Article

Assessment of the Physiological Condition of Spring Barley Plants in Conditions of Increased Soil Salinity

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Abstract: Soil salinity is one of the most important environmental factors threatening agriculture on a global level. Plants show differences in resistance to salt stress, both in terms of species and variety. The aim of the study was to determine the effect of salt stress on photosynthetic efficiency and the activity of plants of two barley varieties—KWS Irina and RGT Planet. Plants grown in a pot experiment were subjected to soil treatment with sodium chloride (NaCl) at concentrations of 0, 50, 100, and 150 mmol·(dm³)^{−1}. Measurements were made four times at intervals of 7, 14, 21, and 28 days after the application of NaCl. The relative chlorophyll content in leaves (CCI) and selected chlorophyll fluorescence parameters (F_v/F_m , F_v/F_0 , and PI) and gas exchange parameters (P_N , E , g_s , and C_i) were assessed. In the final stage of the experiment, a visual assessment of the plants' condition was carried out and the amount of fresh mass (FM) of the above-ground part was determined. The content of sodium and potassium in the vegetative parts of plants was also analysed. Salinity significantly influenced the values of the measured parameters in both of the tested barley genotypes. High salt concentrations in the soil at levels of 100 and 150 mmol NaCl (dm³)^{−1} negatively affected the growth and development of plants by disturbing the process of photosynthesis and other plant gas exchange parameters. The antagonistic effect of sodium in relation to potassium resulted in a decrease in the K⁺ content in the plants, along with an increase in the salinity level.

Keywords: chlorophyll fluorescence; gas exchange; *Hordeum vulgare* L.; plant stress

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1. Introduction

Due to its wide range of applications, barley is one of the most popular species grown in the world. Its role as a raw material for the brewing industry is particularly important, therefore it is justified to undertake research on the reaction of new malting barley varieties for cultivation in conditions of increased soil salinity in the world. Among the abiotic stresses, salinity is one of the most significant threats to cereal crops, which are a strategic commodity [1–3]. It is one of the main processes contributing to soil degradation that threatens the ecosystem. In addition, it is recognised as one of the most important problems at a global level in terms of agricultural production, food security, and sustainable development [1,4–6].

Salinity problems occur in all climatic zones and are a consequence of both the mechanisms of nature (primary salinity) and human activities (secondary salinity). The distribution of salinized soils varies geographically and is largely determined by the type of climate, landscape type, agrotechnics used, irrigation methods, and land management policies. Salt stress is a real threat to agriculture all over the world, especially in countries where crop irrigation is used. It is estimated that the increase in soil salinity amounts to 0.3 to 1.5 million hectares of arable land annually, which reduces the production potential by a further 20 to 46 million hectares [1]. Plants growing in soils with a high concentration of salt have a limited ability to take up water, as well as macro- and micro-elements, as a result of which the seed germination capacity is reduced, and the process of photosynthesis is disturbed, which has a negative impact on plant growth and yield [7–16].

Under salinity conditions, plants activate various biochemical and physiological mechanisms to deal with the resulting stress. These mechanisms include changes in morphology, anatomy, water relations, photosynthesis, hormonal profile, distribution of toxic ions and biochemical adaptation (e.g., antioxidant metabolism response) [7,10,17–20]. The closing of the stomata is the first response of plants to abiotic stress [8,21]. Unfavourable environmental conditions allow them to increase the rate of respiration, which is a necessary condition for the production of ATP in order to activate substances soluble in osmosis, which reduce the osmotic potential of the cell and thus increase its water uptake [22]. It has been found that salt stress affects photosynthesis both in the short and long term. In the short term, salinity may affect photosynthesis by limiting the stomata, leading to a reduction in carbon assimilation [10]. This effect may cause a rapid and significant halt in plant growth, even after several hours of exposure to salt [23]. In the long term, salt stress may affect the photosynthesis process by accumulating salt in young leaves [11] and reducing the concentration of chlorophyll and carotenoids [24–26]. The rate of photosynthesis may decrease due to stomatal closure or other extramedullary restrictions such as disruption of the photosynthetic electron chain or inhibition of the Calvin cycle enzymes such as Rubisco, phosphoenol pyruvate carboxylase, ribulose-5-phosphate kinase, glyceraldehyde, or 3-phosphate dehydrogenase fructose-1,6-bisphosphatase [10,27]. A decrease in stomatal conductivity may prevent excessive water loss as a result of transpiration, while proper regulation of the photosynthesis process may minimize the production of ROS in PS II ($^1\text{O}_2$) and on the reducing side of PSI (O_2^- and H_2O_2) [28].

