	**1000**
75	**4.38**	**1000**

2.3 Experimental Design

Ten sterilized seeds were transferred into sterile petri dishes (9 cm diameter) that have been layered with one piece of Whatman filter paper no. 1 containing 5 ml of deionized distilled water (for control) or 5 ml of different concentration of NaCl solutions including 25mM, 50mM and 75mM. The experiment was arranged in a completely randomized design (CRD) with five replicates per treatment for three cycles [18]. The petri dishes were sealed with parafilm to avoid evaporation and the seeds were left to germinate at room temperature of 25 ± 1ºC.

2.4 Observation

Number of germinated seeds were recorded daily during 10 days for each cycle. Seeds were considered germinated as soon as the roots protruded 2 mm through the pericarp [19]. The seedlings of water spinach were retained for measurements of hypocotyl length, radicle length, fresh weight and dry weight at the end of the experiment.

2.5 Data Collection

2.5.1 Water Uptake Percentage

The percentage of water uptake by the seeds sown were calculated according to the formula [20]

$$WUP = [(\text{Seed fresh weight} - \text{Seed dry weight}) / \text{Seed fresh weight}] \times 100$$

2.5.2 Germination Percentage

Germination percentage of seeds were calculated after 10 days by using the following equation [21]

$$GP = (\text{Number of germinated seeds} / \text{Total number of seeds sown}) \times 100$$

2.5.3 Germination Index

Germination index (GI) of seeds were calculated using the equation [22]

$$GI = \sum (\text{Number of germinated seed} / \text{Days of last count})$$

2.5.4 Mean Germination Time

Mean germination time is calculated according to equation [17]

$$\text{Mean germination time (MGT)} = \sum Dn / \sum n$$

where n is the number of seeds which were germinated on day D, and D is the number of days counted from the beginning of germination.

2.5.5 Relative Injury Rate

Relative injury rate was calculated according to a formula reported by Tsegay and Gebreslassie (2014) as the difference between germination percentage in control and germination percentage in salt treated seeds divided by the germination percentage in control [23].

$$RIR = (\text{Germination percentage in control} - \text{Germination percentage in salt treated seeds}) / \text{Germination percentage in control}$$

2.5.6 Seed Vigor

Seed vigor was calculated based on following equation [22]

$$\text{Seed Vigor} = [(\text{Length of radical} + \text{length of hypocotyl}) / 100] \times GP$$

2.5.7 Seedling Height Reduction

Seedling height reduction (SHR) is defined as the delay in root length and shoot length expressed in percentage and calculated using the equation [24].

$$SHR = [(\text{Plant height in control} - \text{Plant height in salt treatment}) / \text{Plant height in control}] \times 100$$

2.5.8 Seedlings Biomass

The seedlings biomass was weighed by using analytical balance after dried in circulating oven at 80ºC for 24 hours in order to standardize the mass [25].

2.5.9 Salt Tolerance

The salt tolerance (ST) rate was calculated using the standard formula as follows [26]

$$ST = (\text{Seedling dry weight in salt treatment} / \text{Seedling dry weight in control}) \times 100$$

2.5.10 Total Phenolic Content

Total phenolic content in water spinach were determined by using Folin-Ciocalteau reagent based on the method using a gallic acid as a standard phenolic compound [27]. About 1.0 mL of aqueous extract solution containing 1.0 g extract was diluted with 46 mL distilled water in a volumetric flask. Then, 1.0 mL of Folin-Ciocalteau reagent was added and mixed thoroughly. Three minutes later, 3.0 mL of 2% sodium carbonate was added and the mixture was allowed to stand for three hours with sporadic shaking. The absorbance of the blue color that developed was measured at 760 nm by using spectrophotometer. The concentration of phenolic was expressed as mg/g of dry extract. The amount of total phenolics was calculated as gallic acid equivalents (GAE) from the calibration curve obtained from gallic acid standard solution and expressed as mg GAE/g dry weight.

2.5.11 Total Flavonoids Content

Total flavonoid contents in water spinach were determined by using the calorimetric method with slight modification [28]. In brief, 100 µL of aqueous seed extract or standard solution was mixed with 400 µL of ethanol and followed by the addition of the same volume of 2% $AlCl_3$ solution diluted in ethanol. The mixture was incubated for an hour at room temperature. Then, the absorbance was measured at 517 nm. Rutin was used to plot the standard curve and the results were expressed as the mean in mg of rutin equivalents per gram of plant material from triplicate extracts (mg rutin/g dry weight).

2.6 Data Analysis

Statistical analysis was analysed using SPSS Window Version 24. One-way analysis of variance (ANOVA) was used to determine the significance difference among treatments followed by Duncan's Multiple Range Test (DMRT) at p<0.05 for mean comparison.

