Research Article

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# Effect of weather, nitrogen fertilizer, and biostimulators on the root size and yield components of *Hordeum vulgare*

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**Abstract:** In this study, the effect of nitrogen doses (52, 80, 110, 140 kg/ha N) and the application of biostimulant preparations containing *Ascophyllum nodosum* L. algae extract were assessed. During the years 2018–2019, the influence of the preparations on the electrical capacity of the roots ($C_R$) and yield components of spring barley was determined. Root electrical capacitance was determined in growth stages 45–50, 55–65, and 70–75 according to the BBCH-scale. The best phases of vegetation growth for the application of biostimulators with *Ascophyllum nodosum* extract were the barley tillering and elongation phases. This application increased $C_R$ while reducing the amount of N required to achieve similar or higher production of barley yield components compared to high N treatments. The root electrical capacitance, the number of productive tillers, and the number of grains per plant were significantly influenced ($p > 0.05$) by the weather of the year. The number of productive tillers was closely correlated with $C_R$ ($r$ = 0.912**) as well as the number of grains per plant ($r$ = 0.859**) and their weight ($r$ = 0.850**). These relationships were the highest at the beginning of the grain formation (BBCH 70–75). Foliar biostimulation was not very effective in the dry year of 2018. The problem may be the foliar application itself. The effect of foliar application is strongly dependent on weather conditions and may be ineffective in many cases. We recommend the foliar application of effective biostimulants in tillering and elongation phases. They can reduce production costs and environmental pollution by reducing the amount of fertilizer needed while maintaining yields.

**Keywords:** *Ascophyllum nodosum*, root size, foliar application, drought, cereal, electrical capacitance

## 1 Introduction

*Ascophyllum nodosum* is one of the most researched and used algae in agricultural production [1]. Much of the research on the effects of *A. nodosum* extracts on various plant species has been reviewed [1]. The positive effects of *A. nodosum* on plants include better absorption of macro and micronutrients, improved root and above-ground growth, stimulation of gene expressions involved in plant growth and development, and increased tolerance to biotic and abiotic stresses [1–5]. The positive effects are related to phytohormones such as auxins, cytokinins, abscisic acid, and substances with similar effects [1–5]. The content of the individual components is highly variable and differs depending on the extraction methods (acid or basic hydrolysis and others), pH, temperature, and time of the algae harvest [4].

Malting barley (*Hordeum vulgare*) is the primary raw material for beer production and has to achieve high yields with optimal grain quality. The shape, size, weight, test weight, germination of the grain, and the content of nitrogenous substances in the grain are very important [6,7]. The malting industry demands a high quality of malting barley, which also determines its purchase price and use [6,7]. Quality standards are often not met, and malting barley is bought at a low price or used for animals [6,7]. Drought and high temperatures most often cause reduced yield and grain quality [8]. A number of agronomists then use fertilizers with a high nitrogen content to correct quality or yields. It is a negative side of agronomy which has a negative impact on ecology. In addition, a large amount of used fertilizers is not used by plants. Depending on the weather, 65% of the applied nitrogen fertilizer may not be absorbed by crops and end up in the environment [9–11].

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*A. nodosum* can generally stimulate root growth or increase the efficiency of soil nutrient absorption and utilization [1]. An increase in the efficiency of nutrient uptake was also observed in cereals (*H. vulgare*, *Triticum aestivum/durum*, *Zea mays*). It was related to a higher yield of plants and a reduction in the required amount of fertilizers [1–5]. Drought and increased temperature have a negative effect on the root system size (RSS), and they change its architecture [10,11]. Elevated temperature increases the negative impact of drought but can also act on its own. Therefore, even with sufficient precipitation, there may be a decrease in the yield or quality of cereals [10–15]. RSS and architecture play a key role in the absorption of water and nutrients from the soil [12–14]. The growth of primary or lateral roots and the formation of root hairs are related to the availability of water and nutrients [12–14]. A quick response to a negative condition through root growth gives plants an advantage. With this strategy, the plants can find faster groundwater or missing nutrients. Such cereals are more resistant to drought [15,16]. Stimulation or inhibition of root growth, as well as growth direction, is also influenced by the interactions of phytohormones, weather, and variety [13,14,17,18]. The higher the plasticity of the plant root system, the higher the tolerance level against drought stress [12–14]. The root architecture of barley root, its volume or size, and growth direction affect the yield and barley grain quality and can affect the response of plants in various stressful situations. This impact is strongly dependent on the weather, especially on the temperature and the amount of precipitation [12]. In malting barley, a bigger root system increases starch content, malt extract, and proteins during the dry season [11]. On the contrary, in dry conditions a weak root system corresponds to a low grain yield and a significant deterioration in quality [11]. A positive correlation was found between the grain yield of barley and the amount of root hairs in dry conditions [19]. However, a large root system can also be a disadvantage and can reduce grain quality in conditions of excessive precipitation. In these conditions, a shallow but wide root system is more suitable [17]. A narrow and deep root system may be more advantageous in dry conditions as it provides access to water from the deeper soil layers during the grain-filling phase [18]. If there are drought conditions from the beginning of the vegetative stage with the following period of sufficient moisture, the cereals are able to compensate for these negative effects on grain quality and yield [15]. However, in the state of water shortage during flowering and grain filling, the grain yield and its quality are compromised because the plants are no longer capable of any compensation in this phase [20].

