Nitrogen

Article

Does the Amount of Pre-Sowing Nitrogen Fertilization Affect Sugar Beet Root Yield and Quality of Different Genotypes?

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Abstract: There has always been a specific focus on nitrogen fertilization in sugar beet production due to its important effect on sugar beet root yield and quality. For stable sugar beet growth and satisfactory root yield and quality, balanced N fertilization is crucial. Thus, this study aimed to investigate spring N fertilization in two seasons as the following treatments: N_0—control, N_1—only pre-sowing fertilization, and N_2—pre-sowing with topdressing. Four different genotypes were included in the study (Serenada, Colonia, Fred, and Danton). The experiment was set up in a plain area, belonging to the temperate climate zone in Eastern Croatia (Županja and Vrbanja), with the long-term mean (LTM) (March–October) air temperature around 16 °C and the total precipitation of 515 mm. Pre-sowing N fertilization had a smaller impact on root yield in the year with higher precipitation (31% higher than LTM). Therefore, the average yields with pre-sowing fertilization (N_1) and pre-sowing fertilization with top dressing (N_2) were very similar and were only 7% higher than those of the control. In a season with less rainfall (29% less than LTM), pre-sowing fertilization with top dressing (N_2) had a more pronounced effect on the increase in sugar beet root yield, which was 17% higher compared to that of the control treatment. The sugar beet sucrose content and quality parameters (brei impurities, loss of sugar in molasses, extractable sugar) differed when N fertilization was applied among locations in both seasons. The white sugar yield was the highest at N_2 treatment with pre-sowing and topdressing N fertilization. In general, according to the average of all locations and years of research, the Serenada hybrid achieved the highest average root yield (81.1 t ha^{-1}), while Colonia exhibited the highest root sugar content (14.5%) and white sugar yield (9.7 t ha^{-1}).

Keywords: field trial; sugar beet; nutrient supply; hybrids; sucrose content

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1. Introduction

The European Union is the world's leading producer of sugar beets, contributing about 50% of the total world production [1]. In temperate climates, sugar beet root is the main raw material for sugar processing. The average sucrose content in the root mostly varies from 14 to 20% [2–5]. In addition to the main product, white sugar, a wide range of processed products provide opportunities for added value, both directly, as the main product—sugar—and indirectly, as secondary products, i.e., sugar beet leaves and crowns, beet slices, molasses, and saturation sludge. By-products of sugar beet production are valuable components in livestock nutrition [6–8]. The most common by-products are sweetened beet slices, which are still mainly used as livestock feed, either directly, ensiled, or dried. Another use of sweetened slices can be in the form of biofuels, after their fermentation into biogas, or in the direct burning of dry slices, as well as biodegradable plastics, which could be produced by beet biorefineries in the future [9]. These options can produce additional revenues and at the same time, reduce the processing costs of sugar beet factories.

Nitrogen (N) is one of the three most important nutrients for crop growth, along with phosphorus (P) and potassium (K). The growth and development of field crops require adequate fertilization as necessary, which ensures a continuous supply of N and other nutrients [10,11]. Farmers use N fertilizers to increase soil fertility and the yield of field crops. Photosynthesis activity and plant development are crucial for plant growth and stable yield [12]. Nitrogen fertilization has a direct influence on canopy development, green color, and photosynthetic activity, which allows farmers to monitor crop health more quickly and easily [13–18]. An excess or lack of N in sugar beets are direct reflections of the plant phenotype. Thus, on the one hand, a lack of N in sugar beets can be visually identified, as in this case, the canopy cannot be fully developed, and growth is stopped earlier. Nitrogen deficiency symptoms appear first on the oldest leaves, which lose their green color, and the yellowing extends along the leaf vein, with the marginal parts of the leaf remaining green [19–21]. On the other hand, too much nitrogen nutrition promotes the growth of the above-ground part of the plant, resulting in excessive leaf mass of the plants [22,23]. The proportion of harmful nitrogenous compounds increases at the expense of sugar, and thus, the technological quality of the raw material is impaired, the quality of storage is reduced, the quality of root cutting is reduced, and the costs of processing and transportation are generally increased. Thus, the importance of balanced N fertilization is crucial in regards to stable root yield and quality parameters.