The reductions in the photochemical and non-photochemical parameters that occur in plants subjected to salt stress depend on the plant species and the severity of the stress. Under salinity conditions, there is a general decrease in the photochemical quenching parameters and electron transport rate, and an increase in the non-photochemical quenching parameters [19,20,29–31]. The stress response of NaCl is correlated with a decrease in PSII efficiency and an increase in non-photochemical quenching parameters as a mechanism for the safe dissipation of excess energy.

The negative impact of salinity on plants may also consist of the toxic effect of a specific ion on the plant (Na^+ , Cl^-) or exceeding of the tolerance threshold of a given plant species to the total salt concentration, which contributes to an increase in osmotic pressure and a limitation or even loss of water uptake, resulting in the occurrence of physiological drought [32–35]. There are many reports in the literature on plant resistance to cultivation under salt stress, but there is no detailed information on the differentiation of resistance within varieties. Therefore, studies were undertaken to assess the physiological response of two malting barley varieties (KWS Irina and RGT Planet) in response to salinity stress at different NaCl concentrations. The research hypothesis assumes that the increased concentration of NaCl in the soil influences the functioning of the photosynthetic apparatus and the osmotic properties of barley (*Hordeum vulgare* L.) plants.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

The pot experiment was performed under controlled conditions at the University of Rzeszów (Poland). Two varieties of barley, KWS Irina and RGT Planet, were sown in pots (10 cm in diameter) in which 1.5 kg of soil with a clay sand particle size composition and a slightly acidic pH (pH: KCl 6.35; H_2O 6.52) was placed. The total content of compounds in the soil was as follows: phosphorus oxide (P_2O_5) $17.4 \text{ mg} \cdot 100 \text{ g}^{-1}$, potassium oxide (K_2O) $17.0 \text{ mg} \cdot 100 \text{ g}^{-1}$, magnesium (Mg) $8.87 \text{ mg} \cdot 100 \text{ g}^{-1}$, and calcium (Ca) $9.46 \text{ mg} \cdot 100 \text{ g}^{-1}$. The experiments were carried out in four replications in a growth chamber (Model GC-300/1000, JEIO Tech Co., Ltd., Daejeon, Korea) at a temperature of $23 \pm 2^\circ\text{C}$, moisture $60 \pm 3\%$ RH, photoperiod of 16/8 h (L/D), and a maximum light intensity of about $350 \mu\text{mol m}^{-2} \cdot \text{s}^{-1}$. The substrate moisture was maintained at a level of 60% of the field water capacity. The positions of the pots in the experiment were randomised weekly. In the two-leaf stage, an aqueous NaCl solution with concentrations of 50, 100, and $150 \text{ mmol (dm}^3)^{-1}$ was applied

to the soil. Plants untreated with NaCl (0 mmol NaCl (dm³)^{−1}) were used as the controls. Physiological measurements of the barley plants were taken four times on the first or second fully developed leaves at intervals of 7, 14, 21, and 28 days after the application of the NaCl solution to the soil. The plants were harvested 35 days after emergence (on the date of the last measurement).

2.2. Measurement of Gas Exchange

The net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO₂ concentration (C_i) were measured on two fully developed leaves. The photosynthesis measurement system LCpro-SD (ADC Bioscientific Ltd., Herts, UK) was used to measure the photosynthesis of leaves. The LCpro-SD plant leaf photosynthesis chamber has a flow accuracy of $\pm 2\%$ of its range. During the measurement, the light intensity in the measuring chamber was 350 $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$, and the temperature was $23 \pm 2^\circ\text{C}$.