A non-destructive method based on measuring $C_{\mathrm{R}}$ is being used to determine the size of the root system [21]. The electrical capacitance has been used as a non-destructive measure of RSS for 30 years [22]. This measurement does not destroy the roots ,especially, the root hairs. Root hairs less than 0.25 mm in size can represent 95% of the root length [23]. Determination of the RSS takes place indirectly by measuring its $C_{\mathrm{R}}$, which is closely correlated with the length and surface of the roots [21,22,24]. This method makes it possible to measure many plants per day and to repeat the measurements on the same plants in different phenological phases [21]. The advantage is practicality and easy application in field conditions. Under standardized soil conditions and with the constant location of the electrode on the plant above the substrate surface, the method adequately estimates the RSS [23–26]. This method has been used in the breeding of plants against drought, especially cereals [11,21]. Correlations were also found between $C_{\mathrm{R}}$ and the content of starch, protein, malt extract or barley grain yield, overall malt quality, or prediction of grain yield depending on the air conditions [11,21,26]. A disadvantage of this method is that it cannot display root morphology such as branching, distribution pattern, or penetration depth [27].

This research was focused on the evaluation of the combination effect of biostimulators derived from *A. nodosum* and the required amount of nitrogen nutrition to achieve a favorable effect on $C_{\mathrm{R}}$ and the formation of yield components such as the number of ears, tillers, or grains of malting barley.

## 2 Materials and methods

### 2.1 Experimental fields

A small-plot field experiment was performed on fields belonging to the agricultural company Agrospol Velká Bystřice near Olomouc, Czech Republic, from March to August 2018 and 2019. Each treatment was grown on three small plots, which means three repetitions, each 13 $\mathrm{m}^2$. The experimental plots are located at the coordinates of 49°61′ north latitude and 17°35′ east longitude. The average altitude is 240 m. The land is located in a slightly warm and humid climate. The soil is a cambisol type. Weather conditions during the growing periods are given in Figure 1. The previous crop was sugar beet. The incorporation of post-harvest residues (beet tops) was carried out by medium ploughing. The experimental fields were in the same region, in the same soil and coordinates, and in the same pre-crop each year. Fields were facing each other across a field road.

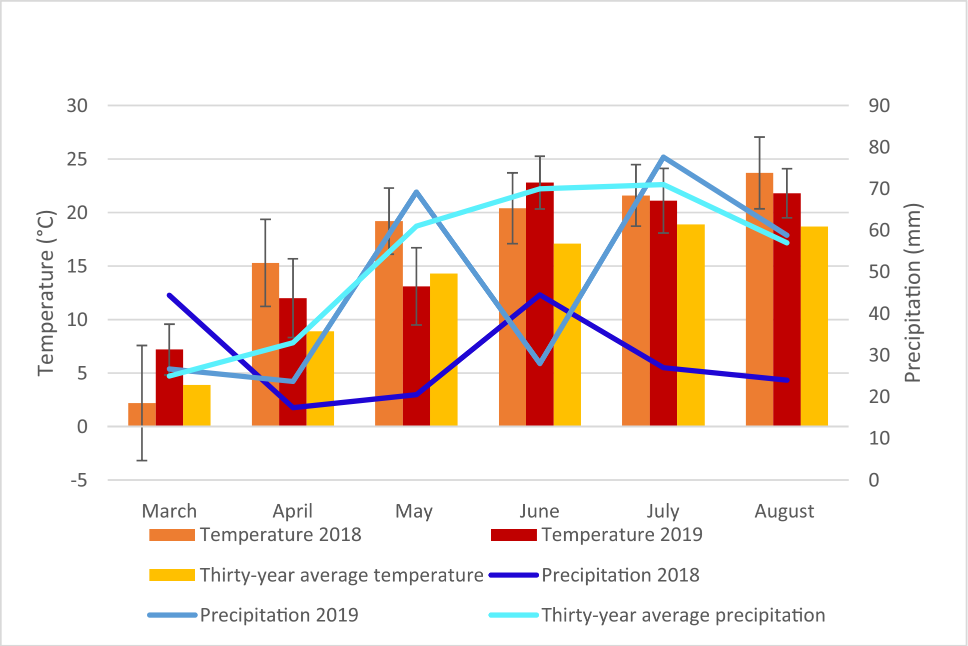

**Figure 1:** Weather conditions during the 2018–2019 growing season.

## 2.2 Experimental treatments, sowing, and mineral content in the soil

At the end of February, prior to planting, the amount of basic minerals in the soil was determined. Subsequently, P, K, and N fertilizers were applied in solid granular form. Amophos containing 52% $P_2O_5$ and 12% $NH_4$ and potassium salt containing 60% $K_2O$ were used for fertilization. The applied dose of P and K was 22.7 kg/ha P and 40 kg/ha. Ammonium nitrate with calcium carbonate (27% N, 20% $CaCO_3$) was applied at a rate of 52 kg/ha N. The final agrochemical characteristics of soil after 4 weeks from application are presented in Table 1. The Francin barley variety was sown. The sowing rate was 3.7 million germinating seeds per hectare. The sowing was carried out on 4 April 2018 and on 26 March 2019. Further fertilization to the selected level of N-nutrition was applied after the emergence of the plants, and ammonium nitrate with calcium carbonate was applied again. The experiments were harvested on 30 July 2018 and on 6 August 2019.