3. RESULTS AND DISCUSSION

3.1 Effect of Salinity on Water Uptake Percentage of *I. aquatica*

Table 2 shows that the water uptake percentage of water spinach seeds under all salt treatments were inversely related with NaCl concentration level. The result indicates that the water uptake percentage of water spinach reduced significantly (p<0.05) at 75 mM. Seeds treated under both 25 mM and 50 mM NaCl solution showed no significant difference as compared with control. As salinity level increases, the water uptake percentage of water spinach decreases gradually. Soils containing excess sodium chloride limits the plant available water which results in dehydration of the cell cytoplasm [29]. The entry of water into the seeds is profoundly influenced by the nature of seed coat. The water uptake percentage of water spinach decreases as the salinity level increases. Researchers stated that water uptake imbalance limits the hydrolysis of food reserve and cause immobilization of food reserve from storage tissue to developing embryo [30]. The result obtained is similar to the findings from salinity study of bean and *Oryza sativa* L. cv. MR219 [31] [32]. Water absorption in cell differentiation and cell division is reduced due to salt stress that eventually lead to osmotic pressure. The concentration of soluble salts with their respectively high osmotic pressures affect plant growth by inhibiting water uptake in the roots [33]. So it can concluded that enhanced in salinity stress reduced the water uptake in *I. aquatica*.

Table 2: Impact of salinity stress on germination properties of *I. aquatica.* Values are mean ± standard error of mean of fifteen replicates (N=15). Superscripts within the means of each column (a-b) with different letters indicate significant differences among the means (Duncan's Multiple Range Test, p<0.05).

Salt Concentration (mM)	Water Uptake Percentage (%)	Germination Percentage (%)	Germination Index	Mean Germination Time	Seed Vigor	Seedling Height Reduction (cm)
0	76.22 ± 2.68a	80.00 ± 4.14ab	0.80 ± 0.04ab	3.86 ± 0.14a	4.78 ± 0.49a	0.00 ± 0.00c
25	71.14 ± 2.90ab	84.67 ± 3.36a	0.85 ± 0.03a	3.63 ± 0.15ab	4.34 ± 0.23ab	10.89 ± 3.30bc
50	66.74 ± 3.71ab	77.33 ± 3.16ab	0.77 ± 0.03ab	3.36 ± 0.15bc	3.70 ± 0.22bc	14.57 ± 4.49b
75	62.36 ± 4.53b	72.67 ± 4.19b	0.73 ± 0.04b	3.09 ± 0.17c	2.84 ± 0.19c	25.88 ± 5.33a

3.2 Effect of Salinity on Germination Percentage of I. aquatica

Seed germination is a crucial and most salt-sensitive plant growth stage that severely affected by increasing salinity. Seed germination begins from the imbibition of dry seeds. The imbibition of seed germination by salinity stress usually followed by toxic ion effects, water uptake deficiency and decreases in nutrient mobilization [34]. In present study, the effects of different NaCl concentrations on seed germination and early seedling growth of *Ipomoea aquatica* were compared. The result shows that there were no statistically significant difference at ($p<0.05$) on germination percentage of *I.aquatica* seeds under different salinity levels as shown in Table 2. Seeds of water spinach germinated the most under very mild salinity level at 25 mM whereas the least germinated seeds were under 75 mM of NaCl solution. The germination percentage of the water spinach seeds were slightly decreased along with increasing salinity level. Seeds treated in control treatment has less germination percentage compared to seeds treated in very mild concentration at 25 mM NaCl solution. Higher salinity level at 50 mM and 75 mM decrease the amount of germinated seeds. Thus, it was observed that the seeds required longer time to germinate as high salinity delayed seed germination.Salt stress restricts water absorption by the seeds and consequently decrease the total germination percentage [35]. decrease in seed germination under salinity stress is due to physicochemical effects or by osmotic-toxic salts that exist in saline conditions [36]. Furthermore, the high concentrations of Na^+ and Cl^- in the environment delay the seed germination by inducing toxicity in seeds [37]. In present study, seeds of water spinach showed no significant difference in germination percentage. However, seeds treated under 25 mM NaCl solution had a slight increment in germination percentage as compared to control. This result agrees with previous studies reported on the halophytes *Salicornia europaea* and *Suaeda maritima* and New Zealand spinach where low levels of salt treatment improved plant growth [38] [39].

3.3 Effect of Salinity on Germination Index of I. aquatica

Germination index are used to indicate the uniformity and speed of the seed germination as proposed by Deinlein et al. (2014). The result shows that the germination index of water spinach under different salinity were not significantly difference at ($p<0.05$). The highest germination index was the seeds treated under 25 mM NaCl solution while the lowest was seeds treated under 75 mM NaCl solution as shown in Table 2. The germination index reflects the germination percentage on each day along the germination period. Higher germination index values indicate higher and faster germination [40]. Mean germination time and germination index could lead to an enhancement in the salt tolerance during germination phase. The present findings showed no significant difference in germination index of water spinach seeds. Seeds treated under low salinity level (25 mM NaCl) has higher germination index as compared to control. Increment in germination index values indicates the decline in phytotoxicity and eventually more mature germinated seeds [41]. Whereas seeds treated under higher salinity levels (50 mM and 75 mM NaCl) showed a decline in germination index. This result is aligning with the findings in *Zea mays* that showed decreased germination index at higher salt concentrations [42].