2.3. Measurement of the Relative Chlorophyll Content

Measurement of the relative chlorophyll (CCI) content was taken using a CCM-200plus handheld chlorophyll meter (Opti-Sciences, Hudson, NH, USA). Measurements were made on three fully developed leaves per pot.

2.4. Measurement of Chlorophyll Fluorescence

To measure chlorophyll fluorescence, a continuous excitation Pocket PEA fluorimeter (Pocket PEA, Hansatech Instruments, King's Lynn, Norfolk, UK) equipped with black shading clips that were applied to the leaf lamina away from the leaf nerve was used. The following parameters were measured: the maximal quantum yield of PSII photochemistry (F_v/F_m), the maximum quantum yield of primary photochemistry (F_v/F_0), and the photosynthetic yield index (PI). The fluorescence signal was collected under actinic red light with a light source peak wavelength of 627 nm and transmitted for 1 s at the maximum available intensity of 3500 μmol (photon) for photosynthetically active radiation (PAR) $\text{m}^{-2}\text{s}^{-1}$. Fluorescence measurements were performed in triplicate in each pot on the medial leaf lamina after 30 min of dark adaptation.

2.5. Determination of Fresh Mass and Assessment of Plant Condition

In the final stage of the study, the visual condition of the plants was assessed. The assessment was carried out on a 9° scale (where 9° indicates no leaf damage and 1° indicates complete leaf damage). The evaluation included the number of leaves that differed in morphological appearance (leaf wilt, necrosis, and chlorosis, etc.). It was assumed that 9° = 0–5%, 8° = 6–15%, 7° = 16–25%, 6° = 26–40%, 5° = 41–60%, 4° = 61–75%, 3° = 76–85%, 2° = 86–95%, and 1° = 96–100% of the above-ground part of plants with visible changes. The above-ground part of the plants were then weighed and their fresh mass (FM) was determined. The weight of the plants from individual varieties of the experiment was compared with the weight of the plants from the control (100%), in accordance with the following equation:

$$\text{FM}_{(\%) } = \frac{m_{ss} \cdot 100}{m_c}$$

where:

FM (%)—calculated weight of fresh plants;

m_{ss} —the mass of the above-ground part of plants subjected to salinity;

m_c —the mass of above-ground part of control plants.

2.6. Analysis of Na and K Content in Leaves and Roots

The total Na⁺ and K⁺ contents were analysed by absorption spectrometry (ASA) [36]. A representative leaf sample was dried and ground in a laboratory mill and was mineralised in an open system in concentrated nitric acid in a Foss Tecator 1012 heating block.

After mineralisation, the samples were quantitatively transferred to volumetric flasks that were then filled with deionised water. The content of the elements in the solutions was determined using a Hitachi Z-2000 atomic absorption spectrometer.

2.7. Statistical Analysis

Statistical analysis was performed using TIBCO Statistica 13.3.0 (TIBCO Software Inc., Palo Alto, CA, USA). In order to check the normality of the distribution at $p = 0.05$, the Shapiro–Wilk test was performed. The homogeneity of variance was also checked. A two-way repeated measures ANOVA (with time assessment as a factor) was then performed. In order to determine and verify the relationships, Tukey's post hoc test was performed with a significance level of $p \leq 0.05$.

3. Results

3.1. The Effect of Salinity on the Relative Content of Chlorophyll in Barley Leaves (CCI)

The concentration of NaCl had a significant effect on the content of chlorophyll in the leaves of the barley varieties tested. On the first date of measurement, along with an increase in salt concentration in the medium, the varieties KWS Irina and RGT Planet reacted with a decrease in the chlorophyll content in the leaves. This relationship was observed at higher salt concentrations. A significant decrease in the chlorophyll content in the leaves compared with the control, of 22.5% (KWS Irina) and 20.2% (RGT Planet), respectively, was observed at a salinity of 100 mmol NaCl (dm³)^{−1}. With a salinity of 150 mmol NaCl (dm³)^{−1}, the decrease was 43.2% (KWS Irina) and 47.3% (RGT Planet), respectively. The exception was the concentration of 50 mmol NaCl (dm³)^{−1}, when the chlorophyll content in the leaves did not differ significantly from the control. On the second date of measurement, a significant decrease in the content of chlorophyll was observed in the tested barley varieties, regardless of the salt concentration. Compared with the control, the greatest decrease, of 44.4% in the KWS Irina variety and 49.7% in the RGT Planet variety, was noted at the concentration of 150 mmol NaCl (dm³)^{−1}. Similar relationships were noted on the third and fourth dates of measurement.