The ten experimental treatments are summarized in Table 2. The doses of nitrogen were 52, 80, 110, and 140 kg/ha N, and it was applied in BBCH 15 (leaf development). The biostimulant preparations called Rooter and Forthial containing the extract of the algae *A. nodosum L.* were applied with and without nitrogen in BBCH 27 (end of tillering) and BBCH 31–34 (stem elongation). The first four treatments are with the lowest nitrogen dose and one or the other biostimulator or both. The next three are just with increasing N dose, and the last three are with increasing N and both biostimulants. The application concentration of the biostimulants was 1% *A. nodosum* extract, and the rate was 1 l/ha (Table 2). The foliar application was applied in the morning. The composition of herbal preparations is listed in Table 3.

## 2.3 RSS

The measurements were performed according to the methodology of Středa et al. [21]. The root electrical capacitance was measured using an LCR 4080 digital multimeter (Voltcraft, Germany) commonly used to measure the condenser electrical capacitance (parallel mode, 1 kHz, 1 V AC). A sharp stainless-steel rod (5 mm diameter, 20 cm long) was inserted 100 mm into the field soil and 100 mm from the base of the stem. The plant stainless-steel clamp (14 cm long) was clamped to all the basal parts (all tillers of one

**Table 1:** Agrochemical properties of the plot during sowing (units mg/kg of soil)

| Year | pH | K | P | Mg | S | Ca | N (Nan) |
|---|---|---|---|---|---|---|---|
| 2018 | 5.72 | 212 | 58.1 | 150 | 10.7 | 2,280 | 178 |
| 2019 | 5.88 | 187 | 72.3 | 114 | 12.6 | 1,470 | 212 |

The nutrient content is determined according to Mehlich III, Experimental treatments.

**Table 2:** Experiment scheme

| Treatment | Dose of N (kg/ha) | | Rooter | Forthial |
|---|---|---|---|---|
| | Before sowing | BBCH 15 | BBCH 27 | BBCH 31–34 |
| 1 | 52 | | | |
| 2 | 52 | | 1 l/ha | |
| 3 | 52 | | | 1 l/ha |
| 4 | 52 | | 1 l/ha | 1 l/ha |
| 5 | 52 | 28 | | |
| 6 | 52 | 58 | | |
| 7 | 52 | 88 | | |
| 8 | 52 | 28 | 1 l/ha | 1 l/ha |
| 9 | 52 | 58 | 1 l/ha | 1 l/ha |
| 10 | 52 | 88 | 1 l/ha | 1 l/ha |

Note: each treatment was repeated three times.

**Table 3:** Composition of biostimulators

| Rooter | | Forthial | |
|---|---|---|---|
| Total phosphorus ($P_2O_5$) | 13.0% | Total nitrogen (N) | 6.02% |
| Total potassium ($K_2O$) | 5.0% | Water-soluble Mg (MgO) | 9.0% |
| Extract of *A. nodosum* | 25% | Extract of *A. nodosum* | 30.5% |
| pH | 1.60–2.6 | pH | 6.7–7.7 |

plant) of the plant 15 mm above ground level. The clamps are more practicable and less destructive than needles through the stem [24]. Each reading was taken after allowing the meter to stabilize for 6 s to obtain a case. The electrical capacitance of the roots is given in nanofarad units. The measurement took place in a standard established stand with an inter-row distance of 12.5 cm. The measured plants were always unified (plucking) in the second row of the experimental plot for each repetition so that the plants did not touch each other, and the results were not affected. There were three repetitions of each treatment with five plants. The measurements were performed at the end of shooting (BBCH 45–50), during the stages from heading to flowering (BBCH 55–65) and at the beginning of grain formation (BBCH 70–75). The number of productive tillers was determined for each plant at the full maturity. The measured plants were harvested, and the number of grains per plant and their weight were evaluated.

## 2.4 Statistical analysis

The results were evaluated using Microsoft Excel and Statistica 12 using multifactor analysis of variance followed by Tukey's *post hoc* test at a significance level of 95% ($p > 0.05$). The relationships between the selected parameters were evaluated by the correlation analysis using Pearson correlation coefficients at the 95% significance level ($p > 0.05$).

# 3 Results

## 3.1 Weather conditions

Both years were warmer than the 30-year-old (Figure 1). In 2018, there was a lack of precipitation throughout the growing season, and it was extremely dry. At first, 2019 was a little dry, but then the rainfall increased to be above the 30-year average. This caused a long dry and warm period followed by high rainfall.

