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Investigating the effect of abiotic stress on germination, physiology and nutrient profile of Raphanus sativus L. (Cherry Belle var.)

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Investigating the effect of abiotic stress on germination, physiology and nutrient profile of *Raphanus sativus* L. (Cherry Belle var.)

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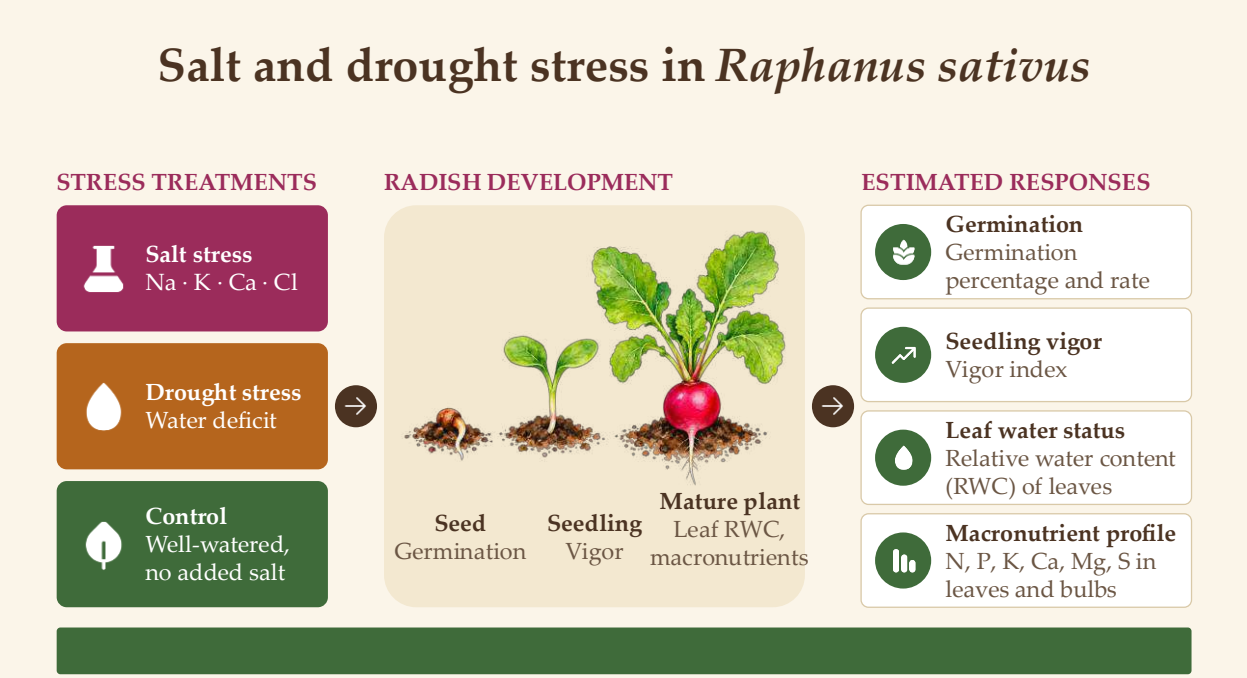
Graphical Abstract



Abstract

Changing weather patterns, chemical fertilizer-based agriculture and anthropogenic activities have resulted in soil salinization and drought, thus affecting crop productivity and nutritional security. The effect of salinity and drought in seed germination and nutrient uptake are agronomic questions pertinent to human nutrition. This study investigated the effects of different cationic salts (NaCl, KCl, and $CaCl_2$ at 100–400 mM) in germination, seedling vigor and physiology of *Raphanus sativus* L. (Cherry Belle var.). The effect of simulated drought (20% PEG 8000) and salt stress (200 mM NaCl) in nutrient uptake and organ-specific partitioning was estimated. $CaCl_2$ exerted the strongest inhibitory effect on germination, seedling water content and seedling vigor. Relative water content of fully developed radish leaves did not show drastic shift in

abiotic stresses as compared to control group. Mineral analysis demonstrated distinct organ-specific nutrient partitioning, with leaves accumulating higher amounts of calcium and magnesium as compared to bulbs. An approximate six-fold sodium accumulation relative to control group was recorded in plant tissues grown under NaCl stress. Potassium concentrations were elevated under abiotic stress reflecting osmotic adjustments. These results offer deeper insights on stress physiology in plants that could be utilized for targeted agronomic strategies to counter abiotic stress.