Analysing the effect of the exposure time of the plants to NaCl in terms of the chlorophyll content in the leaves, it was observed that only on the first and second dates were the measurement results statistically significant for low salt concentrations, which most likely proves the stimulating effect of NaCl at a concentration of 50 mmol (dm³)^{−1} (Figure 1). There were no statistically significant differences between the values obtained on the third and fourth measurement dates.

3.2. The Influence of Salinity on the Parameters of Chlorophyll Fluorescence

In the tested barley varieties, the F_v/F_m changed with increasing the salt concentration. A significant decrease in relation to the control, of 8.5% (KWS Irina) and 13.6% (RGT Planet), was demonstrated at the highest concentration (150 mmol NaCl (dm³)^{−1}). Lower NaCl concentrations did not significantly affect the value of the analysed parameter. The duration of plant exposure to NaCl did not significantly affect the value of the F_v/F_m ratio (Figure 2A).

Figure 1. The effect of the NaCl ($\text{mmol NaCl (dm}^3\text{)}^{-1}$) concentration and measurement date on the chlorophyll content in the leaves (CCI; date 1, 7 days after NaCl application; date 2, 14 days after NaCl application; date 3, 21 days after NaCl application; and date 4, 28 days after NaCl application) * Capital letters indicate significant differences between the means at the measurement dates for particular NaCl concentrations, and lower case letters indicate significant differences between the means at subsequent measurement dates ($p = 0.05$).

Figure 2. Cont.

Figure 2. The effect of NaCl ($\text{mmol NaCl (dm}^3\text{)}^{-1}$) concentration and measurement date on chlorophyll fluorescence parameters: maximal quantum yield of PSII photochemistry (F_v/F_m) (A), maximum primary photochemistry yield (F_v/F_0) (B), and PS II performance index (PI) (C) in the varieties of KWS Irina and RGT Planet (date 1, 7 days after NaCl application; date 2, 14 days after NaCl application; date 3, 21 days after NaCl application; date 4, 28 days after NaCl application) * Capital letters indicate significant differences between the means in the measurement dates for particular NaCl concentrations, and lower case letters indicate significant differences between the means at subsequent measurement dates ($p = 0.05$).

F_v/F_0 was dependant on the salt stress caused by the increased concentration of the aqueous salt solution used in the experiment. The application of NaCl at a concentration of $100 \text{ mmol (dm}^3\text{)}^{-1}$ caused a decrease in F_v/F_0 of an average of 22.2% for the KWS Irina variety and 20.8% for the RGT Planet variety. The lowest values of the parameter were obtained with $150 \text{ mmol NaCl (dm}^3\text{)}^{-1}$ at each measurement date. In the case of the KWS Irina variety, these values were lower by 35.4% on average compared with the control, and in the case of the RGT Planet variety, the difference was 34.4% on average. There were no statistically significant differences between the control sample and the values obtained for plants treated with a $50 \text{ mmol NaCl (dm}^3\text{)}^{-1}$ solution. The described relationships regarding the concentrations used were analogous in the subsequent measurement dates. By analysing the exposure time of plants to the effect of different salt concentrations, it was shown that on the first and second measurement dates, only salt at a concentration of $50 \text{ mmol NaCl (dm}^3\text{)}^{-1}$ had a positive effect on the F_v/F_0 value. Higher salt concentrations ($100 \text{ mmol NaCl (dm}^3\text{)}^{-1}$ and $150 \text{ mmol NaCl (dm}^3\text{)}^{-1}$) caused a significant decrease in the value of the analysed parameter. On the third and fourth measurement dates, a significant decrease in the F_v/F_0 value was noted, regardless of the concentration used (Figure 2B).