The strongly negative weather conditions were significantly reflected in the growth and development of the malting barley during the vegetative stage in 2018. The sowing date was delayed due to the relatively high precipitation level in March. Subsequent severe drought negatively affected the growth and development of barley. This was also reflected in the average values of $C_R$.

The extreme drought in 2018 had a significant effect on the RSS. The unfavorable precipitation conditions and drought from the beginning of the vegetative stage eliminated the effect of nitrogen fertilization and biostimulators in 2018. The vegetative stage was also shortened (late sowing, early harvest), indicating the long-term stress to which the plants were exposed. Thus, the extent of the negative effect was directly dependent on the duration of the stress. By comparison, in 2019, there was a significant ($p > 0.05$) reduction in $C_R$ with minimal changing dynamics during the vegetative stage (Figure 2). The precipitation level was lower at the beginning of the vegetative stage in 2019. Colder May than the 30-year-old and excess precipitation in June and July had a positive effect on plant growth and development. In 2019, more favorable weather conditions corresponded to better dynamics of the RSS. Every aspect of the weather influenced the application of fertilizers or biostimulants and their effects.

## 3.2 Effect of biostimulators, amount of nitrogen on RSS

The highest $C_R$ was recorded at the BBCH 45–50 in most treatments (Figure 2). At the BBCH 55–65, the highest value of $C_R$ was recorded in treatment with the lowest N and

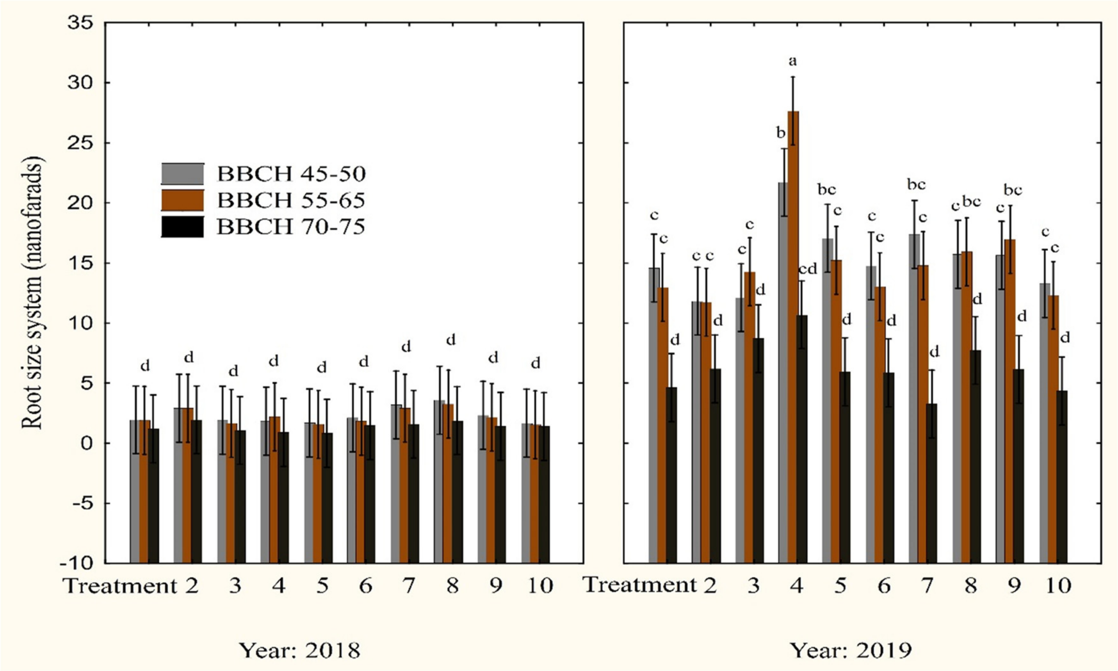

**Figure 2:** Effect of the year and the date of the measurement on the RSS. Vertical error bars denote 0.95 confidence intervals with least squares.

combination of biostimulants (treatment 4). A higher intensity of nitrogen fertilization did not significantly lead to an increase in $C_R$ (Figure 3).

The combination of the highest N dose and biostimulants (treatment 10) caused the smallest RSS and was similar to the control (treatment 1). The BBCH 55–65 showed the highest RSS ($p > 0.05$) in treatment with the lowest N dose and combination of biostimulants, which represents the lowest dose of nitrogen when applying both biostimulators with *A. nodosum* extract. This treatment at BBCH 65–70 (end of flowering) still showed higher root activity. The root activity was gradually decreased at the beginning or during ripening.

### 3.3 Effect of biostimulators, amount of nitrogen on yield components

Drought and higher temperatures in 2018 reduced the formation of productive tillers, the number of grains per plant, and the weight of grains (Figures 4–6). This effect was significant in most treatments ($p > 0.05$). An increase in the number of productive tillers, grains per plant, and grain weight was detected in 2019, especially for treatments with a low N dose and a combination of biostimulants and two higher N doses with both biostimulants. Significantly, the highest effect ($p > 0.05$) was recorded at the lowest nitrogen dose with the application of both biostimulators (treatment 4). A higher intensity of the nitrogen fertilization and application of both biostimulators no longer led to a significant increase in the number of productive tillers, grains per plant, or grain weight.