3.4 Effect of Salinity on Mean Germination Time of I. aquatica

The result shows that the mean germination time of water spinach under different salinity level were significantly difference at ($p<0.05$). Seeds treated under control treatment possess the highest mean germination time whereas seeds treated in 75 mM NaCl solution possess the lowest mean germination time. The time it takes for the seeds to germinate decreases along with increasing salinity level as shown in Table 2. Mean germination time is considered as mean of the lag period for all seeds in a sample between the imbibition of seeds and the first sign of germination [43]. mean germination time increased with salinity stress. However, in present study, the mean germination time of water spinach decreased significantly with increasing NaCl concentration. Seeds of water spinach treated in 75 mM NaCl treatment experienced the lowest mean germination time. It means that seeds of water spinach have develop tolerance and germinate under high salinity. The seeds of water spinach are still capable to germinate if detrimental effect of salinity is not severe during dormancy period. This is contradicting to the findings of in mango and *Lathyrus sativus* and *Pisum sativum* var. *abyssinicum* which showed increasing mean germination time as the salinity level increases [44] [45].

3.6 Effect of Salinity on Seed Vigor of I. aquatica

As the salinity level increases, the general trend of seed vigor of water spinach was associated with significant ($p<0.05$) decrease as shown in Table 2. Seeds of water spinach treated under control treatment presented the best seed performance while the lowest seed vigor was experienced by seeds treated under 75 mM NaCl solution.Seed vigor is a vital index for seed quality that particularly determines the potential for rapid and uniform emergence of plants. High seed vigor is aligned with potential of increasing growth and productivity agricultural productivity. Seed weight and seed nutrient content gives impact to plant growth at seedling period [47]. Earlier studies done to showed a close relationship between seed size or seed weight and early vigor in rice which reveals that rice seed with thin hull and large embryo are preferable for seed vigor [48]. In the present study, Table 2 showed that the vigor of water spinach seeds decreased relative to increasing salinity. Similarly, the decline in seed vigor of melon, *Citrullus colocynthis* as the salt concentration increases [49].

3.7 Effect of Salinity on Seedling Height Reduction of I. aquatica

The seedling height reduction of water spinach seedlings were observed to have a linear relationship with increasing salinity level. The result in Table 2 shows that the seedling height reduction was significantly difference at ($p<0.05$). Moreover, the seedling height reduction in water spinach declined in all salt treatments due to increasing salinity level. The highest values of seedling height reduction were observed in seeds treated under 75 mM NaCl solution. There was no much differences among seedlings treated under control treatment and 25 mM NaCl treatment. However, seedlings treated in higher salinity which is 50 mM and 75 mM NaCl treatment experienced stunted growth and reduced in length. Most of the seedlings in 75 mM NaCl treatment were shrunk and the leaves incompletely developed. Salt stress had significant effects on seedling height reduction. Seedling height reduction in most crop plants grown in saline environments is a common observation [50]. Plant cells usually experienced dehydration and shrink moments after introduced to salinity however their original volume is recovered after few hours [51]. The toxic effects in high salt concentration eventually cause plant to experienced water stress due to an increase in osmotic potential in the rooting medium. The current study revealed that the seedling height reduction of water spinach is significantly increased relative to increasing salinity level. Those seeds treated under 75 mM NaCl solution has the shortest seedling height as compared to control. This result indicates that high salinity suppresses the early seedling growth of water spinach. The reduction in cell elongation and cell division that eventually leads to slower leaf appearance and leaf size greatly caused by salinity [32].

3.8 Effect of Salinity on Hypocotyl and Radicle Length of I. aquatica

Table 3 shows that both hypocotyl and radicle length of water spinach seedlings declined significantly at ($p<0.05$) with increasing salinity level. The result indicates that high salt concentrations negatively affected the length of hypocotyl and radicle of water spinach seedlings as compared to control. The longest hypocotyl and radicle length were experienced by seeds treated under control treatment whereas seeds treated under 75 mM NaCl solution has the shortest hypocotyl and radicle length. The hypocotyl length and radicle length also reduced with increasing salinity. In present study, the result in Table 3 shows radicle of water spinach experienced more reduction in length compared to hypocotyl. Radicle elongation is more sensitive than hypocotyl under salt stress as it is the first organ facing the injurious effect of salinity. After the plant is introduced to salinization, osmotic stress begins outside the radicle that consequently leads to changes in cell-water relations. Sodium ions are found abundantly in roots while chloride ions mostly concentrated in shoots [52]. Generally, water cannot carry most of the water-soluble nutrients to the root as the osmotic pressure declined at the germination atmosphere [53]. This is similar with the finding in chick pea seedlings [54]. The gradual decrease in hypocotyl of water spinach seedlings may be caused by inhibitory effect of NaCl salt in hypocotyl growth similar to the finding in five halophytes [55]. Therefore, salinity had a deleterious effect and the reduction of seedling growth in high salinity may be caused by lower absorption of salt component by seed and also germination process is less responsive to high tissue sodium concentrations than early seedling growth.