In the analysed varieties, PI decreased with the increase in NaCl concentration, regardless of the date of the test. On the first date of measurement, a significant decrease in PI was demonstrated after the application of 100 and $150 \text{ mmol NaCl (dm}^3\text{)}^{-1}$ compared with the control. The decrease was 6.8% and 20.0%, respectively, for KWS Irina and 16.3% and 27.2%, respectively, for RGT Planet. The decrease in PI was still observed on the second measurement date. A significant decrease in the measured parameter, of 38.0% in KWS Irina and 36.6% in RGT Planet, was noted primarily at the highest concentration of NaCl ($150 \text{ mmol (dm}^3\text{)}^{-1}$). At the next measurement dates, salt stress had caused a significant decrease in PI in the barley plants. At a concentration of $100 \text{ mmol NaCl (dm}^3\text{)}^{-1}$, the decrease in relation to the control was 30.1% (date 3) and 30.7% (date 4) in the KWS Irina variety, respectively, and 38.4% (date 3) and 32.4% (date 4) in RGT Planet, respectively. The highest concentration of NaCl used ($150 \text{ mmol (dm}^3\text{)}^{-1}$) resulted in a decrease in the PI value, of 46.8% (date 3) and 52.1% (date 4) in the KWS Irina variety, and 44.5% (date 3) and 46.3% (date 4) in RGT Planet. At the first and second dates of measurement, the use of $50 \text{ mmol NaCl (dm}^3\text{)}^{-1}$ had a positive effect on the values obtained compared with the control, but these differences were not statistically significant. On the third and

fourth dates, the salt in this concentration caused a decrease in the measured parameter in the same way as in the case of the higher concentrations. Analysing the effect of the exposure time of plants to the increased concentration of NaCl in the soil, the use of NaCl at the level of $50 \text{ mmol (dm}^3)^{-1}$ significantly influenced the value of the parameter in the studied barley varieties. On the first and second dates of measurement, the values obtained were higher than the control by an average of 3.2% in KWS Irina and 0.2% in RGT Planet. Along with the salt exposure time, the concentration of $50 \text{ mmol (dm}^3)^{-1}$ caused significant decreases in PI (dates 3 and 4; Figure 2C).

3.3. The Influence of Salt Stress on the Course of Gas Exchange

The study showed the significant effect of salinity on the value of the P_N . An increase in the NaCl concentration in the soil resulted in a decrease of this parameter in the studied varieties. The greatest differences were noted at concentrations of 100 and $150 \text{ mmol (dm}^3)^{-1}$, and they were on average 25.7% and 59.1% (KWS Irina) and 36.3% and 54.5% (RGT Planet), respectively, in relation to the control. In plants treated with $50 \text{ mmol (dm}^3)^{-1}$ salt, no significant reductions in P_N were observed. The effect of plant exposure time on NaCl was visible only in the KWS Irina variety. On the fourth date of measurement, KWS Irina showed a significant decrease in P_N at a concentration of $100 \text{ mmol (dm}^3)^{-1}$ (Figure 3A).

Salt stress influenced plant transpiration. Both varieties reacted with a significant decrease in E at the highest NaCl concentrations. At a concentration of $100 \text{ mmol NaCl (dm}^3)^{-1}$, this decrease was on average 14.0% in KWS Irina and 13.5% in RGT Planet. At a concentration of $150 \text{ mmol NaCl (dm}^3)^{-1}$, a decrease in E values was also observed, of 27.4% (KWS Irina) and 26.5% (RGT Planet). A negative effect of NaCl at the level of $50 \text{ mmol (dm}^3)^{-1}$ was found on the third and fourth dates (Figure 3B).