Keywords: radish; salinity; drought; macronutrients; agriculture

Introduction

Ensuring food and nutritional security for 10 billion human population by 2050 is a major agricultural and environmental challenge [1]. In a changing environment, soil salinization and reduced precipitation leads to abiotic stresses such as salt stress and drought stress in plants. Furthermore, constrains in arable land exacerbates the problem. Irrigation and chemical fertilizer-driven agricultural practices lead to soil salinization while shifting weather patterns contribute to droughts. Abiotic stresses adversely affect the physiology of plants and contribute to osmotic imbalances, generation of reactive oxygen species and alters ionic homeostasis. Plants encounter these challenges by modulating its transcriptional, hormonal and metabolic responses to restore nutrient homeostasis and cell water relations [2]. Such tradeoffs reduce biomass accumulation, yield and productivity of crops [3]. Germination of seeds and establishment of seedlings in soil is critical to crop productivity. The early vegetative seedling stage is the most vulnerable to abiotic stress, thus, investigating the effect of abiotic stress in seed germination is critical to designing successful strategy for agriculture and food security [4]. Germination assays determine osmotic and ion-specific toxicity thresholds while mineral analysis of plant tissues identifies the effect of stress on nutrient uptake. Thus, estimating the effect of abiotic stress in germination of seeds and nutritional profile of edible plant parts evaluates the broader effects of abiotic stress in forestry, agriculture and human nutrition [5]. Such investigations are useful to collect data that can be used to model and predict the effect of drought and salinity in agricultural productivity in a changing environment.

Plants require several nutrients to complete its lifecycle; these nutrients are grouped into macronutrients and micronutrients based on the amount required by plants. Macronutrients such as nitrogen, phosphorus, potassium, calcium, magnesium, and

sulfur and micronutrients such as manganese, molybdenum, boron, iron, zinc, copper, and chlorine serve critical structural and functional roles in plants [6]. Sodium is not considered as an essential nutrient for plants; however, it can be beneficial to plants in minute amounts. Sodium in larger amounts competes with potassium due to its physicochemical similarity and displaces it thus disrupting ion homeostasis [7]. Potassium is the main osmoticum in plant cells and is required for enzyme activation, stomatal regulation, protein synthesis and maintenance of turgor pressure [8]. Calcium is central to cell signaling and acts as a secondary messenger, it is an important structural component of cell walls. Trace amounts of chlorine are required in plant cells for photosynthesis, charge balance and osmotic regulation [9]. Due to excessive use of agricultural lands, monoculture practices, drought, chemical fertilization and irrigation the nutritional profile of soil shows dramatic change over time. Deficiency of soil nutrients are often reported that have downstream effects in productivity while certain nutrients when present in excess amount within soil can cause nutrient deficiencies of competing ions. Fertilizers, industrial runoff, flooding and other anthropogenic activities such as removing ice from roads introduces excess sodium, potassium, calcium and chlorine in agricultural soils. Potassium and calcium are major constituents of inorganic fertilizers and soil amendments [10]. The increased concentration of ions in soil lowers the water potential of soil and exerts ionic stress, disrupts ionic homeostasis, causes oxidative damage, ion toxicity and membrane leakage in plant cells. These affects germination potential of seeds, growth and development of plants and productivity of crops. Thus, evaluating the effects of sodium, potassium, calcium and chlorine is a pertinent question in agricultural research and is particularly relevant in the context of a changing environment.

Radish (*Raphanus sativus* L.), a widely consumed vegetable root crop of the *Brassicaceae* family. It is cultivated and consumed globally due to ease of growing and rich nutrient profile. The whole plant could be consumed and is rich is vitamins, minerals and anti-inflammatory compounds such as glucosinolates [11]. The short life cycle, small space requirement and minimal input required to grow radishes makes it an ideal candidate to investigate the effect of abiotic stress in plants, particularly root crops. Extensive research pertaining to salt stress in plants has been reported, however, most research focused on the effect of sodium chloride. The individual effect of different salt such as NaCl, KCl and $CaCl_2$ on germination of seeds can illustrate the cation-specific effect on germination and seedling vigor. Furthermore, investigating the effect of abiotic stress

on nutritional profile of plant tissues reflects the effect of a changing environment in human diet. Selecting radish plants as an experimental candidate gives unique advantages to evaluate organ-specific mineral partitioning between source (photosynthetic leaves) and sink (bulb). The effect of abiotic stress on *Raphanus sativus* L. (Cherry Belle var.) in early developmental stage and at maturity is less understood [12]. Understanding physiological aspects such as osmotic status along with nutrient sequestration offers deeper insights on the effects of abiotic stress. The physiological and the biochemical data could be utilized concurrently with molecular investigation to identify molecular targets for stress alleviation, thus advancing nutritional security [13]. Estimation of final germination percentage is important in establishing the baseline viability and osmotic tolerance limits of seeds exposed to different salts at varying concentrations. Vigor index indicates the mobilization of reserves from seeds to developing tissues of seedlings and biomass accumulation thus reflecting early growth performances [14]. Measuring water content of seedling tissues and relative water content of mature leaves reveals the capacity of seedlings and whole plants to maintain osmotic balance and turgor pressure under abiotic stress. Finally, mineral analysis of leaves and bulbs evaluates the effect of abiotic stress in nutrient uptake and storage in edible plant parts thus highlighting the broader implications of such stresses in human diet [15].

