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Osmotic Stress or Ionic Composition: Which Affects the Early Growth of Crop Species More?

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Abstract: Salinization is a key soil degradation process. An estimated 20% of total cultivated lands and 33% of irrigated agricultural lands worldwide are affected by high salinity. Much research has investigated the influence of salt (mainly NaCl) on plants, but very little is known about how this is related to natural salinity and osmotic stress. Therefore, our study was conducted to determine the osmotic and ionic salt stress responses of selected C3 and C4 cultivated plants. We focused on the early growth stages as those critical for plant development. We applied natural brine to simulate natural salinity and to compare its effect to NaCl solution. We assessed traits related to germination ability, seedlings and plantlet morphology, growth indexes, and biomass and water accumulation. Our results demonstrate that the effects of salinity on growth are strongest among plantlets. Salinity most affected water absorption in C3 plants (28% of total traits variation), but plant length in C4 plants (17–27%). Compensatory effect of ions from brine were suggested by the higher model plants' growth success of ca 5–7% under brine compared to the NaCl condition. However, trait differences indicated that osmotic stress was the main stress factor affecting the studied plants.

Keywords: salinity; barley; oat; millet; corn; traits assessment

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1. Introduction

Salinization is one of the most critical processes of soil degradation on Earth [1]. It is caused by low precipitation, irrigation with saline water, a rising water table and inadequate irrigation. More than 6% of the world's total soil land area is saline [2]. It has been estimated that, worldwide, 20% of total cultivated lands and 33% of irrigated agricultural lands are affected by high salinity [3]. Moreover, current predictions indicate that salinity is expected to be responsible for loss of arable land of up to 50% by the year 2050 [4,5]. Salt stress usually inhibits crop growth and decreases yield. The adverse effects of salinity on plants include osmotic stress related to the difficulty of water absorption, and ion toxicity, which is associated with both nutrient constraints and oxidative stress [3]. Several physiological processes such as photosynthesis, respiration, starch metabolism and nitrogen fixation are also affected under saline conditions, leading to crop productivity losses [6]. Excess NaCl inhibits plant growth in both shoots and roots [7,8]. The ability of plants to tolerate salt is determined by multiple biochemical pathways that facilitate retention and/or acquisition of water, protect chloroplast functions and maintain ion homeostasis [9].

Crop plant species differ significantly in their growth response to salinity. The goal of improving salinity tolerance in crop plants is to develop cultivars that can grow and produce economic yields under moderately saline conditions [10]. The complex nature of salt-stress tolerance is an essential factor that interacts with the difficulties in breeding salt-tolerant crop varieties [11]. In general, cereal crops are most sensitive to salinity stress during the vegetative and early reproductive stages and less sensitive during flowering and

grain filling steps [10]. However, plant genotypes responses to salt may vary at different growth stages. For example, differences in salt tolerance responses among rice genotypes at different growth stages were detected by Zeng et al. [12].

Although salt stress affects all plant growth stages, seed germination and early growth stages are more sensitive in most plant species [13–15]. Seed germination is a major factor limiting the establishment of plants under saline conditions. These conditions may cause significant reductions in the rate and percentage of germination. Numerous studies have been carried out to determine salinity effects on seed germination and seedling growth [14,16–18]. Under salt stress, seed germination differs between plant species, and significant variation is observed within cultivars [16,19]. For example, the early seedling stage of rice is among the stages most sensitive to salt stress, so studies on this stage of growth could provide a basis for improving tolerance across the life cycle of the plant [20]. Final germination percentage corresponds positively with the dry weight of germinated seedling at transplanting and biomass production under salinity [21]. The early phase of plant growth, such as seed germination and seedling establishment, could be very good indicators of potential grain yield harvested at the late stage [22]. For the final plant yield, plantlet development is also crucial [23]. When salt stress reduces growth in an early phase of plantlet growth, the yield is strongly reduced and plant products have low quality and quantity [13]. Many studies have reported that salinity reduces seed germination, seedling emergence, leaf elongation, and biomass accumulation at early growth stages [24–26]. Therefore, our study focused on germination, seedling growth and plantlet development.

Another factor affected by salinity is photosynthesis. Photosynthetic rates are usually lower in plants exposed to salinity, and especially to NaCl [27]. The C4 photosynthesis mechanism of carbon fixation is a modified version of the ancestral (C3) photosynthetic pathway [28]. Because C4 plants can reduce photorespiration, the water saving that this provides means that C4 photosynthesis has been assumed to have advantages under conditions (like heat, drought and salinity) that promote photorespiration [28,29]. In C4 plants, long-term exposure to salinity is correlated with photosynthesis potential and plant succulence [30]. Under salinization, the inclusion of CO_2 into organic compounds in C3 plants increases their similarity to C4 plants for photosynthetic metabolism [30]. Taxa with C4 carbon fixation are often found in salt-affected areas [31]. However, the association between the photosynthetic pathway and salt dependence needs to be tested much more deeply.

Limited information is available on the most common cultivated plants' responses to osmotic stress versus ionic toxicity stress under saline conditions. Salinity consists of two main elements: an osmotic component and an ionic component related to the accumulation of toxic ions at high concentrations (Na^+ and Cl^-) [32]. Hyperosmotic stress caused by excessive salt is responsible for the primary stress signals. In turn, secondary signals are generated by ions and their toxicity effects on cells. They include oxidative stress and damage to cell components such as the membrane lipid layer, proteins and nucleic acids [33]. In agreement with Munns [34], the decrease in germination under saline conditions results from the cumulative effect of osmotic and ionic factors.