The application of the NaCl solution to the soil in various concentrations significantly influenced g_s . On the first date of measurement, both varieties reacted with an increase in g_s at a concentration of $50 \text{ mmol NaCl (dm}^3)^{-1}$. The increase in relation to the control was 14.2% for KWS Irina and 43.7% for RGT Planet. In both varieties, on the first measurement date, significant falls in g_s compared with the control were noted at the concentration of $150 \text{ mmol NaCl (dm}^3)^{-1}$. These amounted to 61.9% (KWS Irina) and 62.5% (RGT Planet). On the subsequent measurement dates (dates 2, 3, and 4), along with an increase in salt concentration, decreases in the measured parameter were noted at a concentration of $50 \text{ mmol NaCl (dm}^3)^{-1}$, but they were not significant. A significant relationship was demonstrated for the higher salt concentrations—100 and $150 \text{ mmol NaCl (dm}^3)^{-1}$. The average decrease in g_s value during the entire period of salt stress at a concentration of $100 \text{ mmol NaCl (dm}^3)^{-1}$ was 36.0% in KWS Irina and 31.1% in RGT Planet, while at $150 \text{ mmol NaCl (dm}^3)^{-1}$, it was 71.9% in KWS Irina and 69.5% in RGT Planet (Figure 3C).

The conducted research has shown the influence of salt on C_i . Higher salt concentrations (100 and $150 \text{ mmol NaCl (dm}^3)^{-1}$) caused a significant decrease in C_i for each measurement date. In the KWS Irina variety on the first measurement date, the decrease was 15.1% and 29.5%, and in the RGT Planet variety 15.5% and 21.5%, with a salinity of 100 and $150 \text{ mmol NaCl (dm}^3)^{-1}$, respectively. Significant decreases in the value of C_i were also observed in relation to the control at subsequent measurement dates (longer duration of salt stress). On the last date of measurement (date 4), the decrease in the value of C_i was the highest in relation to the control, and amounted to 20.2% and 20.5% in KWS Irina and 22.9% and 24.8% in RGT Planet with a salinity of 100 and $150 \text{ mmol NaCl (dm}^3)^{-1}$, respectively. The average decrease in C_i during stress over the entire study cycle was 17.4% and 26.1% in the KWS Irina variety, and 25.3% and 30.1% in RGT Planet, respectively, which was statistically proven (Figure 3D).

Figure 3. The effect of NaCl ($\text{mmol NaCl}(\text{dm}^3)^{-1}$) concentration and measurement date on gas exchange parameters: net photosynthetic rate (P_N) (A), transpiration rate (E) (B), stomatal conductance (g_s) (C), and intercellular CO_2 concentration (C_i) (D) in the KWS Irina and RGT Planet varieties (date 1, 7 days after NaCl application; date 2, 14 days after NaCl application; date 3, 21 days after NaCl application; date 4, 28 days after NaCl application) * Capital letters indicate significant differences between the means in the measurement dates for particular NaCl concentrations, and lower case letters indicate significant differences between the means at subsequent measurement dates ($p = 0.05$).

3.4. The Effect of Salt Stress on the Fresh Mass of Barley Plants and Plant Condition

The use of salt in various concentrations resulted in a reduction in the FM of the above-ground part of the plant and in deterioration of the plant condition in both barley varieties. A stronger response of the plants to salinity was observed at higher salt concentrations of 100 and 150 mmol NaCl (dm³)⁻¹. The application of salt with a concentration of 150 mmol NaCl (dm³)⁻¹ resulted in a reduction of FM of 13.8% in the KWS Irina variety and 16.9% in the RGT Planet variety compared with the control, and the condition of the plants was assessed at 6.5° and 6° in the studied varieties, respectively (Figure 4).

Figure 4. The effect of salt stress on the condition of the plants and the content of the fresh mass of barley plants in the KWS Irina and RGT Planet cultivars.

3.5. Ion Content in Roots and Leaves (Na⁺ and K⁺)

The concentration of NaCl in the soil influenced the content of sodium and potassium in the leaves and roots of both of the tested barley varieties. With an increase in the salinity level, the potassium content in the tested parts of the plants decreased, while the percentage of sodium increased. The greatest differences in the content of K⁺ and Na⁺ in relation to the control sample were noted at the NaCl concentration of 150 mmol (dm³)⁻¹. Compared with the control, the potassium content decreased with 150 mmol NaCl (dm³)⁻¹ in the leaves by 2.8% dry mass (DM) in the KWS Irina variety and 3.8% DM in RGT Planet, and in the roots by 0.28% and 0.31% DM, respectively. At the highest concentration of NaCl, compared with the control, the increase in sodium content in KWS Irina was 1.67% DM in the leaves and 0.59% DM in the roots, while in RGT Planet it was 2.13% DM (leaves) and 0.33% DM (roots) (Figure 5A,B).