Materials and Methods

Germination

Radish (*Raphanus sativus*) seeds of Cherry Belle variety distributed by the seed company Burpee (USA) were procured from Walmart (East Main Street, Russellville, Arkansas). Two Whatman paper (grade 1) were placed in a petri plate of 90 mm diameter. Four solutions of concentrations 100 mM, 200 mM, 300 mM and 400 mM were made for each of the three salts of NaCl, KCl and $CaCl_2$. 10 ml of a specific salt solutions were transferred to petri plates using a plastic pipette and 15 seeds were placed equidistantly in each plate for germination experiments. Seeds broken or with unhealthy appearance were discarded and visually healthy-looking seeds were selected for germination experiments. Each salt treatment consisted of a control group and four treatment group of salt concentrations 100 mM, 200 mM, 300 mM and 400 mM respectively and had 3 replicates ($5 \times 3 = 15$ plates). Each salt treatment had 15 plates and the whole germination experiment had 45 petri plates (3×15). The germinating seeds were placed

in dark at room temperature and germination data was recorded every day for the next 5 days. Fresh weight of the 15 seeds in each petri plate was recorded after 5 days. Following which they were oven dried at 65°C for 2 days until a constant mass was achieved and dry weight of the 15 seeds in each petri plate was recorded. Final germination percentage (FGP), seedling tissue water content (WC%), mean dry weight per seed (MDW) and vigor index by weight (VI) were estimated using the equations as mentioned below.

Equations

Final Germination Percentage (FGP)

$$FGP = (\text{Total number of seeds germinated at day 5} \div 15) \times 100$$

Seedling tissue water content (WC%)

$$WC\% = \{(FW - DW) \div FW)\} \times 100$$

Mean Dry Weight (MDW)

$$\text{MDW per seed} = \text{Total dry weight of seeds in a plate} \div 15$$

Vigor index by weight (VI)

$$VI = FGP \times \text{MDW per seed}$$

Harvesting tissues for mineral analysis

The effect of salt stress and drought stress in radish plants at maturity, were estimated. Plastic pots of 6 inches diameter (0.25 gallons) were used to grow radish plants. The pots were filled with soil from Miracle Gro Potting Mix and 4 seeds were centrally spaced and planted. A plastic plate was placed at the base of the pots to prevent loss of nutrient through drainage while the pots had adequate outlets to support drainage and healthy plant growth. The seeds were planted at a depth of 0.5 inches from the soil surface. The experiment was set such that there were 12 pots consisting of 4 replicates each for the control, salt stress (200 mM NaCl) and drought stress (20% PEG 8000) treatments. All 12 plants were placed in an environmental chamber with 16 hour light and 8 hour dark photoperiod with a day temperature of 26°C and night temperature of 21°C. For the first 3 days all pots were watered with 100 ml of nanopure grade water (resistivity of ≥17.1 MΩ·cm), following which they were labelled with plastic strips and

permanent markers indicating treatment type and replicate number. The pots were watered alternate days with 100 ml of nanopure water or 200 mM NaCl solution or 20% PEG 8000 solutions according to their treatment labels. Each pot was fertilized with 0.5 grams of Miracle Gro all-purpose fertilizer at 10 days, 20 days and 30 days after sowing. Thinning of plants were done 7 days after sowing (DAS) to leave only the healthiest plant in the pot. The pots were rotated and their positions were changed every week to reduce the effect of microenvironment and position in the growth of plants. As the plants grew the environment chamber became crowded and the leaves of the radish plants were lighter in color compared to their normal green color. Thus after 3 weeks, the pots were relocated to a garden (near McEver Hall, Arkansas Tech University) and were subjected to natural light, temperature and precipitation. Due to summer heat of 2025, the plants were watered on alternate days with 100 ml of nanopure water and were concurrently subjected to their usual treatment solutions. All plants were harvested after 5 weeks from the first day of sowing and the bulb was dissected from leaves of each plant and their fresh weight (FW) was immediately recorded. The plant tissues were oven-dried at 65°C for 2 days until a constant mass was achieved and dry weight was recorded. The dried samples were sent for ICP-OES analysis to the Central Analytical Lab at the Department of Poultry Science in the University of Arkansas. Nutritional profile of samples were reported in parts per million (ppm) on a dry weight basis.

Relative water content

Fully expanded radish leaves from each of the 12 pots were harvested on the same day after 4 weeks from the day of sowing. One leaf was dissected from the base of the petiole of each plant and immediately weighed to determine the fresh weight (FW). The cut end of the petiole was placed in a beaker containing deionized water and the entire setup was covered with a plastic bag. The leaves were taken out from water, blot-dried and weighed to estimate the turgid weight (TW). The leaves were oven-dried at 65°C for 2 days until a constant mass was achieved and dry weight (DW) of leaves were recorded. Relative water content (RWC) was determined using the following equation: $RWC\ \% = \{(FW - DW) \div (TW - DW)\} \times 100$.