The cations component of total soluble salts in soils generally include Na^+, Ca^{2+} and Mg^{2+} and the anions are Cl^-, $SO_4{}^{2-}$ and carbonates ($CO_3{}^{2-}$, $HCO_3{}^-$). However, Na^+ as an anion and Cl^- as a cation are found in most saline soils [35]. Therefore, many studies have investigated the influence mainly of NaCl in many concentrations on plants [36–40]. So, the contribution of different soil ions to growth reduction under salt stress is still less understood than that of Na^+ in crops. This reflects that most research on salinity stress has focused on Na^+, with little regard to any other ion toxicity [41]. In the case of *Salicornia europaea* L., good germination of large seeds occurred under NaCl between 0.5 and 2%, under Na_2SO_4 and 2NaCl + KCl + $CaCl_2$ between 0.5 and 3%, and under $2Na_2SO_4$ + K_2SO_4 + $MgSO_4$ between 0.5 and 5% [42]. Fatemi et al. [43] indicated that salinity stresses induced by 200 mM and 400 mM KCl and polyethylene glycol (PEG, the nonpenetrant osmotic agent) significantly reduced the fresh weight of the halophyte grass

Aeluropus littoralis (Gouan) Parl. compared to a control [43]. The results also showed that potassium chloride was more toxic than sodium chloride, and the K^+ ion affected plant growth more than the Na^+ ion [43]. Another study of five selected landscape ornamental species demonstrated that plant growth decreased when irrigated with increasing NaCl + $CaCl_2$ concentrations [44]. However, there is still a lack of proven results on NaCl experiments and natural plant growth conditions. Because natural brine can simulate the composition of saline soil solutions and be used to examine the synergistic effect of different salts on seed germination and plant growth, we decided to apply this solution in our research.

Our study was conducted to determine the osmotic and ionic composition salt stress responses of selected cultivated plants at early growth stages. We hypothesized that the salinity effects would differently be dependent on: (a) plant species, (b) stage of growth, (c) type of photosynthetic processes (C3, C4) and (d) the ionic composition of salt. Therefore, we decided to investigate the C3 plants barley (*Hordeum vulgare* L.) and oat (*Avena sativa* L.) and the C4 plants millet (*Panicum miliaceum* L.) and maize (*Zea mays* L.). We applied natural brine (to simulate natural salinity with multiunit composition) and pure NaCl solution, both with similar osmotic potential. In this way, differences in plant growth between these two treatments we interpreted as ionic effects, whereas the lack of differences as an osmotic effect in relation to the nonsaline control.

2. Materials and Methods

2.1. Model Plants

Barley (*H. vulgare*) is an annual member of the grass family and is a major grain grown in temperate climates globally [45]. Oat (*A. sativa*) is the eighth most widely grown crop globally and is produced under a broad spectrum of soil and climatic conditions [46]. Millet (*P. miliaceum*) is one of the oldest crop species in Europe and Asia. Due to its C4 photosynthetic type, millet is thermophilic, like maize [47]. Maize (*Z. mays*) is a cereal grain that has become a basic food in many parts of the world, with maize's total production surpassing that of wheat or rice. All tested species have a high consumer value for humans.

Seeds of barley variety ELLA and maize variety OPOKA were provided by Plant Culture in Smolice [48]. Seeds of oat variety BINGO and millet HASHAKI seeds came from Plant Culture in Strzelce [49].

2.2. Experimental Solutions

Experiments were conducted at Nicolaus Copernicus University in Torun, Poland in 2019. To test the ionic effect, we applied natural brine and NaCl solution with the same osmotic potentials. The differences between these two treatments we interpreted as caused by ionic difference between solutions (ionic effect). The lack of differences in growth between NaCl and brine we interpreted as osmotic effect in relation to the nonsaline control. Distilled water treatments served as the experiments' control of the salinity effect. The natural brine was taken from salt spring no. 16 in the Health Resort of Ciechocinek, Central Poland. The physico-chemical properties of this chloride-sodium, iodine, thermal water (5.34%) are as follows: electrical conductivity 82 $dS \cdot m^{-1}$, color 5 $mgPt \cdot dm^{-3}$, flavor salty, pH 6.60, temperature 32 °C. The detailed ionic composition is shown in Table S1. First, we performed a preliminary experiment. Based on its results we selected 150 mM NaCl solution (out of 0, 50, 100, 150, 200 mM NaCl) as giving the first symptoms of salt stress for all tested plant species at germination and seedling stages. Seed germination was assessed based on ISTA's principles for seed sampling [50]. Therefore, we applied 150 mM concentration to compare brine and pure NaCl solution effects.

However, to have the same salt concentration and osmotic potential of 150 mM NaCl solution and brine we first measured electrical conductivity (EC) and then calculated the necessary dilution of the brine. The EC of 150 mM NaCl amounted to 15 $dS \cdot m^{-1}$. For experiments, we obtained brine of the same EC, i.e., 15 $dS \cdot m^{-1}$. We calculated osmotic potential of these solutions based on osmotic pressure (OP) according to Soil Survey

Staff [51]: OP (atm) ≈ 0.36·EC (dS·m^{-1}). According to this formula osmotic pressure of 15 dS·m^{-1} solutions is equal to ca 5.4 atm and, therefore, osmotic potential to −5.4 atm.

2.3. Germination and Seedlings Development

To test germination and seedlings properties, the seeds were sown on Petri dishes containing filter paper. As was mentioned already, we watered seeds with three variants of the solution: distilled water (control), brine and NaCl of EC equal to 15 dS·m^{-1} and similar osmotic potential. For each species and each salt treatment we used three replicates of 20 seeds. Germination and growth of seedlings were observed under controlled conditions: 16 h/8 h day/night period and temperature 25 °C over seven days. Germinated seeds were counted after 24 h and then each day. The criterion of germination was a root length of 1 mm.

We calculated the percentage of seed germination (GP) according to the formula:

$$GP = (\textit{number of germinating seeds} \div \textit{number of sown seeds}) \times 100 \quad (1)$$

Moreover, we measured root length (RL) shoot length (SL) and total seedling length (TL).

Seedling vigor index (SVI) was calculated according to the Abdul-Baki and Anderson [52] formula:

$$SVI = \%\ \textit{germination seeds} \times \textit{length of seedling} \quad (2)$$

2.4. Plantlet Growth Assessments

To study early growth limitations of model plants we established a pot experiment. We sowed each model plant's seeds in palettes (28 pots/palette) containing a sterile mixture of sand and vermiculite (1:1). Before sowing seeds, each palette was saturated with a suitable sterile solution (500 mL for each palette). As before, three salinity variants were taken into account: distilled water (the control), brine and NaCl solution of EC equal to 15 dS·m^{-1}. In *H. vulgare* and *A. sativa*, we sowed 28 seeds (one in each pot). Because of lower germination rates, we increased the number of seeds to 56 seeds for *Z. mays* (two in each pot) and 140 for *P. miliaceum* (five in each pot). Finally, we obtained one growing plant in each pot.