Nitrogen plays an important role in sugar beet growth, yield formation, and quality parameters. Even though N is found in the soil in organic and inorganic compounds, for sugar beets, N is mainly added by fertilization in its mineral form: NH_4^+ and NO_3^- [24]. Both forms of nitrogen experience the well-known "nitrogen cycle" process. Plant nutrient uptake depends on agroecological conditions, the variety grown, and the yield achieved. Nitrogen can be easily washed out of the soil if conditions of high soil moisture occur (heavy rain or continuous wet weather). According to Bilir and Saltali [25], losses of N as a result of excessive or incorrect application can be up to 70%. In Germany, Eckei et al. [26] state that for wheat and sugar beets, the highest N_2O and N_2 emissions were detected when the crops were in the early growth stages and when the intake of N and water from the soil was lower. Thus, excessive N fertilization applied close to seeds can lead to toxicity problems. On the contrary, P and K are slowly moved through the soil (~2 cm/year), so when they are applied shallowly into the soil, the roots will grow slower [27,28]. Later in the growing season, beet roots absorb water poorly because the roots prevent sufficient absorption of water and nutrients from the deeper layers of the soil.

Soil selection is also an important factor in sugar beet production [29–32]. High sugar beet yields can be achieved in soils with high fertility, deep arable layers, good water–air relationships, good structures, and slightly acidic, neutral to alkaline reactions [33–35]. Over the past decades, the excessive use of inorganic nitrogen fertilizers has affected soil conditions, led to soil and microbial community imbalances, and affected freshwater and terrestrial ecosystems [36]. Optimal fertilization is the most important factor in achieving high sugar beet yields. The term "optimal fertilization" means that the sugar beets have an optimal amount of nutrients available during the entire growing season, so it is necessary to conduct a soil analysis and to apply fertilizers according to the accepted recommendations. Fertilization should be carried out in such a way as to maintain harmony in the soil and to satisfy crop needs. High nutrient requirements characterize sugar beet, and it is known that an excessive amount of N reduces root sucrose content and increases the proportion of brei impurities [37–39]. According to Brinar et al. [40], N is stored in about the same amount in the roots and root crowns in sugar beet, while K, Ca, Mg, and S mostly remain in the root crown. On the contrary, P is stored in the root. Other elements used in sugar beet nutrition, Na and Cl, as well as microelements, especially B and Mg, are important for sugar beets [41–43].

The recommendations for N fertilization for sugar beet are different concerning soil analysis, but for high yields, beets mostly require from 80 to 120 kg N ha^{-1} [44–48]. The formulation of mineral fertilizer NPK 15:15:15 promotes a shallow intake of nutrients and an inadequate ratio of P and K, and adding it before sowing should be avoided. Farmers often use NPK 15:15:15 as a pre-sowing fertilization, mostly due to its availability on the market. Rerhou et al. [49] achieved the maximum sugar beet root yield and technological quality by applying 20 t ha^{-1} of compost to improve soil fertility.

Balanced fertilization, especially in regards to nitrogen, is very important when growing sugar beets. Many farmers use excessive doses of N fertilizers, or in contrast, due to increased prices and lack of availability of fertilizer on the market, do not apply adequate fertilization for sugar beet production. In order to determine the optimal amount of N required, this study aimed to analyze the effect of different N pre-sowing fertilization on sugar beet yield and quality parameters of different genotypes.

2. Materials and Methods

2.1. Field Trial

The field trial was set up according to a randomized block design plan for two years (2014 = Season A, and 2015 = Season B) and at two locations in eastern Croatia: Vrbanja (45°01′ N; 18°59′ E) and Županja (45°08′ N; 18°42′ E). The soil of the location belongs to the riparian black soil type, which usually exhibits a humus horizon depth from 40 to 60 cm. Fine soil particles are in the upper 20 cm, and the dry transitions horizon is 40–50 cm deep, speckled with clay and rusty iron deposits, with coarse soil particles [50,51].