Results

Final germination percentage (FGP) of *Raphanus sativus* (Cherry Belle var.) seeds declined progressively with increasing concentrations of NaCl, KCl and $CaCl_2$ (Figure

1). Control groups established the baseline viability of seeds with FGP ranging from 86.67 to 91.11. A 100 mM salt concentration did not show distinct cation-based differences in FGP, however, at 200mM the difference was obvious with $CaCl_2$ completely inhibiting seed germination while KCl maintaining a relatively high FGP 73.33 (± 11.54). Further increases in salinity to 300 mM severely impaired germination with seeds under KCl stress performing marginally better than the seeds under NaCl induced stress. At 400 mM there were no visible germinating seeds except for 1 seed that germinated in one of the replicates under KCl induced stress. Thus, KCl exerted the least inhibitory effect on seed germination while $CaCl_2$ proved to be the most inhibitory among the three salts tested. Water content in seedling tissues (WC%) decreased across all salt treatments with increase in salinity. The pattern of decrease in water content was similar in monovalent salts such as NaCl and KCl while divalent salt such as $CaCl_2$ caused the sharpest decrease in water content (Figure 2). In controls, the water content varied from 86.37 % (± 2.5) to 93.76% (± 0.29). $CaCl_2$ showed a sharp decline from 100 mM concentration while both NaCl and KCl showed sharp decline in water content from 200 mM. The lowest seedling water content was recorded at 400 mM for all three salts with $CaCl_2$ recording the lowest value of 59.64% (± 2.58).

The weight-based seedling vigor index declined progressively with increase in salinity thus, reflecting combined reductions in germination percentage and seedling dry matter accumulation (Figure 3). KCl showed a more uniform decline compared to other salts. The controls recorded a value from 1.73 (± 0.28) to 0.88 (± 0.17). $CaCl_2$ at 100 mM did not show much difference with the control but drastically declined to a value of 0 at 200 mM or higher concentrations. Vigor index was severely suppressed and declined to near zero at 300 mM for both NaCl and KCl and became 0 at 400 mM indicating complete failure of germination. Across all concentrations, KCl consistently conserved higher seedling vigor compared to NaCl or $CaCl_2$. Salinity and drought reduced leaf relative water content (RWC%) in mature *Raphanus sativus* (Cherry Belle var.) leaves. However, surprisingly the margin of reduction was not high when compared to the control and were similar between salt and drought stresses (Figure 4). Control leaves maintained the highest hydration status, exhibiting a mean RWC of 81.19% (± 2.4), while the values for salt and drought stresses were 75.14 (± 3.8) and 76.73 (± 2.2) respectively.

ICP-OES based mineral analysis estimated the macronutrient content in leaves and bulbs of *Raphanus sativus* under salt and drought stress with control group estimating

the baseline. The amount was expressed on dry-mass basis in g/Kg (Table 1) and was converted to concentrations at millimolar level (Table 2) to better visualize the physiological context. In the control group calcium was significantly higher in leaves (25.74 g/Kg) compared to bulbs (4.65 g/Kg) and magnesium content in leaves (4.62 g/Kg) and bulbs (1.47 g/Kg) showed similar trends in the control group. In salt and drought stressed plants, the patterns for calcium and magnesium did not change and the values were comparable to the control group. Potassium accumulation in leaves and bulbs did not show significant differences; a pattern that was also found in phosphorous. Highest accumulation of potassium was recorded in drought stressed leaves (56.43 g/Kg) while lowest accumulation of potassium was recorded in the leaves of the control group (40.80 g/Kg). Both salt stress and drought stress increased potassium content in leaves and bulbs. Drought stressed bulbs recorded the highest accumulation of phosphorous (7.39 g/Kg) and the lowest values for phosphorous accumulation was recorded in salt stressed bulbs (5.50 g/Kg). Sulfur accumulation ranged from 7.11 g/Kg in drought stressed leaves to 4.0 g/Kg in bulbs of the control group. Leaves contained more sulfur than bulbs regardless of the treatment group or control. Salt stressed leaves (9.78 g/Kg) and bulbs (6.46 g/Kg) showed significant higher (approximately 6 times) accumulation of sodium as compared to the control group or drought stressed plants.

Discussion

The cation-specific effects of salt stress in seed germination and seedling vigor were evaluated in this study. The other important aspect of the study being an estimation of the effects of NaCl stress and drought stress in mineral distribution in source (leaf) and sink (bulb) of a vegetable root crop (*Raphanus sativus*). Seed germination and seedling vigor in *Raphanus sativus* (Cherry Belle var.) are governed by osmotic potential and cation identity. $CaCl_2$ stress showed a much restrictive effect on germination and seedling vigor as compared to NaCl and KCl, while KCl had the least inhibitory effect on germination and seedling vigor among the three salts tested. Comparing FGP and WC% of all three salts and all 4 concentrations of salt including the control group reveals that WC% is the critical factor dictating seed germination. WC% values at mid to high 80 has minimal negative effects while a value less than 70 shows drastic negative effects in germination. This is aligned with established physiological principles because water is required for metabolic activities and mobilization of seed reserved to