All variants were put in a growth chamber at 25 °C and 16-h light period under a sodium lighting system (100 µmol m^2·s^{-1} PAR (photosynthetically active radiation)). The palettes were watered each day with an equal amount (150 mL) of solution. We applied distilled water for the first 14 days then Hoagland's solution to ensure homogeneity of salinity and nutrient supply. The plants were grown for 42 days (Figure S1) then the plants were washed from the substrate.

We calculated growth success (GS) as:

$$GS = NP \div NS, \quad (3)$$

where NP is the number of surviving plantlets and NS is the number of sown seeds.

Moreover, we assessed the number of leaves (NoL), root length (RL), stem length (SL) and total plant length (PL). Then we assessed biomass accumulation as fresh (W_f) and dry weight (W_d). The dry weight (W_d) was assessed after 72 h drying at 85 °C. The tissue water content (TWC) was calculated according to the formula of Black and Pritchard [53]:

$$TWC = ((W_f - W_d) \div W_f) \times 100 \quad (4)$$

We also calculated the tissue water content in roots (TWC_r) and shoots (TWC_s) separately.

Moreover, we included the following growth indexes:

$$\text{Specific Leaf Area (SLA)} = A \div W_l, \quad (5)$$

where A is leaf area and W_l is dry leaf weight,

$$\text{Leaf Weight Ratio (LWR)} = W_l \div W_d, \quad (6)$$

and

$$\text{Root Weight Ratio (RWR)} = W_r \div W_d, \quad (7)$$

where W_r is dry root weight.

2.5. Statistical Analysis

Statistical significance between treatments was assessed at the $p < 0.05$ level using one-way ANOVA with Tukey's honestly significant difference (HSD) post hoc test [54]. Because we obtained a relatively small number of plantlets after the pot experiment, to make assessment more independent of observation number, and to select the most affected traits, we applied Canonical Variate Analysis as a discriminant analysis with a forward selection procedure and Monte Carlo permutation test [55].

3. Results

3.1. Osmotic and Ionic Effects on Germination

The percentage of seed germination (GP) of *H. vulgare* in three variants of the experiment was not significantly different between control, brine and NaCl (Figure 1). In all treatments it was over 90%. We observed the same in the case of *A. sativa* and *P. miliaceum*. However, millet GP was the lowest among the model plants and reached only ca 40%. We noted germination inhibition under salt stress in the case of *Z. mays*. In the control solution, GP amounted to ca 98% and was significantly higher compared to brine (63%) and NaCl (78%) solutions (Figure 1). We did not detect significant differences between osmotic and ionic effects (brine vs NaCl solution).

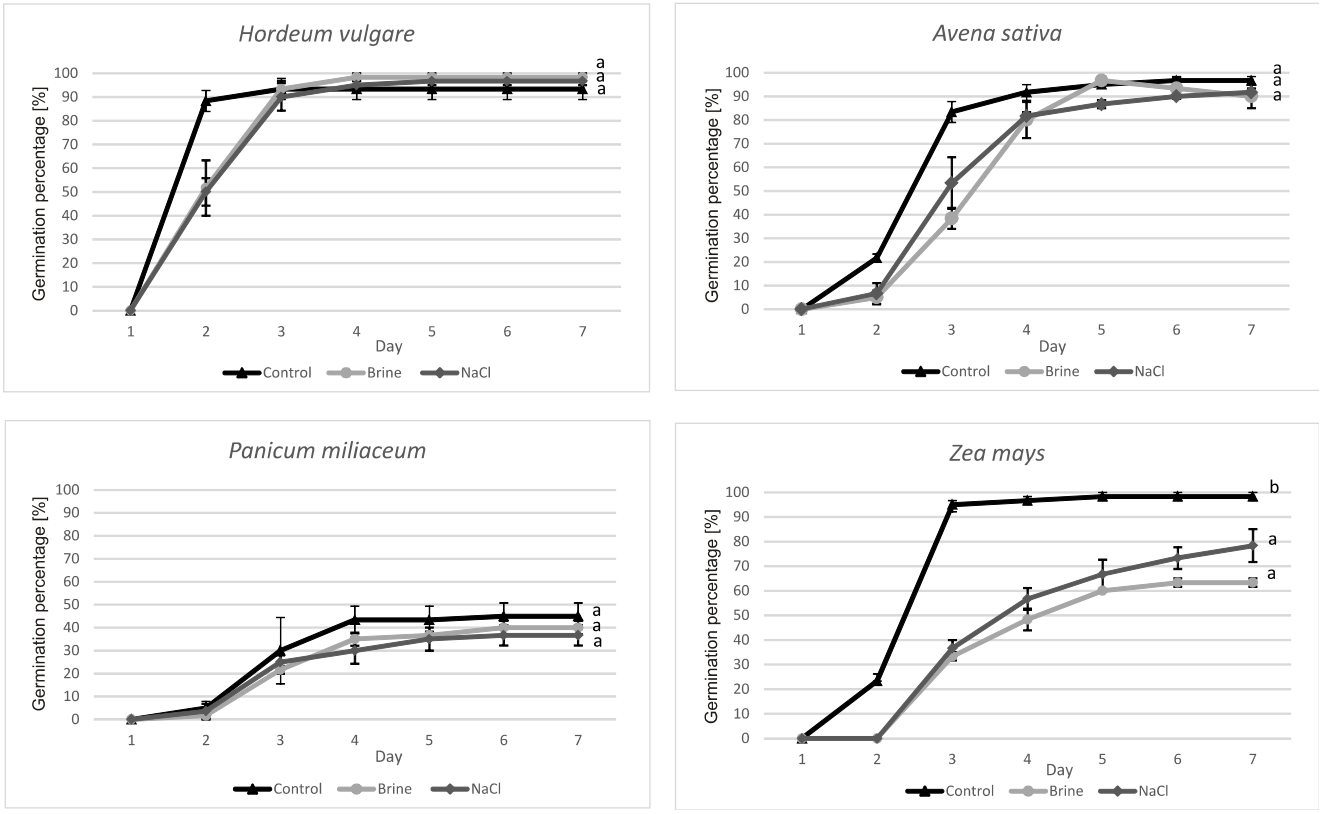

Figure 1. Germination percentage (GP) of model plants. Significant differences ($p < 0.05$) are marked by different letters. Average values ($\overline{X}$) with standard error (SE) are given ($\overline{X} \pm$ SE), $n = 3$.