Soil samples were obtained for chemical analysis from the planned plots in autumn, after harvesting the pre-crop and before setting up the experiment, (Table 1). The soil analysis was conducted in the Agropedological Laboratory in Virovitica (Agrokontrolla d.o.o., Zagreb). According to the soil analysis, the soil was weakly acidic to a neutral reaction. Organic matter was determined by the dichromate method [52]. According to the ammonium-lactate method (AL method) by Egner-Rheim-Domingo [53], the soil of the experimental field in Županja was well supplied with phosphorus and was lacking in potassium in Season A, while the soil in Vrbanja was very poor in phosphorus and poor in potassium. In the second year of research, the soil in Županja was highly supplied with phosphorus and potassium, while the soil in Vrbanja was lacking in phosphorus and well supplied with potassium.

Table 1. Chemical properties of the soil.

	pH (KCl)	Organic Matter (%)	AL-P_2O_5	AL-K_2O
Season A				
Županja	6.92	1.77	16.11	9.91
Vrbanja	5.85	1.53	7.78	17.59
Season B				
Županja	6.89	2.20	35.71	35.12
Vrbanja	6.03	1.47	12.57	23.28

AL-P_2O_5 and AL-K_2O—available P and K in mg 100 g^{-1} soil.

To determine the content of mineral nitrogen in the soil and to make a recommendation for nitrogen fertilization, an N_{min} analysis of mineral nitrogen in the soil was carried out before sowing (Table 2). The N_{min} sampling was performed before sowing from two depths, 0–30 and 30–60 cm, which is usual for the field crops.

Table 2. Mineral nitrogen (kg ha^{-1}) of the experimental sites.

	Soil Depth			
	0–30 cm		30–60 cm	
	$N–NH_4$	$N–NO_3$	$N–NH_4$	$N–NO_3$
Season A (2014)				
Županja	8.45	26.23	1.91	38.80
Vrbanja	6.27	25.90	2.02	41.36
Season B (2015)				
Županja	12.66	31.65	0.86	31.19
Vrbanja	14.66	32.10	6.75	28.14

After autumn plowing (30–40 cm), the winter furrow was closed in spring, and the soil was prepared for sowing. In both seasons of the study, sugar beet sowing was carried out in optimal terms (Table 3), and the length of the vegetation period for each year of the study was approximately the same. The usual agrotechnical care measures for sugar beet production were carried out in both locations. There were several weeds (*Cirsium arvense* (L.) Scop., *Echinochloa crus-galli* L., *Ambrosia elator* L., *Sorghum halepense* (L.) Pers., *Abutilon theophrasti* Medik. and *Setaria glauca* L. P. Beauv.) that were encountered during vegetation, but all weeds were removed manually upon appearance. During vegetation, interrow cultivation was performed twice, which helped remove the weeds and additionally aerated the soil. Since there were no pest attacks, no pest protection was carried out in any year. Protection against leaf spot (*Cercospora beticola* Sacc.) was carried out on four occasions in Season A due to the higher intensity of infection in both locations, while in Season B, the protection was carried out on three occasions.

Table 3. Pre-crop and days of vegetation.

	Season A		Season B	
	Županja	**Vrbanja**	**Županja**	**Vrbanja**
Pre-crop	wheat	soybean	maize	wheat
Sowing	18 March	18 March	2 April	25 March
Harvest	14 October	16 October	23 October	9 October
Vegetation period	205	199	211	213

The interrow spacing in sowing was 0.5 m in 6 rows. The basic plot of each treatment of N pre-sowing fertilization and hybrid was 60 m^2 in four repetitions. Thus, the experiment was conducted on a total of 960 m^2. The pneumatic seeder is intended for sowing pelleted (1.3 U/ha) sugar beet seed. Four sugar beet hybrids (H), genetically tolerant to sugar beet leaf spot (*Cercospora beticola* Sacc.) and Rhizomania, were included in the research: Colonia, Serenada (Kleinwanzlebener Saatzucht—KWS, Einbeck, Germany), Danton, and Fred (Strube GmbH & Co. KG, Söllingen, Germany).