propel cell division, cell expansion and growth [16, 17, 18]. Calcium had the most pronounced effect because in plant cells cytosolic calcium is strictly regulated at nanomolar levels as it acts as a secondary messenger [19]. Calcium is also a key component of cell wall as it is required for calcium pectate formation and cross linking [20]. Protrusion of radicle and growth requires loosening of cell wall [21]. High concentration of calcium needs to be stored in apoplast rather than symplast and this makes the cell wall more rigid. Thus, high concentration of calcium reduces germination percentage and seedling vigor index. Potassium being the major osmoticum in plant cells, KCl stress had least adverse effect in germination [22]. Vigor index showed a steadier decline in potassium stressed seeds as compared to other salts. Such behavior could be explained by the fact that plant cells require higher amount of potassium than calcium or sodium. Thus, plant cells would adapt more efficiently to an increase in environmental potassium compared to calcium or sodium.

Surprisingly, mature *Raphanus sativus* leaves did not show drastic difference in relative water content (RWC%) in stressed conditions as compared to the control group. The difference in RWC% was marginal between salt and drought treatment groups. This anomalous observation could be explained by the experimental setup. Since the plants were placed in natural environment after three weeks from the days after sowing, they were exposed to natural light, temperature and precipitation. Besides this, to counter summer heat, all plants regardless of the treatment groups and treatment regimens were watered with nanopure water every alternate day after three weeks and until they were harvested. Thus, some of the effects of abiotic stress that could have further differentiated the RWC% values among the different treatment groups and the control group were shielded. The approximate 6-fold increase in sodium content in salt-stressed plants as compared to the other groups reveals the mobility of sodium in plants. Sodium has physicochemical similarities with potassium, thus can be transported through non-selective cation channels and through certain potassium transporters [23, 24, 25]. Thus, can substitute potassium in osmotic regulation. The higher concentration of magnesium in leaves as compared to bulbs can be explained by the fact that magnesium is a key atom of chlorophyll and serves as a cofactor for Rubisco [26, 27, 28]. Magnesium levels in abiotic stressed leaves are not reduced significantly compared to control group because maintaining a minimum threshold of magnesium is essential for photosynthesis and transport of photosynthates to the bulb. Calcium levels in leaves remain tightly conserved across treatment groups and control indicating xylem-driven

calcium transport and the stabilizing of cell membranes under stressed conditions [29, 30]. The increased concentration of potassium in salt and drought stressed plants indicates potassium-driven osmotic adjustments to sustain water uptake and maintain relative water content of leaves [31, 32, 33].

This study illustrates the cation specific effects of salt stress in *Raphanus sativus* (Cherry Belle var.) where besides osmolarity, the cationic properties and cross-linking abilities of calcium play a critical role. The role of abiotic stresses in nutrient partitioning is reported, and this indicates the putative impact of changing environment in human nutrition. These findings offer deeper insights to generate agronomic strategies for crop management and tailored fertilizer formulations to address salt and drought stresses [34, 35, 36]. There were only two biological replicates used for ICP-OES mineral analysis and this was the major limitation of the study. Thus, the ICP-OES data is presented without any standard deviation and in the form of a table representing the averages of those two biological replicates for each sample. Future research includes an integrated approach to estimate the effect of soil amendments in abiotic stresses, biochemical assays, identification of molecular targets and utilizing synthetic biology to alleviate salt and drought stress in plants.

Author Contributions

A.A conceptualized, designed experiments, investigated, wrote the original draft, reviewed, edited and submitted the manuscript. Several undergraduate students participated in collecting germination data and growing plants in environmental chamber.

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Conflicts of Interest

The author declares no conflicts of interest.

Data availability statement

The corresponding author can be contacted for access to the data presented in this study upon request.