### 3.4 Correlations of yield components and RSS

The $C_R$ correlated very closely with the number of productive tillers as well as the number of grains and the grain weight per plant (Figures 7–9). The correlations were very strong from the first measurement at BBCH 45–50 and slowly increased. At BBCH 45–50 and BBCH 55–65 (graphs not presented) correlation of productive tillers with $C_R$ was 0.777 and 0.818. The correlation of grain number with $C_R$ was 0.828 and 0.8614 at BBCH 45–50 and BBCH 55–65. The correlation of grain weight with $C_R$ was 0.808 and 0.844 at BBCH 45–50 and BBCH 55–65. The strongest correlations were at BBCH 70–75 ($r = 0.912^{**}$), ($r = 0.859^{**}$) for tillers and grain number. The correlation of grain weight was ($r = 0.850^{**}$).

## 4 Discussion

Drought is one of the most serious negative factors, which affects root growth and RSS. In the short term, plants

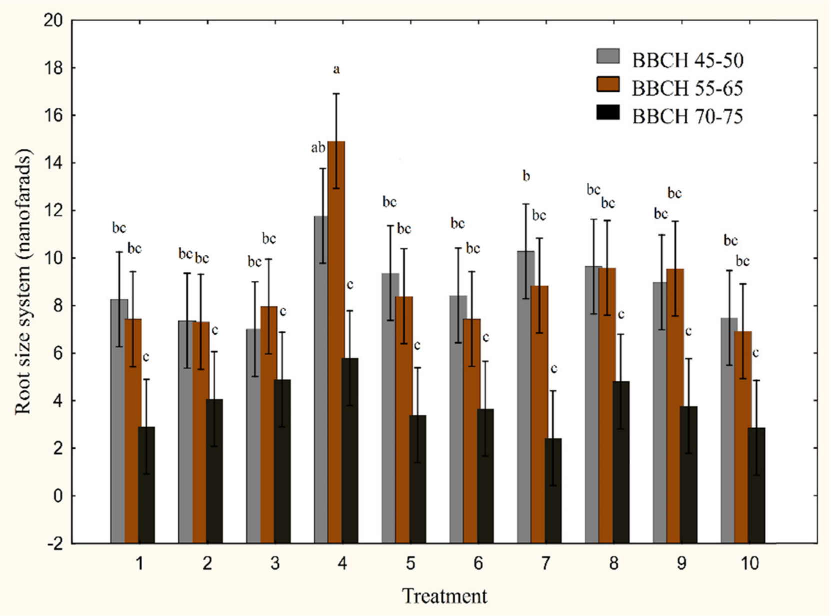

**Figure 3:** Effect of the treatment and the measurement date on the RSS. Averages of 2 years of data, vertical error bars denote 0.95 confidence intervals with least squares.

stimulate root growth to depth, a strategy to find groundwater [12–14]. On the other side, the long-term drought has an extremely negative effect on the growth, structure, and development of roots and whole plants [8,10]. The extent of the negative effect on barley growth was directly dependent on the duration of the stress. This fact has been confirmed by many other authors [15]. Low root growth could also be attributed to high temperatures, which can affect root activity and morphology even with sufficient soil moisture [10]. High temperatures and heat thus fundamentally increase the negative effect of drought [9–12]. In 2018, both factors occurred, so the system of root size reached low values. Impacts of biostimulants and nitrogen were also on low values. In general, biostimulants should help

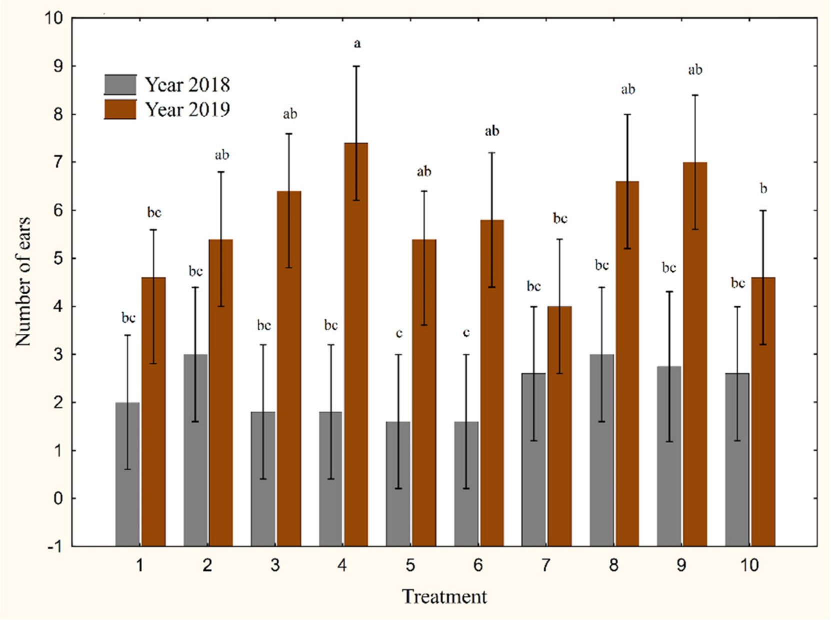

**Figure 4:** Effect of the year and the treatment on the number of ears per plant. Vertical error bars denote 0.95 confidence intervals with least squares.