3.2. Effects of NaCl and Brine on Seedling Stage

We observed growth limitation under the saline condition of *H. vulgare*, *A. sativa* and *Z. mays* (Figure 2). Growth parameters RL, SL, TL and SVI were significantly lower compared to the nonsaline control (Table 1). In the case of *H. vulgare*, all measured parameters were ca 30% lower. Growth limitation was higher in the case of *A. sativa*, of which all measured parameters were ca 60% lower compared to the control. Almost the same was noted based on *Z. mays* measurements. However, the maize root length was more affected (65% shorter) than stem length (ca 50% shorter) than control. The vigor index (SVI) was ca 70% lower (Table 1). We did not find such growth limitations in the case of *P. miliaceum* (Figure 2). Seedling parameters did not differ significantly (Table 1).

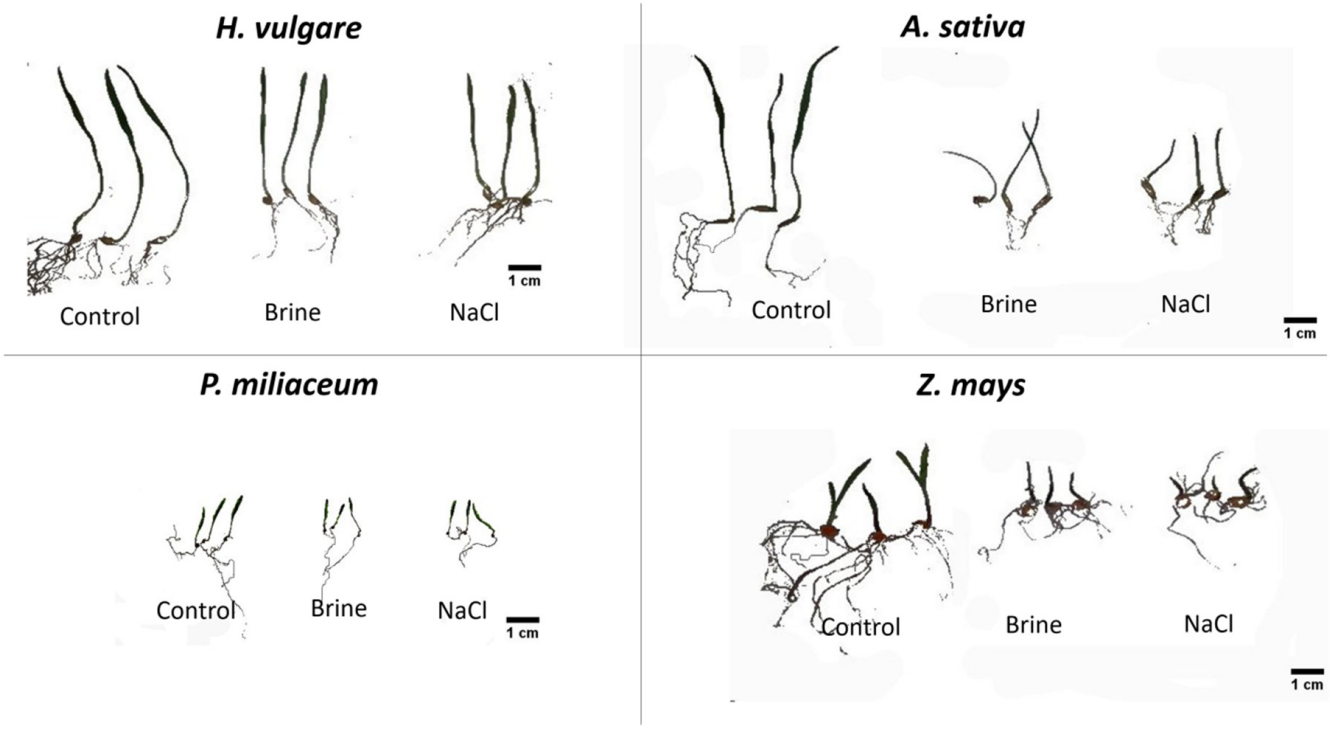

Figure 2. Growth of seven-day-old seedlings in control, brine and NaCl solutions. Only three individuals of each treatment are shown.

We also did not identify significant differences between brine and NaCl solutions (Table 1), so we did not detect the ionic impact of early growth on seedlings, but only osmotic influence.

3.3. Effect of NaCl and Brine on Plantlet Development

After six weeks of plant growth, 25 plants of *H. vulgare* in control, 17 plants in brine solution, and 15 plants in NaCl were alive. Hence, GS was 89, 61 and 54%, respectively. The morphological plant traits NoL, RL and PL were significantly lower in brine and NaCl solution than control (Table 2). The biomass average values of W_f and W_d were significantly lower in brine and NaCl solution compared to the control (Table 2). In SLA and RWR we did not note statistically significant differences between control, brine and NaCl solutions (Table 2). The results of the discriminant analysis revealed that the most critical features for discrimination between treatments were the water contents in plants: TWC (28.6% of total variation explained), W_f (22.2%) and TWC_s (8.9%) (Figure 3, Table S2). The NaCl variant plants had significantly lower tissue water content, especially in shoots and, therefore, lower fresh biomass than that of brine and control variants (Table 2).

Table 1. Osmotic and ionic composition effects on seedlings of model plants after seven days of growth. Average values ($\overline{X}$) with standard error (SE) are given ($\overline{X} \pm$ SE).