Abbreviations

DAS – days after sowing

DW – dry weight

FW – fresh weight

FGP – final germination percentage

ICP-OES - Inductively Coupled Plasma-Optical Emission Spectrometry

MDW – mean dry weight

MΩ·cm - megaohm-centimeter

PEG - Polyethylene glycol

RWC – relative water content

TW – turgid weight

VI – vigor index

WC – water content

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Figures

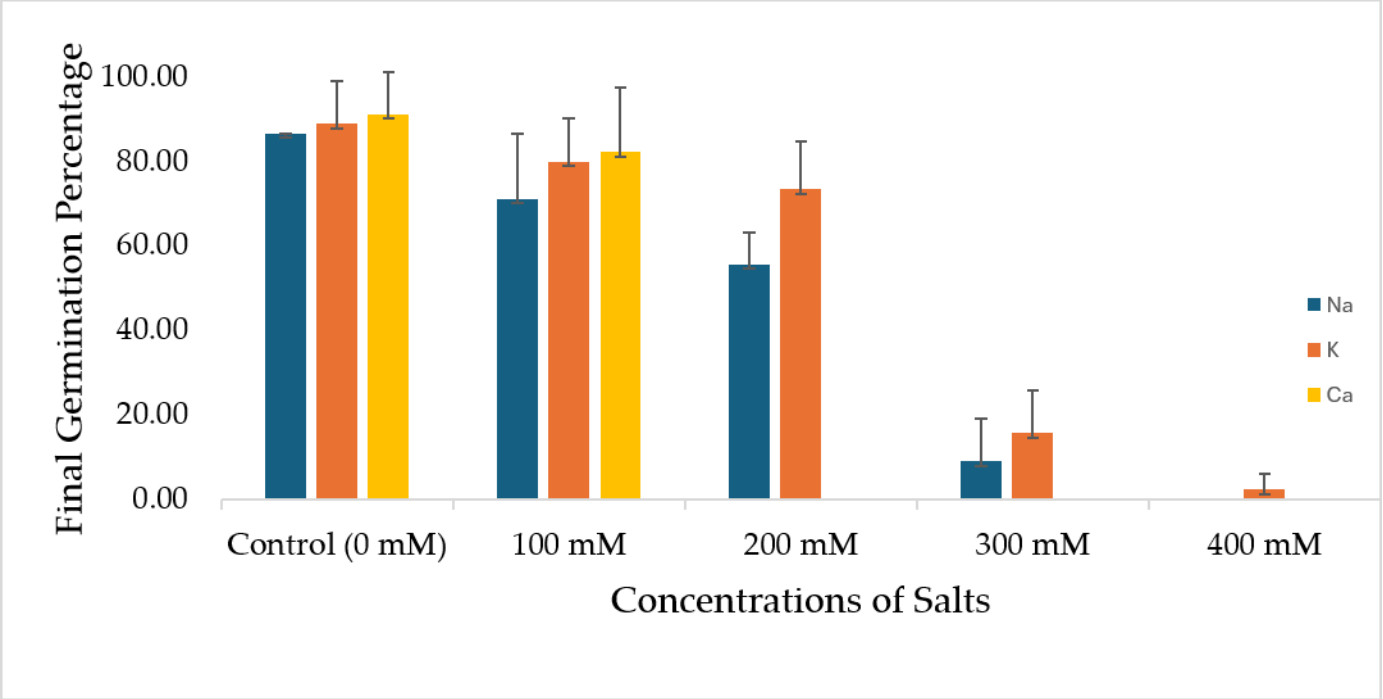

Figure 1 – Final germination percentage of radish seeds treated with different concentrations of NaCl, KCl and $CaCl_2$.