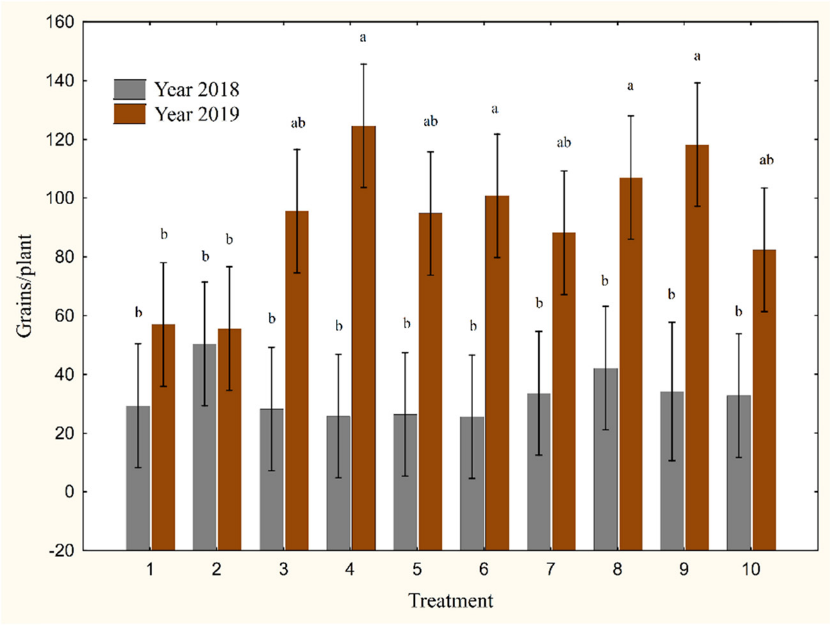

**Figure 5:** Effect of the year and the treatment on the number of grains per one plant. Vertical error bars denote 0.95 confidence intervals with least squares.

plants with abiotic stress [28,29]. In these extreme conditions, a problem could be in time of application. Many studies indicate that humidity is important for the good absorption of various substances by leaves [30,31]. In some cases, foliar nutrition is applied in the morning and other times in the evening, but there is often no record of the time of application or weather conditions [30,31]. Low humidity, drought, light, and heat can affect foliar intake [30]. In case of a long period of drought and heat, the morning humidity rapidly decreases, and the evaporation of drops from leaves accelerates. The condition of the plants is also important. Crops under heat or water stress show less response to foliar applications. Those are the conditions of 2018. It follows that in extremely unfavorable conditions, it will be necessary to change the application time. At least 70% moisture, ideal 21°C and no wind is

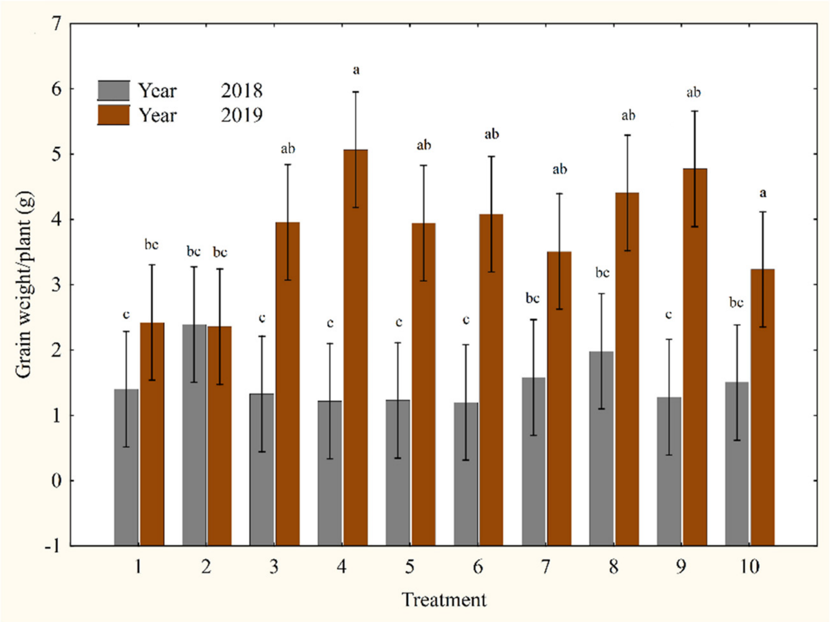

**Figure 6:** Effect of the year and the treatment on grain weight per one plant. Vertical error bars denote 0.95 confidence intervals with least squares.