	RL	SL	TL	SVI
		Hordeum vulgare		
Control	$73.5^{b} \pm 0.8$	$112.7^{b} \pm 1.8$	$186.2^{b} \pm 2.5$	$17357^{b} \pm 645$
Brine	$47.4^{a} \pm 1.6$	$79.9^{a} \pm 1.7$	$127.3^{a} \pm 3.2$	$12524^{a} \pm 438$
NaCl	$52.9^{a} \pm 4.9$	$80.1^{a} \pm 1.3$	$133.0^{a} \pm 4.6$	$12788^{a} \pm 384$
ANOVA	$p < 0.01$	$p < 0.001$	$p < 0.001$	$p < 0.001$
		Avena sativa		
Control	$83.5^{b} \pm 6.5$	$76.6^{b} \pm 0.9$	$160.1^{b} \pm 7.5$	$15461^{b} \pm 514$
Brine	$30.4^{a} \pm 5.0$	$34.2^{a} \pm 3.4$	$4.6^{a} \pm 8.2$	$5839^{a} \pm 856$
NaCl	$30.6^{a} \pm 7.5$	$32.5^{a} \pm 8.4$	$63.1^{a} \pm 15.8$	$5745^{a} \pm 1375$
ANOVA	$p < 0.01$	$p < 0.01$	$p < 0.001$	$p < 0.001$
		Panicum miliaceum		
Control	$43.6^{a} \pm 4.8$	$19.6^{a} \pm 1.2$	$63.2^{a} \pm 4.7$	$2893^{a} \pm 547$
Brine	$48.3^{a} \pm 10.6$	$20.8^{a} \pm 2.1$	$69.0^{a} \pm 12.2$	$2693^{a} \pm 303$
NaCl	$50.6^{a} \pm 12.3$	$23.0^{a} \pm 1.2$	$73.6^{a} \pm 13.5$	$2817^{a} \pm 860$
ANOVA	NS	NS	NS	NS
		Zea mays		
Control	$151.9^{b} \pm 11.7$	$47.7^{b} \pm 3.3$	$199.7^{b} \pm 15.0$	$19606^{b} \pm 1332$
Brine	$52.9^{a} \pm 6.0$	$25.7^{a} \pm 3.8$	$78.5^{a} \pm 8.3$	$4994^{a} \pm 625$
NaCl	$53.2^{a} \pm 3.6$	$23.1^{a} \pm 0.3$	$76.3^{a} \pm 3.8$	$5991^{a} \pm 683$
ANOVA	$p < 0.001$	$p < 0.01$	$p < 0.001$	$p < 0.001$

RL = root length, SL = stem length, TL = total seedling length, SVI = vigor index. Differences between groups based on Tukey's range test are marked by different letters. Values within a group in a column denoted by different letters are significantly different based on Tukey post hoc comparisons at $p < 0.05$. NS—not significant.

In the case of *A. sativa*, after six weeks we obtained 15 plants in control, five plants in brine and only three plants in NaCl solution. GS was ca 53, 18 and 11%, respectively. ANOVA results demonstrated only differences in NoL, RWR and TWC_s between treatments (Table 2). We found an interesting relationship between number of leaves for *A. sativa* and type of irrigation. We observed that brine decreased NoL in comparison to control and NaCl. Discriminant analysis and forward selection of traits revealed that the most essential features for discrimination between compared treatments were the water content in roots TWC_r (27.8% variation explained) and roots development indicated as RWR (18.1%) (Figure 3, Table S2). Significantly higher values of TWC_r were typical for NaCl treatment plants, whereas higher RWR was typical for brine treatment (Figure 3, Table 2).

The GS of *P. miliaceum* was 21% (30 plantlets survived) in the control, 14% (19 individuals) in brine and 9% (12 individuals) in the NaCl treatment. ANOVA results demonstrate that NoL, RL, SL, PL, and RWR traits were significantly affected by salinity (Table 2). However, the most important features for discrimination between treatments were PL (17.4% of total traits variation explained) and SL (13.8%) (Figure 3, Table S2). Plantlets were longer in brine than in the NaCl treatment (Table 2).

After six weeks of growth, the GS of *Z. mays* was 41% (23 plantlets survived) in control, and 5% (three individuals) in brine and NaCl treatments. Most of the observed differences in traits were assessed by ANOVA as not significant. However, these results were affected by the small number of surviving individuals in the two salt treatments (Table 2). Only TWC was denoted as significantly affected by salinity and was higher under control conditions. Discriminant analysis, which bases the p significance level on number of permutations, together with a forward selection of traits, revealed that the most important features for discrimination between treatments were PL (26.5% of total traits variation explained) and SLA (14.6%) (Table S2, Figure 3). A higher value of PL was typical for plantlets in the control treatment, whereas SLA was typically higher in NaCl.

Table 2. Effect of NaCl and brine on selected growth parameters of model plants together with ANOVA results. Average values ($\overline{X}$) with standard error (SE) are given ($\overline{X} \pm$ SE).