3.9 Effect of Salinity on Seedling Biomass of I. aquatica

The result in Table 3 showed inconsistent values of seedling biomass of water spinach with increasing salinity level. There was no significant difference at ($p<0.05$) recorded for seedling biomass at all salt treatments. The biomass values marked a slight increment from 0 mM to 50 mM salinity and followed by a decline at 75 mM salinity. The highest seedling biomass was obtained from the seeds treated under 50 mM salinity while the least values obtained from the seeds treated under 75 mM salinity.Water spinach seedlings in present study showed inconsistent values of biomass. This is conflicting with the finding in wheat cultivar which showed reduction in seedling biomass at salt concentration increased [56]. Such reduction in biomass is related with enlarging and injuring of hypocotyls and less or slow mobilization of reserve foods. Salinity consist of both osmotic and specific ionic effects on seedling growth. The metabolism of plants is negatively affected by the toxic ion accumulation particularly Na^+ and Cl^-. The uptake of crucial nutrients such as phosphorus (P) and potassium (K) is restricted by high salt concentrations which in turn influence seedling growth [57]. In order to adapt with saline conditions, plants exhibit the ability to prevent the influx of Na^+ from the roots to the leaves as high amount of sodium could affect nutrient balance and osmotic regulation which eventually causes specific ion toxicity. Osmotic effect resulting from high salinity cause plant inefficiency to absorb water as homeostasis in plant's water status negatively interrupted [58]. This is explained that there where excessively high accumulation of Na^+ ions in saline environments reduces plant water potential which cause plant cells unable to retrieve the turgidity and thus inhibits plant growth [59] [60].

Table 3: Impact of salinity on hypocotyl length, radical length, seedling biomass , total phenolics and total flavonoids of *I. aquatica* . Values are mean ± standard error of mean of fifteen replicates (N=15). Superscripts within the means of each column (a-b) with different letters indicate significant differences among the means (Duncan's Multiple Range Test, p<0.05).

Salt Concentration (mM)	Hypocotyl Length (cm)	Radicle Length (cm)	Seedling Biomass (g)	Salt Tolerance	Total Phenolic Content (mg GAE/g dry weight)	Total Flavonoid Content (mg rutin/g dry weight)
0	3.31 ± 0.14a	2.79 ± 0.15a	0.048 ± 0.003a	100.00 ± 0.00a	0.82 ± 0.19d	0.31 ± 0.06d
25	2.90 ± 0.14b	2.28 ± 0.09b	0.050 ± 0.003a	137.69 ± 29.28a	1.41 ± 0.08c	0.83 ± 0.05c
50	2.69 ± 0.12b	2.09 ±0.07bc	0.056 ± 0.003a	161.11 ± 35.73a	2.05 ± 0.10b	1.29 ± 0.07b
75	2.05 ± 0.10c	1.86 ± 0.06c	0.046 ± 0.002a	194.06 ± 51.54a	2.65 ± 0.12a	1.57 ± 0.13a

3.10 Effect of Salinity on Salt Tolerance of I. aquatica

Salt tolerance is frequently studied by adding NaCl to the growth medium of plant in order to induce the salt stress [61]. The tolerance of water spinach seeds towards salinity was studied using different level NaCl solution as shown in Table 3. The result shows that water spinach seeds were tolerant in all salinity level. Salt tolerance of water spinach seeds has linear relationship with increasing salt concentrations. However, the values of salt tolerance increased non-significantly ($p<0.05$) in all salt treatments as compared to control. Salt tolerance is commonly evaluated as the percentage of biomass production in saline treatment compared to control condition over a continuous period of time. Each crop plants have different responses towards salinity during germination stage. Water spinach is considered as halophyte or salt-tolerant plant that could withstand salinity. Work reported that New Zealand spinach has higher salt tolerance compared to water spinach as the growth of New Zealand spinach increased under salt stress [39]. In the present study, salt tolerance of water spinach increased as well as the salinity level increases. This indicates that water spinach is a salt-tolerant species and capable to germinate under saline conditions. Salt tolerance screening at germination period portray little basis for the future assessment of crop salt tolerance as most of germination studies are organized in laboratory with Petri-dish like containers moisten with solution of various salinity levels [32]. The crop plants that exhibit salt tolerant mechanism during germination stage could turned out to be salt-sensitive during vegetative stage [23].