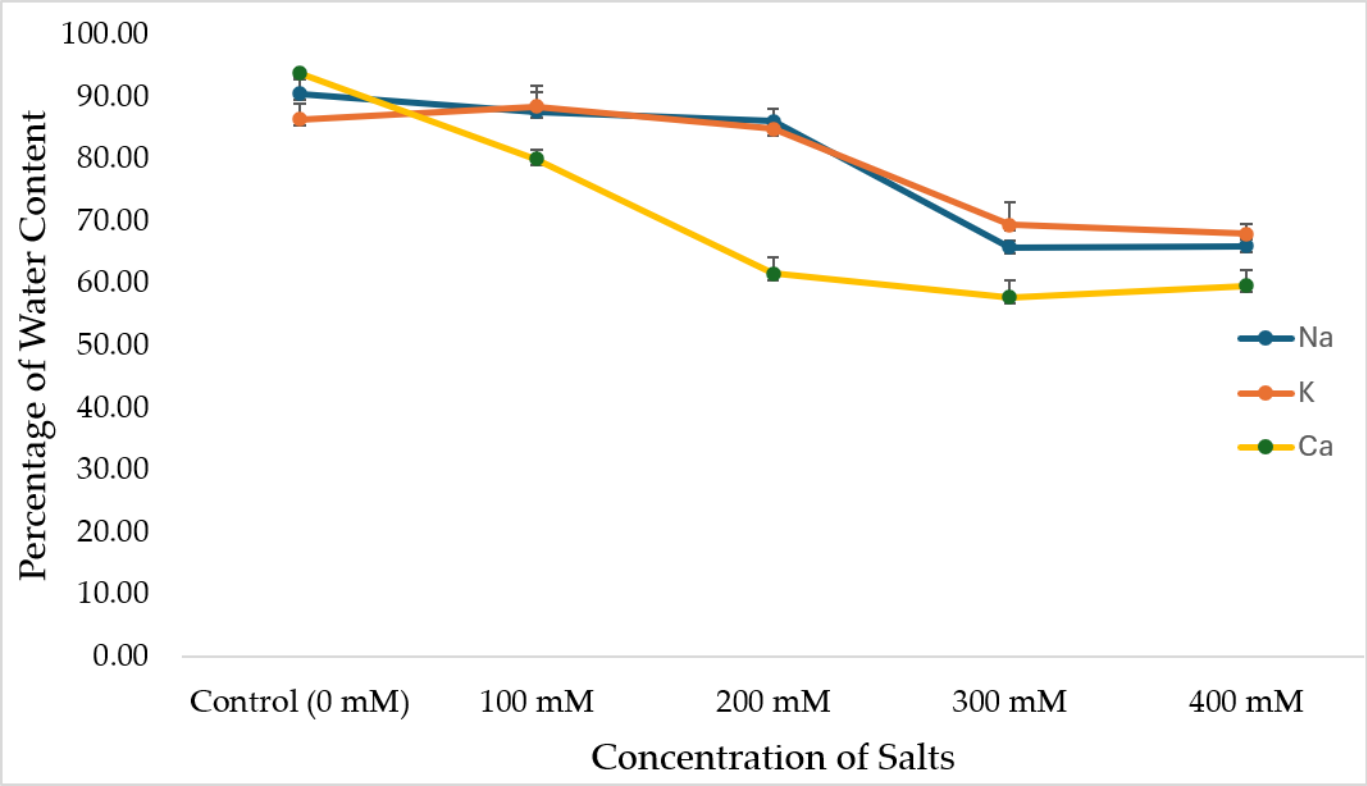

Figure 2 – Water content percentage of seedlings grown in different concentrations of NaCl, KCl and $CaCl_2$.

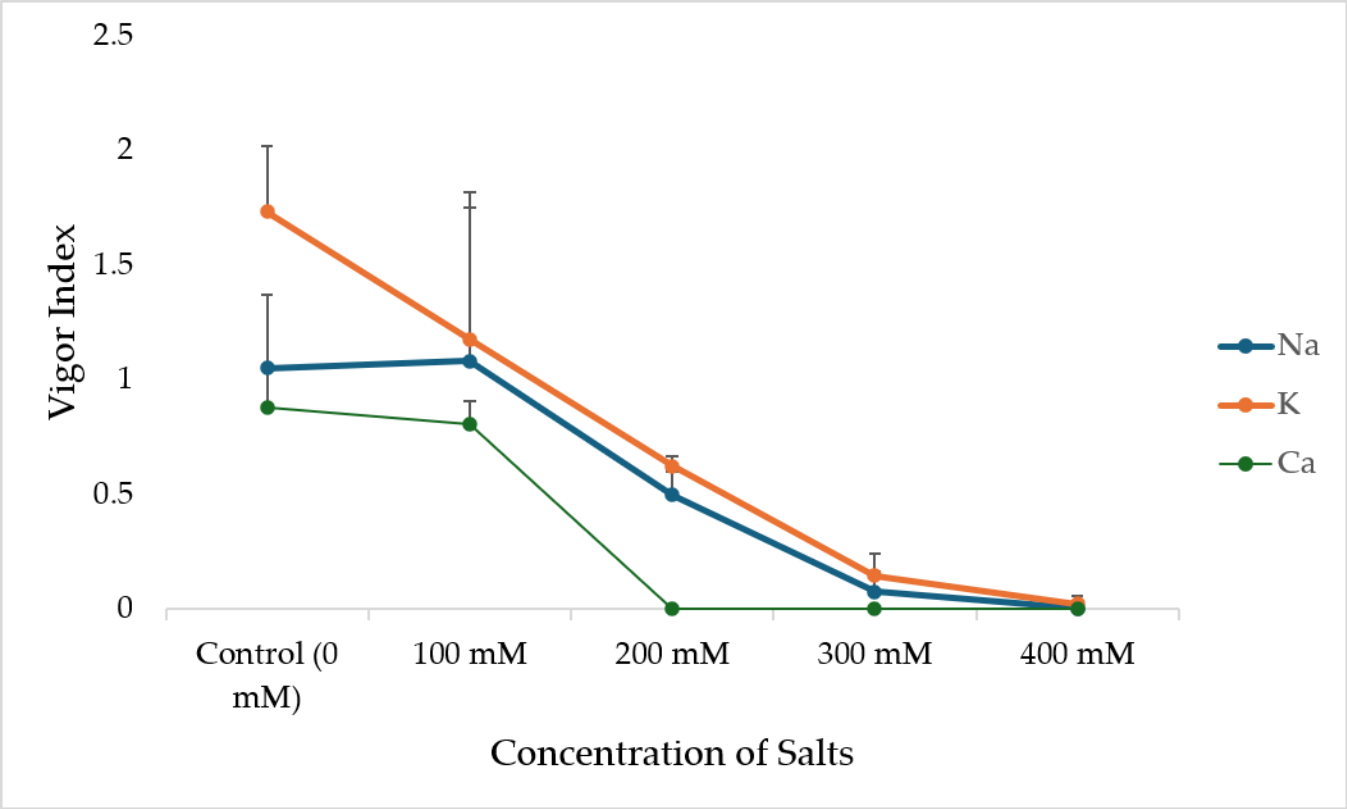

Figure 3 – Vigor index (weight based) of seedlings grown in different concentrations of NaCl, KCl and $CaCl_2$.