Figure 5. Cont.

Figure 5. Potassium (K⁺) and sodium (Na⁺) content in the leaves (A) and roots (B) of barley plants depending on the NaCl (mmol NaCl (dm³)^{−1}) concentration used. Capital letters indicate significant differences between the means in the for particular NaCl concentrations ($p = 0.05$).

4. Discussion

Barley is one of the most tolerant crops under conditions of salinity stress, but with a higher NaCl level, its growth and yield decrease. There are many reports in the literature about the negative influence of salinity on the course of physiological processes in plants as well as a lot of information about the substances used to alleviate the effects of salt stress in plants, for example by reducing the accumulation of reactive oxygen species (ROS) by activating antioxidant enzymes [16,37–43].

Chlorophyll is one of the most important features reflecting the health condition of plants, and it is related to the availability of water and the level of plant nutrition [44]. The concentration of NaCl at the level of 50 mmol (dm³)^{−1} in the initial phase of the experiment had a positive effect on the content of chlorophyll in the leaves and the values of the parameters F_v/F_0 , PI, P_N , E , g_s , and C_i , regardless of the cultivar. The results of other researchers have shown that moderate amounts of Na⁺ can improve plant growth [45,46]. High concentrations of NaCl (100 and 150 mmol NaCl (dm³)^{−1}) in the conducted experiment caused significant decreases in the content of chlorophyll in the leaves, parameters of chlorophyll fluorescence, and gas exchange in both of the tested varieties at each measurement date. A decrease in the level of chlorophyll at a salinity level of 100 mmol NaCl (dm³)^{−1} was also noted in studies carried out on various barley varieties by El-Esawi et al. [47]. The reduction of photosynthetic pigments in most stressed plants may be due to the disintegration of the thylakoid membrane, with a greater degradation of chlorophyll than synthesis through the formation of proteolytic enzymes responsible for the degradation of chlorophyll, as well as through damage to the photosynthetic apparatus [48].

Chlorophyll fluorescence is an effective tool for analysing the photosynthetic capacity of plants and their health under normal or stressful conditions, including salinity stress [49]. The measurement of chlorophyll fluorescence is considered to be a precise method that enables the detection of changes in the general bioenergetic conditions of photosynthetic organisms [50–53]. Salinity at the level of 100 and 150 mmol NaCl (dm³)^{−1} caused in the tested varieties significantly decreases in chlorophyll fluorescence for parameters such as F_v/F_m , F_v/F_0 , and PI. The F_v/F_m ratio reflects the photochemical yield of PSII. Usually, a decrease in photosynthesis efficiency is the first symptom of the negative impact of stresses on a plant, which can affect the maximum PSII quantum yield and is proportional to the F_v/F_m ratio, reflecting the efficiency of light in primary photosynthetic reactions. NaCl stress may disrupt the biochemistry of photosynthesis, limiting the efficiency of two photosystems due to disruption of the integrity of chloroplasts. Under the influence of salt stress, photochemical efficiency and electron transport in PSI and PSII decrease as a result of changes in the structure of thylakoids, limiting the biosynthesis of chlorophyll [48,54,55]. Similar decreases in studies conducted on barley were noted by Kalaji et al. [56]. In our own study, along with the time of exposure of plants to high NaCl

concentrations in the soil, the decreases in the parameters tested were higher than in the control for the analysed cultivars. A negative effect of the duration of salt stress was also noted in studies by Bayuelo-Jiménez et al. [57].