3.11 Effect of Salinity on Total Phenolic Content (mg GAE/g dry weight) and Total Flavonoid Content (mg rutin/g dry weight) of I. aquatica

The results in Table 3 shows that total phenolic content and total flavonoid content under all salt concentrations were directly related with increasing salinity level. The values of total phenolic content and total flavonoid content were significantly difference at ($p<0.05$) relative to the control. Water spinach seedlings were observed to contain more phenolic contents compared to flavonoids. Seeds treated under 75 mM NaCl concentration has the highest values for both total phenolic and total flavonoid contents as compared to other NaCl concentrations.Phenolic compounds are vital components of many crops including fruits and vegetables where it contributes color, flavor and sensory properties and also possess important effects on oxidative stability. Generally, phenolic compound is grouped into different classes which is flavonoids and non-flavonoids. Total phenolic content and total flavonoid content of water spinach seedlings in present study are significantly decreased relative with increasing salinity. This is conflicting with the finding in rapeseed where the total phenolic content increased with increasing salinity expressed based on dry weight basis [62]. A study done on artichoke leaves also showed an increase in total phenolic and flavonoids content as salinity increases [63]. However, findings in radish and broccoli showed a decrease of total phenolic content in sprouts at increasing salinity but expressed based on fresh weight basis [64] [65]. Eventually, the total flavonoids content reduced at higher salinity levels. Increment in total phenolic content relative to salinity is one of the responds of seedlings to encounter adverse effects of salinity during germination period [62]. Plants deflects carbohydrates synthesis to produce secondary metabolites. The uptake of phosphorus and potassium which are known as main substances of secondary metabolites such as polyphenols decreases at higher salt concentration [66].

4. CONCLUSION

Salt stress through advancement of osmotic pressure adversely affects the germination and early seedling growth of *Ipomoea aquatica*. High salinity level causes reduction in water uptake percentage, seed vigor, total phenolic content, total flavonoid content and mean germination time of the seeds. Seeds of water spinach develop tolerance and encounter the germination delay in high salinity throughout this experiment. Germination percentage and germination index reduced non-significantly with increasing salinity and the result showed seeds treated under 25 mM NaCl solution experienced the highest values for both parameters. Apart from that, seedling biomass and relative injury rate showed inconsistent values relative to increasing salinity. Seedling height reduction, hypocotyl length and radicle length were negatively affected by increasing NaCl concentrations. Salt tolerance of water spinach increased non-significantly with increasing salinity as the seedlings established tolerance during early seedling growth.

COMPETING INTERESTS

Declaration of competing interest should be placed here. All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. If no such declaration has been made by the authors, SDI reserves to assume and write this sentence: “Authors have declared that no competing interests exist.”.

REFERENCES

1. Göthberg, A., Greger, M., & Bengtsson, B. E. (2002). Accumulation of heavy metals in water spinach (Ipomoea aquatica) cultivated in the Bangkok region, Thailand. *Environmental Toxicology and Chemistry*, *21*(9), 1934-1939.
2. Prasad, K. N., Prasad, M. S., Shivamurthy, G. R., & Aradhya, S. M. (2006). Callus induction from Ipomoea aquatica Forsk. leaf and its antioxidant activity. *Indian Journal of Biotechnology*, *5*(1), 107–111.