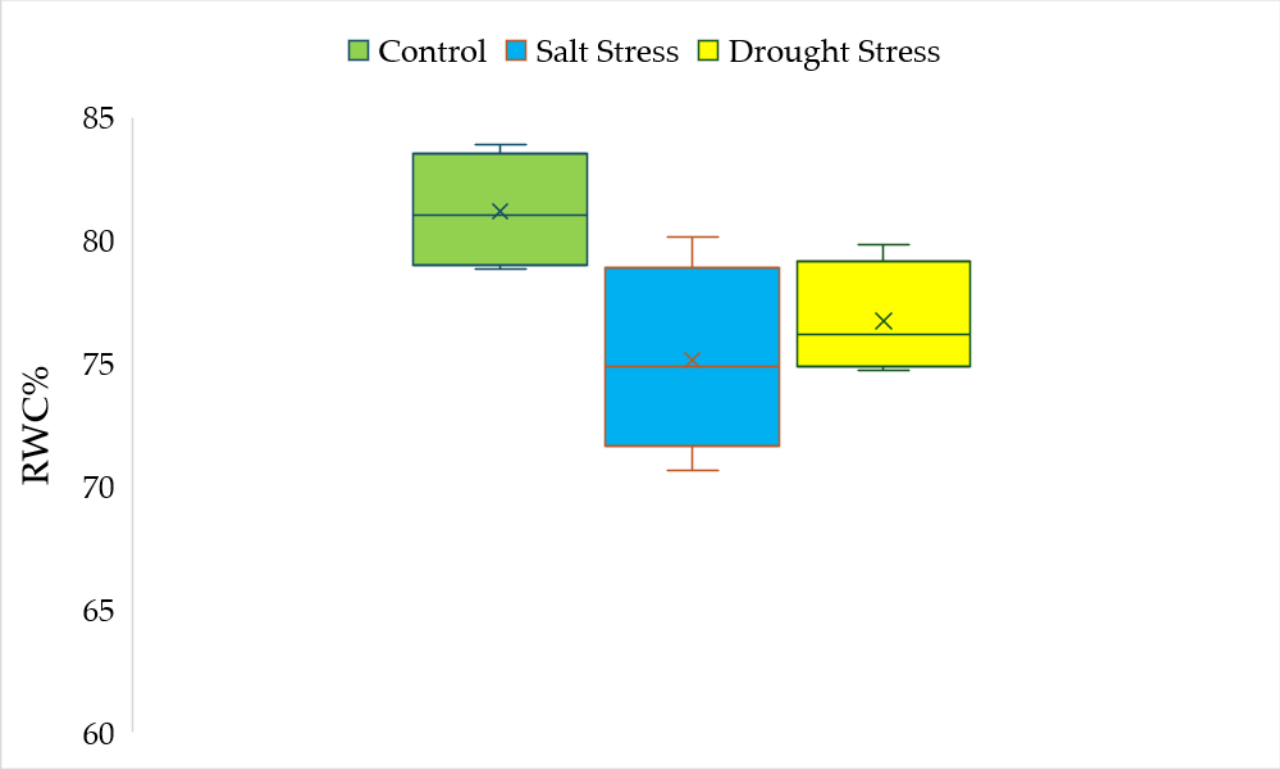

Figure 4 – Relative water content of fully developed radish leaves in salt and drought stressed conditions.

Tables

Samples	Ca (g/Kg)	K (g/Kg)	Mg (g/Kg)	P (g/Kg)	S (g/Kg)	Na (g/Kg)
Leaf (Control)	25.74	40.80	4.62	7.09	6.70	1.42
Bulb (Control)	4.65	42.66	1.47	6.23	4.00	1.11
Leaf (Salt)	25.24	44.70	3.84	5.99	5.86	9.78
Bulb (Salt)	4.14	46.11	0.96	5.50	4.46	6.46
Leaf (Drought)	25.66	56.43	4.17	6.57	7.11	1.49
Bulb (Drought)	4.09	45.79	1.16	7.39	4.60	0.95

Table 1 – Dry weight based mineral analysis of fully developed bulbs and leaves of radish grown under abiotic stress.

Samples	K mM	Mg mM	P mM	S mM	Na mM	Ca mM
Leaf (Control)	93.43	17.27	20.59	18.94	5.55	58.88
Bulb (Control)	70.22	3.87	12.92	7.96	3.11	7.42
Leaf (Salt)	100.72	13.94	17.05	16.13	37.54	55.52
Bulb (Salt)	81.88	2.76	12.36	9.64	19.50	7.18
Leaf (Drought)	143.39	17.06	21.06	21.91	6.47	63.64
Bulb (Drought)	100.43	4.10	20.60	12.32	3.53	8.78

Table 2 – Concentration of elements (mineral analysis) in fully developed bulbs and leaves of radish grown under abiotic stress.