	n	NoL [n]	RL [mm]	SL [mm]	PL [mm]	W_f [g·plant^{-1}]	W_d [g·plant^{-1}]	SLA [cm^2·g^{-1}]	LWR [g · g^{-1}]	RWR [g · g^{-1}]	TWC [%]	TWC_s [%]	TWC_r [%]
						Hordeum vulgare							
Control	25	$5.4^{b} \pm 0.2$	$169^{b} \pm 10$	$127^{a} \pm 14$	$296^{b} \pm 12$	$1.422^{b} \pm 0.127$	$0.178^{b} \pm 0.001$	$569^{a} \pm 25$	$0.506^{a} \pm 0.016$	$0.156^{a} \pm 0.008$	$87^{b} \pm 0.3$	$88^{b} \pm 0.4$	$74^{b} \pm 3$
Brine	17	$4.5^{a} \pm 0.2$	$125^{a} \pm 5$	$126^{a} \pm 7$	$251^{a} \pm 7$	$0.936^{a} \pm 0.007$	$0.099^{a} \pm 0.007$	$625^{a} \pm 19$	$0.556^{b} \pm 0.001$	$0.168^{a} \pm 0.001$	$88^{b} \pm 0.5$	$88^{b} \pm 0.2$	$81^{b} \pm 3$
NaCl	15	$4.5^{a} \pm 0.2$	$114^{a} \pm 5$	$129^{a} \pm 16$	$243^{a} \pm 12$	$0.762^{a} \pm 0.080$	$0.112^{a} \pm 0.001$	$566^{a} \pm 15$	$0.548^{ab} \pm 0.010$	$0.179^{a} \pm 0.013$	$84^{a} \pm 0.6$	$86^{a} \pm 0.4$	$58^{a} \pm 7$
ANOVA		$p < 0.001$	$p < 0.001$	NS	$p < 0.01$	$p < 0.001$	$p < 0.001$	NS	$p < 0.05$	NS	$p < 0.001$	$p < 0.001$	$p < 0.01$
						Avena sativa							
Control	15	$5.6^{b} \pm 0.2$	$106^{a} \pm 13$	$161^{a} \pm 21$	$266^{a} \pm 23$	$0.614^{a} \pm 0.092$	$0.088^{a} \pm 0.011$	$547^{a} \pm 22$	$0.564^{a} \pm 0.012$	$0.079^{a} \pm 0.010$	$86^{a} \pm 0.8$	$87^{a} \pm 0.8$	$17^{a} \pm 4$
Brine	5	$4.4^{a} \pm 0.5$	$113^{a} \pm 29$	$145^{a} \pm 21$	$258^{a} \pm 25$	$0.479^{a} \pm 0.145$	$0.080^{a} \pm 0.023$	$503^{a} \pm 40$	$0.528^{a} \pm 0.018$	$0.134^{b} \pm 0.014$	$82^{a} \pm 1$	$84^{a} \pm 1$	$15^{a} \pm 4$
NaCl	3	$5.7^{b} \pm 0.3$	$158^{a} \pm 9$	$169^{a} \pm 9$	$327^{a} \pm 12$	$0.787^{a} \pm 0.063$	$0.133^{a} \pm 0.007$	$488^{a} \pm 20$	$0.536^{a} \pm 0.007$	$0.092^{ab} \pm 0.003$	$83^{a} \pm 0.4$	$84^{a} \pm 0.5$	$47^{b} \pm 7$
ANOVA		$p < 0.05$	NS	NS	NS	NS	NS	NS	NS	$p < 0.05$	NS	NS	$p < 0.013$
						Panicum miliaceum							
Control	30	$3.1^{a} \pm 0.1$	$89^{b} \pm 6$	$25^{b} \pm 1$	$86^{b} \pm 3$	$0.088^{a} \pm 0.004$	$0.0088^{a} \pm 0.0003$	$1087^{a} \pm 88$	$0.433^{a} \pm 0.011$	$0.379^{b} \pm 0.014$	$88^{a} \pm 0.8$	$92^{a} \pm 0.1$	$60^{a} \pm 3$
Brine	19	$3.4^{b} \pm 0.1$	$50^{a} \pm 5$	$20^{a} \pm 1$	$75^{b} \pm 3$	$0.089^{a} \pm 0.004$	$0.0093^{a} \pm 0.0002$	$907^{a} \pm 47$	$0.471^{a} \pm 0.012$	$0.322^{a} \pm 0.015$	$89^{a} \pm 0.4$	$92^{a} \pm 0.2$	$62^{a} \pm 2$
NaCl	12	$3.2^{b} \pm 0.1$	$48^{a} \pm 4$	$22^{b} \pm 2$	$71^{a} \pm 4$	$0.075^{a} \pm 0.006$	$0.0079^{a} \pm 0.0004$	$1074^{a} \pm 82$	$0.449^{a} \pm 0.014$	$0.348^{ab} \pm 0.015$	$89^{a} \pm 0.5$	$92^{a} \pm 0.4$	$63^{a} \pm 3$
ANOVA		$p < 0.05$	$p < 0.001$	$p < 0.05$	$p < 0.05$	NS	NS	NS	NS	$p < 0.05$	NS	NS	NS
						Zea mays							
Control	23	$4.7^{a} \pm 0.3$	$150^{a} \pm 15$	$210^{a} \pm 31$	$352^{a} \pm 41$	$2.010^{a} \pm 0.394$	$0.407^{a} \pm 0.033$	$860^{a} \pm 40$	$0.202^{a} \pm 0.0030$	$0.710^{a} \pm 0.004$	$76^{b} \pm 2$	$91^{a} \pm 0.2$	$53^{a} \pm 4$
Brine	3	$2.7^{a} \pm 0.3$	$76.3^{a} \pm 17$	$30.0^{a} \pm 15$	$106^{a} \pm 15$	$0.700^{a} \pm 0.138$	$0.292^{a} \pm 0.018$	$813^{a} \pm 262$	$0.031^{a} \pm 0.011$	$0.939^{a} \pm 0.020$	$56^{a} \pm 7$	$87^{a} \pm 5$	$46^{a} \pm 5$
NaCl	3	$3.0^{a} \pm 0.6$	$71.3^{a} \pm 9$	$70.3^{a} \pm 32$	$142^{a} \pm 36$	$0.757^{a} \pm 0.132$	$0.275^{a} \pm 0.024$	$1158^{a} \pm 12$	$0.049^{a} \pm 0.022$	$0.915^{a} \pm 0.036$	$62^{ab} \pm 6$	$91^{a} \pm 1$	$48^{a} \pm 3$
ANOVA		NS	NS	NS	NS	NS	NS	NS	NS	NS	$p < 0.05$	NS	NS

n = number of cases, NoL = number of leaves, RL = root length, SL = shoot length, PL = total plant length, W_f = fresh biomass, W_d = dry biomass, SLA = specific leaf area, RWR = root weight ratio, LWR = leaves weight ratio, TWC = tissue water content, TWC_s = tissue water content in shoots, TWC_r = tissue water content in roots. Statistical significance was assessed at the $p < 0.05$ level using one-way ANOVA. Values within a group in a column denoted by different letters are significantly different based on Tukey post hoc comparisons at $p < 0.05$. NS—not significant.