3. Chauhan, H., Singh, J., & Sharma, D. (2017). Genetic Variability and Heritability Estimation in Water Spinach (Ipomoea aquatica Forsk.) Genotypes. *Int. J. Curr. Microbiol. App. Sci*, *6*(9), 3018-3024.
4. Prasad, K. N., Divakar, S., Shivamurthy, G. R., & Aradhya, S. M. (2005). Isolation of a free radical-scavenging antioxidant from water spinach (*Ipomoea aquatica* Forssk). *Journal of the Science of Food and Agriculture*, *85*(9), 1461-1468.
5. Doka, G. I., Tigni, S. E., & Yagi, S. (2014). Nutritional composition and antioxidant properties of Ipomoea aquatica (Forsek) leaves. *J. For. Prod. Industries*, *3*, 204-210.
6. Ors, S., & Suarez, D. L. (2017). Spinach biomass yield and physiological response to interactive salinity and water stress. *Agricultural Water Management*, *190*, 31-41.
7. Shabala, S., & Munns, R. (2012). Salinity stress: Physiological constraints and adaptive mechanisms. In S. Shabala, N. Farrar, G. Spearing, A. Lainsbury, & F. Chippendale (Eds.), *Plant Stress Physiology* (pp. 59–93). CAB International.
8. Tanji, K.K., Wallender, W.W. (2012). Nature and extent of agricultural salinity and sodicity. In Wallender, W.W., Tanji, K.K. (Eds.), *Agricultural salinity assessment and management* (pp. 10-25). ASCE, Reston, VA, USA.
9. Rengasamy, P. (2002). Transient salinity and subsoil constraints to dryland farming in Australian sodic soils: an overview. *Australian Journal of Experimental Agriculture*, *42*(3), 351-361.
10. Aydinşakir, K., Büyüktaş, D., Nazmi, D. İ. N. Ç., & Karaca, C. (2015). Impact of salinity stress on growing, seedling development and water consumption of peanut (*Arachis hypogaea* cv. NC-7). *Mediterranean Agricultural Sciences*, *28*(2), 78-84.
11. Parihar, P., Singh, S., Singh, R., Singh, V. P., & Prasad, S. M. (2015). Effect of salinity stress on plants and its tolerance strategies: a review. *Environmental Science and Pollution Research*, *22*(6), 4056-4075.
12. Hassen, A., Maher, S., & Cherif, H. (2014). Effect of salt stress (NaCl) on germination and early seedling parameters of three pepper cultivars (*Capsicum annuum* L.). *Journal of Stress Physiology & Biochemistry*, *10*(1).
13. Parihar, P., Singh, S., Singh, R., Singh, V. P., & Prasad, S. M. (2015). Effect of salinity stress on plants and its tolerance strategies: a review. *Environmental Science and Pollution Research*, *22*(6), 4056-4075.
14. Gul, B., Ansari, R., Flowers, T. J., & Khan, M. A. (2013). Germination strategies of halophyte seeds under salinity. *Environmental and Experimental Botany*, *92*, 4-18.
15. Dantas, B. F., Ribeiro, L. D. S., & Aragão, C. A. (2007). Germination, initial growth and cotyledon protein content of bean cultivars under salinity stress. *Revista Brasileira de Sementes*, *29*(2), 106-110.
16. Hassen, A., Maher, S., & Cherif, H. (2014). Effect of salt stress (NaCl) on germination and early seedling parameters of three pepper cultivars (*Capsicum annuum* L.). *Journal of Stress Physiology & Biochemistry*, *10*(1).
17. Olayinka, B. U., Ayanduro, E. T., Abdulrahaman, A. A., & Etejere, E. O. (2016). Effects of salinity and ethylenediamine tetra acetic acid (edta) on the germination of tomato (*Solanum lycopersicum* L.) seeds. *Science World Journal*, *11*(4), 1-3.
18. Arias, C., Serrat, X., Moysset, L., Perissé, P., & Nogués, S. (2018). Morpho-physiological responses of alamo switchgrass during germination and early seedling stage under salinity or water stress conditions. *BioEnergy Research*, *11*(3), 677-688.
19. Gairola, K. C., Nautiyal, A. R., & Dwivedi, A. K. (2011). Effect of temperatures and germination media on seed germination of *Jatropha curcas* L. *Advances in Bioresearch*, *2*(2), 66-71.
20. Han, Z., Ku, L., Zhang, Z., Zhang, J., Guo, S., Liu, H., ... & Dong, L. (2014). QTLs for seed vigor-related traits identified in maize seeds germinated under artificial aging conditions. *PloS one*, *9*(3), e92535.
21. Arias, C., Serrat, X., Moysset, L., Perissé, P., & Nogués, S. (2018). Morpho-physiological responses of alamo switchgrass during germination and early seedling stage under salinity or water stress conditions. *BioEnergy Research*, *11*(3), 677-688.
22. Tsegay, B. A., & Gebreslassie, B. (2014). The effect of salinity (NaCl) on germination and early seedling growth of *Lathyrus sativus* and *Pisum sativum* var. *abyssinicum*. *African Journal of Plant Science*, *8*(5), 225-231.
23. Morris, B., Charles, S., & Son, S. (2003). Legumes. *Encyclopedia of Food and Culture*, *3*, 1-2.
24. Nimbolkar, P. K., Kurian, R. M., Upreti, K. K., Laxman, R. H., & Varalakshmi, L. R. (2018). Seed germination and seedling growth responses of polyembryonic mango (*Mangifera indica* L.) genotypes to salinity stress. *International Journal of Chemical Studies*, *6*(2), 3641-3648.
25. Kaymakanova, M. (2009). Effect of salinity on germination and seed physiology in bean (*Phaseolus vulgaris* L.). *Biotechnology and Biotechnological Equipment*, *23*(sup1), 326-329.