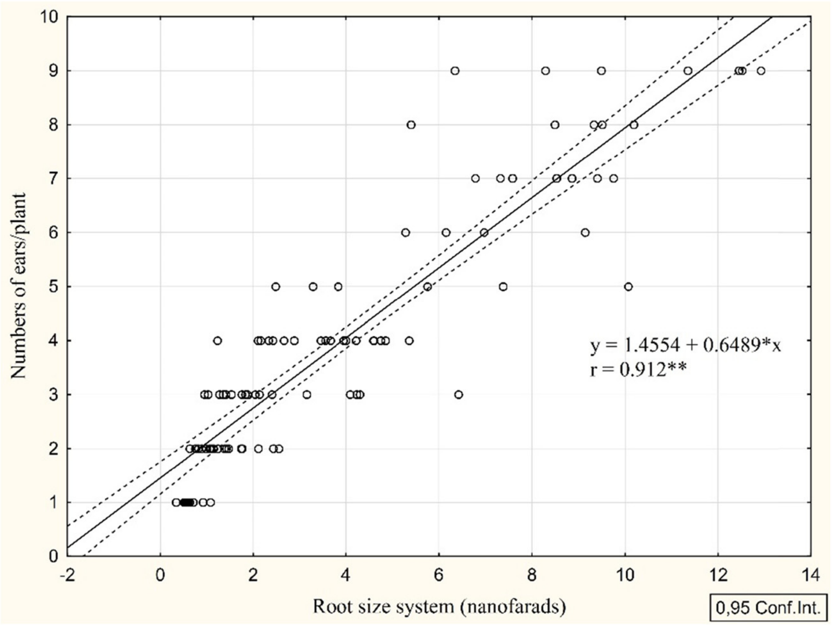

**Figure 7:** Relationship between RSS and number of ears. BBCH 70–75, averages of 2 years of data.

recommended [31]. Those conditions favor tissue permeability. Therefore, the very early morning or even late evening application may be better for the plants depending on the conditions [30,31]. Solid nitrogen fertilizer is not a suitable form for fertilizing in the dry season. Its solubility is at a low level and cannot be absorbed by the roots without water. These are important findings for agronomy.

The presence or absence of nitrogen in the soil also affects the architecture and direction of plant root growth [32]. Initially, the root of some treatments showed a higher $C_R$ but later significantly reduced. The reason could be that the plants rather established lateral roots located shallowly below the surface with regard to the application of N-fertilizers in the surface area [33,34]. These roots can be more