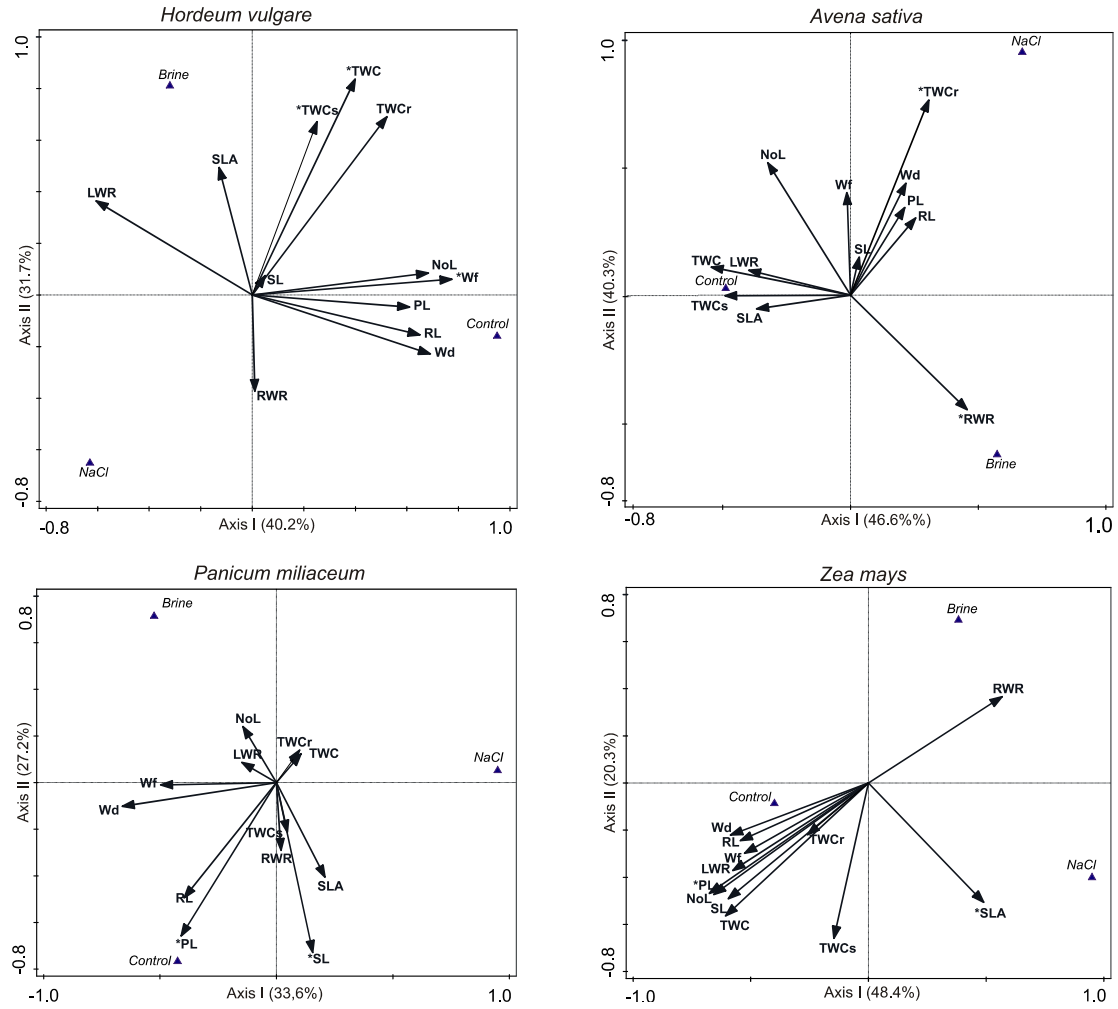

Figure 3. Results of discriminant analysis between control brine and NaCl treatments. Significant traits in group discrimination are denoted by an asterisk * ($p < 0.05$). Abbreviations as in Table 2.

4. Discussion

Our study focused on three phases of plant development that are responsible for successful crop establishment: germination, seedling emergence and plantlet early growth. It is important to note that germination is the first and one of the most important and sensitive stages of the plant life cycle [10,16,56]. Salinity inhibits germination of plant seeds in one of two ways: at higher salinities germination is stopped without loss of viability, whereas at lower salinities the delay of germination can cause some stress to seeds but does not prevent germination [57]. Lower salinity levels prolong germination, while higher levels can reduce the final percentage of seed germination [17,58]. The effect of salinity on germination of model plants was also distinct in our study. It is well recognized that salt stress negatively relates to seed germination and vigor [16]. For all plants, a decrease in seed germination was observed in the first days of the experiment. Munns [34] proved that after adding NaCl, plants usually obtain osmotic homeostasis relatively quickly, within several hours, or at least within the first day following salt stress [34]. Ionic stress, one of the elements of salinity stress, typically begins after one to three days of NaCl application despite an instant influx of Na^+ ions and transport to the shoots. The concentration of Na^+ must reach some toxic level in the cell protoplasts of shoots [34]. We did not observe such a phenomenon during germination because, in our case, after three to five

days the germination rates of *H. vulgare*, *A. sativa* and *P. miliaceum* were similar in all investigated treatments.

Similar results at a salinity of 150 mM NaCl were reported by Piernik et al. [17] for fodder beet (*Beta vulgaris* L.) and by Szymańska et al. [59] for rape (*Brassica napus* L.). Its higher optimum temperature of germination may cause the small success of germination for *P. miliaceum* independent of growth medium compared to the other species. According to Kamkar et al. [56], the GP of millet is affected by increased temperature. The highest germination occurred at 35 °C [56]. We observed limitation of germination under salinity treatment only in *Z. mays*, but we did not find a significant difference between brine and NaCl variants. In this case, the negative influence of salinity stress compared to the control may be associated with water intake [60]. A high concentration of NaCl and other salts in the solution increases osmotic potential, and water is not absorbed from the solution, which may delay and decrease germination rate [61]. High absorption of Na^+ and Cl^- ions by seeds can also be toxic, and inhibits the rate of germination, thereby decreasing germination percentage [62].

Our results based on the parameters of seven-day-old seedlings of oat, barley and maize demonstrated significant growth limitation of seedlings under saline conditions. This is in line with previous research [14,16–18]. We observed a reduction in all monitored traits. The shoot and root length are the most important salt stress indicators because roots are in contact with the soil and absorb water, and the shoot provides it to the rest of the plant [7,8]. Some experts have thought that plant shoots are more sensitive to salinity than roots, such as in *Areca catechu* L. [63]. In contrast, others have suggested that plant roots are more sensitive to salinity than shoot, as in corn [64]. However, we did not find significant differences in seven-day seedling traits in the case of *P. miliaceum*. This species is generally considered tolerant to salinity and an alternative crop for salt-affected areas [65].