26. Baba, S. A., & Malik, S. A. (2015). Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. *Journal of Taibah University for Science*, *9*(4), 449-454.
27. Shukla, S., & Mehta, A. (2017). Antioxidant, total phenolics and total flavonoid content of the aqueous extract of *Caesalpinia bonducella* Seeds. *Chiang Mai Journal of Science*, *44*(3), 929-938.
28. Tsegay, B. A., & Gebreslassie, B. (2014). The effect of salinity (NaCl) on germination and early seedling growth of *Lathyrus sativus* and *Pisum sativum* var. *abyssinicum*. *African Journal of Plant Science*, *8*(5), 225-231.
29. Deivanai, S., Xavier, R., Vinod, V., Timalata, K., & Lim, O. F. (2011). Role of exogenous proline in ameliorating salt stress at early stage in two rice cultivars. *Journal of Stress Physiology & Biochemistry*, *7*(4), 157-174.
30. Qados, A. M. A. (2011). Effect of salt stress on plant growth and metabolism of bean plant *Vicia faba* (L.). *Journal of the Saudi Society of Agricultural Sciences*, *10*(1), 7-15.
31. Kalhori, N., Ying, T., Nulit, R., Sahebi, M., Abiri, R., & Atabaki, N. (2018). Effect of four different salts on seed germination and morphological effect of four different salts on seed germination and morphological characteristics of *Oryza sativa* L. cv. MR219. *International Journal of Advanced Research in Botany*, *4*(1), 29-45.
32. Jouyban, Z. (2012). The effects of salt stress on plant growth. *Technical Journal of Engineering and Applied Sciences*, *2*(1), 7-10.
33. Hameed, A., Rasheed, A., Gul, B., & Khan, M. A. (2014). Salinity inhibits seed germination of perennial halophytes *Limonium stocksii* and *Suaeda fruticosa* by reducing water uptake and ascorbate dependent antioxidant system. *Environmental and Experimental Botany*, *107*, 32-38.
34. Gul, B., Ansari, R., Flowers, T. J., & Khan, M. A. (2013). Germination strategies of halophyte seeds under salinity. *Environmental and Experimental Botany*, *92*, 4-18.
35. Sarker, A., Hossain, M. I., & Kashem, M. A. (2014). Salinity (NaCl) tolerance of four vegetable crops during germination and early seedling growth. *Int. J. Latest Res. Sci. Technol*, *3*(1), 91-95.
36. Pradheeban, L., Nissanka, N. A. A. S. P., & Suriyagoda, L. D. B. (2014). Clustering of rice (*Oryza sativa* L.) varieties cultivated in Jaffna District of Sri Lanka based on salt tolerance during germination and seedling stages. *Tropical Agricultural Research*, *25*(3), 358-375.
37. Moghaieb, R. E., Saneoka, H., & Fujita, K. (2004). Effect of salinity on osmotic adjustment, glycinebetaine accumulation and the betaine aldehyde dehydrogenase gene expression in two halophytic plants, *Salicornia europaea* and *Suaeda maritima*. *Plant Science*, *166*(5), 1345-1349.
38. Yousif, B. S., Nguyen, N. T., Fukuda, Y. A. S. U. K. O., Hakata, H. I. R. O. A. K. I., Okamoto, Y., Masaoka, Y. O. S. H. I. K. U. N. I., & Saneoka, H. (2010). Effect of salinity on growth, mineral composition, photosynthesis and water relations of two vegetable crops; New Zealand spinach (*Tetragonia tetragonioides*) and water spinach (*Ipomoea aquatica*). *Int J Agric Biol*, *12*(2), 211-216.
39. Fuller, M. P., Hamza, J. H., Rihan, H. Z., & Al-Issawi, M. (2012). Germination of primed seed under NaCl stress in wheat. *ISRN Botany*, *2012*.
40. Tsegay, B. A., & Gebreslassie, B. (2014). The effect of salinity (NaCl) on germination and early seedling growth of *Lathyrus sativus* and *Pisum sativum* var. *abyssinicum*. *African Journal of Plant Science*, *8*(5), 225-231.
41. Khayatnezhad, M., & Gholamin, R. (2011). Effects of salt stress levels on five maize (*Zea mays* L.) cultivars at germination stage. *African Journal of Biotechnology*, *10*(60), 12909-12915.
42. Demir, I., Ermis, S., Mavi, K., & Matthews, S. (2008). Mean germination time of pepper seed lots (Capsicum annuum L.) predicts size and uniformity of seedlings in germination tests and transplant modules. *Seed Science and Technology*, *36*(1), 21-30.
43. Nimbolkar, P. K., Kurian, R. M., Upreti, K. K., Laxman, R. H., & Varalakshmi, L. R. (2018). Seed germination and seedling growth responses of polyembryonic mango (*Mangifera indica* L.) genotypes to salinity stress. *International Journal of Chemical Studies*, *6*(2), 3641-3648.
44. Tsegay, B. A., & Gebreslassie, B. (2014). The effect of salinity (NaCl) on germination and early seedling growth of *Lathyrus sativus* and *Pisum sativum* var. *abyssinicum*. *African Journal of Plant Science*, *8*(5), 225-231.
45. Haileselasie, T. H. (2012). The effect of salinity (NaCl) on germination of selected grass pea (*Lathyrus sativus* l.) landraces of Tigray. *Asian Journal of Agricultural Sciences*, *4*(2), 96-101.
46. Wen, D., Hou, H., Meng, A., Meng, J., Xie, L., & Zhang, C. (2018). Rapid evaluation of seed vigor by the absolute content of protein in seed within the same crop. *Scientific Reports*, *8*(1), 5569.