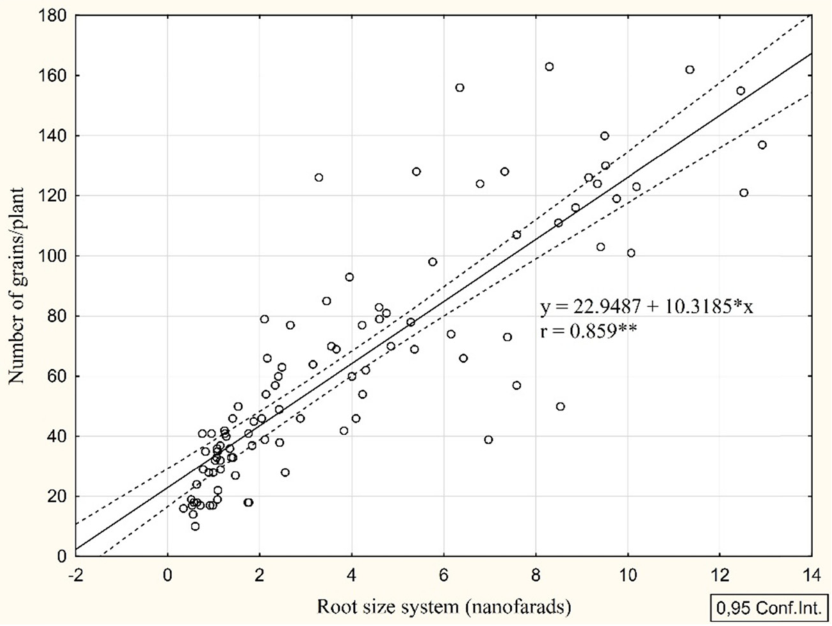

**Figure 8:** Relationship between RSS and several grains on a plant. BBCH 70–75, averages of 2 years of data.

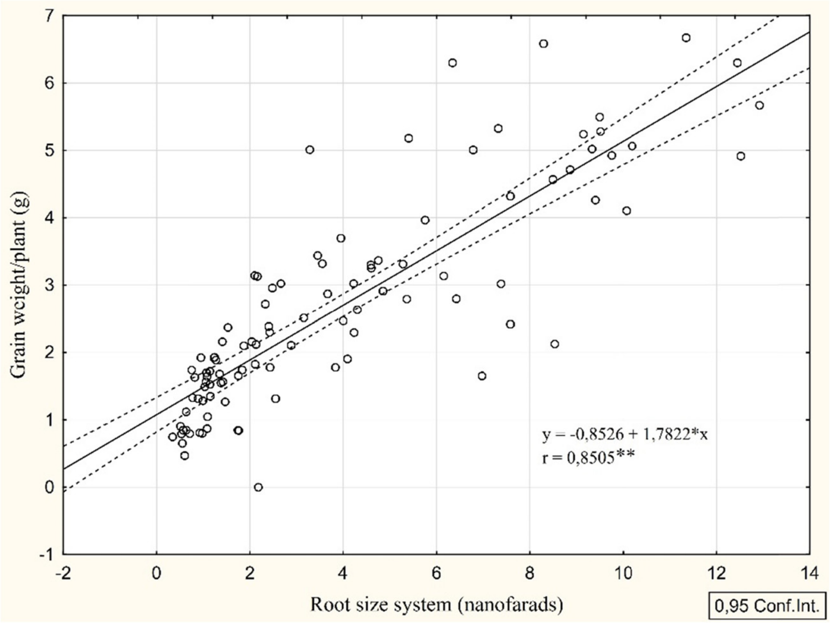

**Figure 9:** Relationship between RSS and grain weight of 1 plant. BBCH 70–75, averages of 2 years of data.

sensitive to drought or heat and die faster. The combination of the highest N dose and biostimulators caused the smallest RSS. Improving nutrient absorption with *A. nodosum* can also have a negative effect at high doses of nitrogen [3]. Separate increasing N-nutrition did not have a visible negative effect in this experiment. Drought in 2018 also showed a significant impact on the reduced formation of productive tillers, the number of grains per plant, and the weight of grains. Similar conclusions were reached by Ihsan et al. [35].

The RSS was larger in the favorable or optimal conditions of 2019. The application of both biostimulants at BBCH 27 and BBCH 30–34 and the lowest dose of N caused a larger root system, and we obtained a better result of the yield components than with a higher dose of N. A reduced amount of N to achieve better yield results was confirmed by studies aimed at increasing the efficiency of nitrogen utilization and absorption by means of *A. nodosum* [1,2,5,9]. The use of *A. nodosum* extracts can reduce cultivation costs and environmental pollution while maintaining favorable yields [5,9]. Measuring the size of the root system showed the demonstrable dependence between the RSS and the number of tillers, grains per plant, and grain weight. The results show the best time for foliar biostimulation is BBCH 27 and BBCH 30–34 (end of tillering-stem elongation). A similar result was reached by another study [28]. In 2019, the root activity decreased at the beginning of ripening. This is a normal state; cereals begin to form grain, and root activity ceases. The application of both biostimulators caused an increase in the RSS even during BBCH 65–70, thus still retaining the potential activity of the root. Cseresnyés et al. [36], showing that saturation of $C_R$ at anthesis can be used to adequately predict grain yield (R2: 0.585–0.686). In another study [26], they showed strong correlations between $C_R$ during flowering and grain mass, grain number, leaf area index, and total chlorophyll in the flag. The $C_R$ correlation is also related to starch content, protein, malt extract, or its quality [11,21]. In our experiment, correlations of yield components with $C_R$ were also very strong. At BBCH 70–75, the correlations were $r = 0.912$ for the number of productive tillers, $r = 0.859$ for the number of grains per plant, and their weight $r = 0.850$. Prolongation of root activity during grain maturation can improve grain quality and yield under optimal conditions. If the conditions are favorable in the grain formation and the root is still active, the grain filling time can be extended and subsequently reflected in grain yield and quality. Otherwise, a negative effect on grain yield can be observed [15,16].

## 5 Conclusion and future perspectives

The architecture of the root in relation to the yield and quality of plants is often overlooked precisely because it is obscured in the soil. Determining the size of the root system by measuring $C_R$ appears to be a practical method for monitoring the active part of the root, which also indirectly reflects its size, activity, or volume. The size of the root influences the formation of yield-producing elements of barley and affects the quality of the grain. The application of biostimulators with *A. nodosum* extract during tillering and elongation can help to increase $C_R$, prolong the root activity, and, at the same time, influence the yield components of barley. Productive tillers were closely correlated with $C_R$ ($r$ = 0.912**), the number of grains per plant ($r$ = 0.859**), and their weight ($r$ = 0.850**) at BBCH 70–75. The application of biostimulators with the alga *A. nodosum L.* and with the lowest dose of N (52 kg/ha) produced similar or higher amounts of yield components compared to high doses of N (80, 110, 140 kg/ha). This reduces the application dose of N. That can positively influence the ecology and economy of growing cereal. The plan of foliar application or biostimulation is also important. It can be ineffective in some cases. In the dry season, we recommend applying the foliar fertilizer in the evening hours to prolong the absorption time and reduce the risk of evaporation of the drops. The measuring of $C_R$ can be used for the prediction of some quality parameters of plants or in monitoring the effect of different preparations on the root. Subsequently, it would be appropriate to determine the architecture of the root. Determining the optimal architecture and RSS will be important in breeding future varieties or in better understanding the impact of different stimulants and fertilizers. In case of unfavorable conditions and predictions, it will be possible to intervene in time and influence the plasticity of the root and its growth, as well as the final quality and yield of grain using these preparations.

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**Data availability statement:** The data generated during the study are available from the corresponding author on reasonable request.

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