Regarding all model plants, we did not find differences between brine and NaCl treatments. Hence, ionic composition did not significantly affect seedling development. This is the opposite of findings reported by Panuccio et al. [66] that the seedlings of quinoa were differently affected by treatments in respect to salt type and concentration. However, in our case the predominant ions in both solutions were Na^+ and Cl^- (Table S1). Therefore, ionic composition would not affect growth in such a short period of seedling development.

We obtained different results after six weeks of plantlet growth. First, we noticed very low growth success of tested species, especially in *P. miliaceum* and *Z. mays*. According to, for example, Konuşkan et al. [14] and Cuartero et al. [15], germination and early seedling growth is more sensitive to salinity than are later stages of growth. Our results were opposite to these findings. This is evidenced by the small number of plants surviving in brine and NaCl treatments. However, the observed reduction in survived individuals' growth rate under salinity is in line with previous research e.g., [67]. The negative action of salinity can be distinguished at several levels, such as shoot, root and tissues [7]. The number of leaves (NoL), the length of root (RL) and the total plant length of *H. vulgare*, *P. miliaceum* and *Z. mays* were lower in brine and NaCl solution compared to control. These results are consistent also with those obtained by Tsegay and Gebresslasie [68], who observed a significant reduction in the number of leaves and the length of roots under salinity stress between seven cultivars of two tested crops, and with the findings of Alam et al. [69], Anuradha [70], Mu et al. [71] and Munns [34]. Decreases in root and shoot lengths of cultivar plants under salt stress may be due to limited metabolites delivery to young growing tissues, because metabolic production is significantly perturbed at high salt stress either by low water uptake or ionic toxicity [72]. We also noticed a reduction in total fresh weight (W_f) and total dry weight (W_d) for *H. vulgare* and *Z. mays* under salt treatments. These results are in agreement with those obtained by Sozharajan and Natarajan [62]. They reported that biomass accumulation was significantly higher in a control crop than in NaCl conditions [62]. This also corresponds with the conclusion of Gururaja Rao et al. [73], who stated that salinity reduced biomass production [74]. However, total biomass can be affected by ion accumulation in tissues [75] and can, therefore, be

even higher under salinity, as observed for *A. sativa* and *P. miliaceum*. After six weeks, the lowest effect of salinity on plantlet growth was noticed for *A. sativa*. No effect of NaCl stress on the survival rate of oat seedlings was reported by Mu et al. [71]. This result can be explained by the fact that oat can accumulate much higher sodium ion levels than wheat and other seasonal crops [75]. However, Halima et al. [74] proved a progressive decrease in root and shoot lengths in *A. sativa* with increasing NaCl level. These different results of plantlet development may also depend on the species cultivar under consideration [16,65]. Discriminant analysis, taking all investigated traits into consideration, revealed that the most affected traits of C3 plants *H. vulgare* and *A. sativa* were related to water management. These results agree with the general assumption that C3 photosynthesis is negatively affected by salinity stress measured as changes in leaf water potential or relative water content [76]. *H. vulgare* had a problem with water absorption under the NaCl treatment, which was expressed by lower tissue water content in shoots. *A. sativa* had a different strategy, by accumulating water in roots under this treatment. In the case of the C4 plants *P. miliaceum* and *Z. mays*, water absorption was not affected, but the most affected traits included morphological parameters such as plant length. C4 plants are characterized by resistance to water deficit [77]. One of the main reactions to salt or drought stress is the closing of stomata, which reduces transpiration, allowing water to be saved [76]. Thanks to higher stomatal resistance in C4 plants, a low transpiration rate occurs. It is well known that C4 plants use water more efficiently than C3 plants [78].

Our findings demonstrate that crop plant species differ significantly in their growth response to salinity, as also confirmed by Arzani [10]. It was evident from the present study that crop plants varied markedly in their sensitivity to salt stress. *H. vulgare* had higher growth success than the rest of the species under salinity treatments. According to Shahid et al. [79], a plant's growth is reduced by salinity. Still, it may fluctuate from species to species depending on their tolerance and, as already mentioned, may vary depending on genotype [16]. Almodares et al. [80] reported that some plants are more sensitive to salinity because the mechanism of tolerance to high salt concentrations is not yet fully developed. Moreover, differences in the tested crop plants' cell membrane stability and macro molecule stability under salinity might also be possible causes of differential responses [81].

The current knowledge about brine's impact as a solution of variable ionic compositions on plants is rudimentary. We observed that brine decreased NoL in comparison to the control and NaCl for *A. sativa*. The same reduction in the number of leaves was noticed by Sánchez-Lizaso et al. [82] after seagrass was treated with brine. This result may suggest that other ionic components of the brine (besides sodium and chlorine ions) may have influenced the number of leaves. On the other hand, we found that brine can stimulate NoL of *P. miliaceum* compared to NaCl and control treatments. Moreover, the reduction in total plant length obtained for millet after irrigation with NaCl versus brine and control may suggest a compensatory effect of ions from brine balancing the negative influence of Na^+ or Cl^- ions. Higher toxicity to NaCl plants compared to brine may also be suggested by the lowest growth success of each model plant under NaCl conditions. To test these hypotheses, additional more detailed studies are necessary. However, any clearer trait difference for the model plants between brine and NaCl treatments indicates that osmotic stress is the main stress factor affecting the studied plants' growth and development. This is in line with the findings of García-Morales et al. [83], Kumari et al. [84], Osakabe et al. [85] and Debez et al. [86], who found that NaCl acts mainly by an osmotic effect. However, more detailed physiological studies are needed to compare the ionic composition of brine and single NaCl effects.

5. Conclusions

Our results demonstrate that salinity limits growth in plantlets more than in germination and seedling stages. Salinity most affects plantlets' water absorption in C3 species (28% of total traits variation) and plantlet length in C4 species (17–27%). It can be concluded that plants are more affected by the osmotic potential of the compared solutions than by

their ionic composition. However, more detailed physiological studies are needed to disentangle the ionic composition of brine and single NaCl effects on plants development.

Supplementary Materials: The following are available online at https://www.mdpi.com/2073-4395/11/3/435/s1, Figure S1: Plantlets of investigated model plants at the end of pot experiment after 42 days of growth, Table S1: Ionic composition of salt spring no. 16 of the Health Resort Ciechocinek, Table S2: Results of discriminant analysis, forward selection and Monte Carlo permutation test.

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