47. Huang, M., Zhang, R., Chen, J., Cao, F., Jiang, L., & Zou, Y. (2017). Morphological and physiological traits of seeds and seedlings in two rice cultivars with contrasting early vigor. *Plant Production Science*, *20*(1), 95-101.
48. Saberi, M., Gharmakhe, H. N., Heshmati, G., Barani, H., & Shahriari, A. (2017). Effects of different drought and salinity levels on seed germination of *Citrullus colocynthis*. *Ecopersia*, *5*(3), 1903-1917.
49. Hakim, M. A., Juraimi, A. S., Begum, M., Hanafi, M. M., Ismail, M. R., & Selamat, A. (2010). Effect of salt stress on germination and early seedling growth of rice (*Oryza sativa* L.). *African Journal of Biotechnology*, *9*(13), 1911-1918.
50. Läuchli, A., & Grattan, S. R. (2007). Plant growth and development under salinity stress. In *Advances in molecular breeding toward drought and salt tolerant crops* (pp. 1-32). Springer, Dordrecht.
51. Afzali, S. F., Shariatmadari, H., & Hajabbasi, M. A. (2011). Sodium chloride effects on seed germination, growth and ion concentration in chamomile (*Matricaria chamomilla*). *Iran Agricultural Research*, *29*(2), 107-118.
52. Goraghani, S., Reza, H., Heidary, G. A., & Solaimani Sardo, M. (2013). Effects of salinity and drought stresses on seed germination and seedling growth of desert wheatgrass Agropyron desertorum. *Journal of Rangeland Science*, *4*(1), 14-22.
53. Gao, W. R., Wang, X. S., Liu, Q. Y., Peng, H., Chen, C., Li, J. G., ... & Ma, H. (2008). Comparative analysis of ESTs in response to drought stress in chickpea (*C. arietinum* L.). *Biochemical and Biophysical Research Communications*, *376*(3), 578-583.
54. Keiffer, C. H., & Ungar, I. A. (1997). The effect of extended exposure to hypersaline conditions on the germination of five inland halophyte species. *American Journal of Botany*, *84*(1), 104-111.
55. Rahman, M., Soomro, U. A., Haq, M. Z. U., & Gul, S. (2008). Effects of NaCl salinity on wheat (*Triticum aestivum* L.) cultivars. *World Journal of Agricultural Sciences*, *4*(3), 398-403.
56. Nasim, M., Qureshi, R., Aziz, T., Saqib, M., Nawaz, S., Sahi, S. T., & Pervaiz, S. (2008). Growth and ionic composition of salt stressed *Eucalyptus camaldulensis* and Eucalyptus teretcornis. *Pakistan Journal of Botany*, *40*(2), 799-805.
57. Nordin, M. N., Che Lah, M. K., & Jahan, M. S. (2015). Effects of different salinity levels on rice production. *Australian Journal of Basic and Applied Sciences*, *9*(5), 524-530.
58. Ologundudu, A. F., Adelusi, A. A., & Akinwale, R. O. (2014). Effect of salt stress on germination and growth parameters of rice. *Notulae Scientia Biologicae, 6*(2), 237.
59. Amirjani, M. R. (2010). Effect of NaCl on some physiological parameters of rice. *European Journal of Biological Sciences*, *3*(1), 6-16.
60. Kumar, V., & Khare, T. (2016). Differential growth and yield responses of salt-tolerant and susceptible rice cultivars to individual (Na^+ and Cl^-) and additive stress effects of NaCl. *Acta Physiologiae Plantarum*, *38*(7), 170.
61. Falcinelli, B., Sileoni, V., Marconi, O., Perretti, G., Quinet, M., Lutts, S., & Benincasa, P. (2017). Germination under moderate salinity increases phenolic content and antioxidant activity in rapeseed (*Brassica napus* var *oleifera* Del.) sprouts. *Molecules*, *22*(8), 1377.
62. Rezazadeh, A., Ghasemnezhad, A., Barani, M., & Telmadarrehei, T. (2012). Effect of salinity on phenolic composition and antioxidant activity of artichoke (*Cynara* scolymus L.) leaves. *Res J Med Plant*, *6*, 245-252.
63. Yuan, G., Wang, X., Guo, R., & Wang, Q. (2010). Effect of salt stress on phenolic compounds, glucosinolates, myrosinase and antioxidant activity in radish sprouts. *Food Chemistry*, *121*(4), 1014-1019.
64. Guo, L., Yang, R., Wang, Z., Guo, Q., & Gu, Z. (2014). Effect of NaCl stress on health-promoting compounds and antioxidant activity in the sprouts of three broccoli cultivars. *International Journal of Food Sciences and Nutrition*, *65*(4), 476-481.
65. Wong, C. C., Li, H. B., Cheng, K. W., & Chen, F. (2006). A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. *Food Chemistry*, *97*(4), 705-711.