At the highest salt concentrations, there was a significant decrease in gas exchange parameters of P_N , E , g_s , and C_i . Usually, plants close their stomata when stressful conditions arise in order to conserve water, consequently reducing the conductivity of the stomata and photosynthesis [27,58]. In the long term, salinity causes ionic toxicity due to an imbalance of nutrients in the cytosol. In addition, salt stress also manifests itself as oxidative stress at a subcellular level, which is mediated by reactive oxygen species (ROS) [59]. All these responses to salinity contribute to the detrimental effect of salinity on plants [8]. Scientific reports indicate the existence of differences in the level of resistance to salinity between varieties [40,47,60–63]; however, in our own research no significant differences were found between the analysed cultivars. The results of the conducted research indicate that the KWS Irina and RGT Planet varieties react to salinity in a way characteristic of barley genotypes, with a lower tolerance compared with the genotypes considered tolerant to salt stress, losing more than 40% of their total chlorophyll content at the highest salt concentrations [60,64].

To understand the mechanisms of salinity tolerance, it is important to determine the concentrations of Na^+ and K^+ in plants. In the experiment carried out in plants, along with an increase in soil salinity, decreases in the K^+ content and an increase in Na^+ in the leaves and roots was observed. The negative effect of salinity is a consequence of Na^+ interference in the assimilation of K^+ [65–67]. Glycophytes typically react to salt stress by trying to limit the build-up of toxic ions in the leaves, reducing their absorption through the roots or blocking their transport to the above-ground part of the plant. These mechanisms are effective only at a low or moderate salinity, and after exceeding a certain threshold—depending on the tolerance of each specific genotype—there is an increase in Na^+ concentration in the leaves [68]. Salinity stress is responsible for an excessive accumulation of Na^+ while reducing the uptake of essential nutrients, such as K^+ , from the roots to the above-ground part responsible for photosynthesis [69]. Salinity largely affects the roots due to their direct contact with the soil. Therefore, the underground parts of plants are primarily responsible for protection against the adverse effects of salinity [54]. Increased Na^+ concentration in the root zones has an antagonistic effect on K^+ uptake. As a consequence, a deficiency of K^+ causes growth inhibition, and thus also a reduction in plant weight [40]. The conducted studies confirm the results of many researchers who noted the harmful effects of high levels of sodium chloride on plants [62,70–72].

Authors should discuss the results and how they can be interpreted from the perspective of previous studies and from the working hypotheses. The findings and their implications should be discussed in the broadest context possible. Future research directions may also be highlighted.

5. Conclusions

The aim of the experiment was to evaluate the effect of salt stress on the efficiency and activity of the photosynthetic apparatus of plants of two barley varieties at different NaCl concentrations. The relative chlorophyll content in leaves and selected parameters of chlorophyll fluorescence and gas exchange were assessed. At a salt concentration of 50 mmol $NaCl \cdot (dm^3)^{-1}$, the analyzed varieties were characterized by similar values of the relative content of CCI chlorophyll, fluorescence parameters (F_V/F_m , F_V/F_0 , and PI) and gas exchange parameters (P_N , E , g_s , and C_i) compared with the controls. Moreover, the concentration of 50 mmol $NaCl \cdot (dm^3)^{-1}$ in the initial phase of the experiment had a positive effect on the value of some of the parameters tested and did not worsen the condition of the plants. Likewise, plant health and dry matter content were most favourable at this salt concentration. Higher concentrations (100 and 150 mmol $NaCl \cdot (dm^3)^{-1}$) had a negative effect on the physiological apparatus of plants already at the first time of measurement, and for the duration of the experiment, the differences in the values of the parameters

tested between them increased. The research showed a significant influence of salinity on the P_N value. The increase in NaCl concentration in the soil resulted in a decrease of this parameter in the studied varieties. Salt stress also influenced plant transpiration. Both varieties responded with a significant decrease in E at the highest NaCl concentrations. Similarly, in the case of g_s , decreases in the measured parameter were noted with increasing the salt concentration. Moreover, higher salt concentrations caused a significant decrease in C_i on each measurement day. A similar dependence was observed in the case of the chlorophyll fluorescence parameters, which changed with the increasing salt concentration and the duration of the experiment. The tested brewing varieties, analysed in terms of physiological parameters, are not suitable for cultivation in areas with a high degree of salinity. However, research should be carried out on the possibility of introducing other varieties of this species to cultivation in areas with a higher salt content in the soil.

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