2.2. Presowing Nitrogen Fertilization

Mineral fertilization with nitrogen was carried out in the spring at three fertilization levels: N_0—no fertilizer added, control; N_1—pre-sowing at 300 kg ha^{-1} with NPK 15:15:15; and N_2—pre-sowing at 300 kg ha^{-1} with NPK 15:15:15, with calcium ammonium nitrate (CAN, 27% N) topdressing. In both seasons, topdressing was performed in May with the same amount of CAN (200 kg ha^{-1} CAN at the Županja site and 165 kg ha^{-1} CAN at the Vrbanja site). The total amount of applied P_2O_5 and K_2O fertilizer in both locations and seasons was 45 kg ha^{-1}.

2.3. Weather Data

Data from the State Hydrometeorological Institute [54] for the meteorological station Gradište were used to collect the meteorological data in the years of the study (air distance to Županja 8.7 km and to Vrbanja 25.5 km).

According to the data of the Gradište meteorological station (Table 4), the air temperature in Season A in the sugar beet growing season (March–October) was, on average 17.1 °C. In general, during the growing season, the mean monthly air temperatures did not deviate greatly from the multi-year average. During sowing in Season A, the air temperatures were optimal for sugar beet germination, and the beet developed well until the canopy closed the rows. Temperatures during the summer months did not deviate significantly from the long—term mean, but although they were higher than optimal (about 18 °C), the plants developed well thanks to the sufficient amount of precipitation in that period. The amount of precipitation in Season A, amounted to a total of 676.8 mm of precipitation during the sugar beet growing season. May of Season A was extremely rainy, with as much as 170% more precipitation than the long-term mean. Such a distribution of precipitation in May prolonged the rooting of the beet due to a greater saturation of the soil with water.

Table 4. Average monthly environmental parameters measured in Season A and Season B—mean air-temperature (°C), amount of precipitation (mm), insolation (h), and relative humidity (%) compared to the long-term mean, LTM (1981–2010).

Month	**Season A (2014)**				**Season B (2015)**				**LTM (1981–2010)**			
	°C	**mm**	**h**	**%**	**°C**	**mm**	**h**	**%**	**°C**	**mm**	**h**	**%**
March	10.6	39.0	178.6	70	7.8	45.9	167.2	67	7.1	48.4	152.6	69
April	13.3	87.8	174.0	73	12.7	24.3	234.7	55	12.1	54.7	183.2	67
May	16.8	165.0	241.0	74	18.2	98.7	235.5	66	17.2	61.7	242.9	67
June	20.8	46.2	284.6	66	21.1	25.8	280.0	63	20.1	85.1	270.3	70
July	22.8	83.3	284.9	75	24.9	9.5	344.9	59	21.9	85.1	298.9	69
August	21.6	94.2	274.0	78	24.0	48.7	284.0	65	21.4	58.1	275.5	70
September	16.8	96.2	135.7	83	18.2	102.7	185.0	74	16.8	62.6	191.5	75
October	13.8	65.1	146.7	84	11.5	89.9	108.0	84	11.8	59.3	148.6	78
March–October	17.1	676.8	1719.5	75	17.3	445.5	1839.3	67	16.1	515.0	1763.5	71

In Season B, the average air temperature in the growing season from March to October was 17.3 °C. The canopy closed the rows at the beginning of June (around 10 June). After June, beet growth was difficult, primarily due to very high average monthly air temperatures in July and August 2015, which were as high as 24.9 °C and 24.0 °C. There was less precipitation than that noted in Season A, so in the vegetation period, the total amount of precipitation in the vegetation was 445.5 mm. After the dry summer of Season B, in September, there was a total of 102.7 mm of rainfall, which positively affected leaf re-growth.

On average, the relative air humidity was 75% in Season A and 67% in Season B, which positively affected sugar beet growth. In general, Season A had slightly more cloudy days compared to Season B. In total, in the sugar beet vegetation period, the duration of sunshine of Season A was 44 h less, whereas, in Season B, there were 76 h more sunshine when compared to the long-term mean (1981–2010) of 1763.5 h (from March to October). The sunshine duration in August, September, and October is important for the accumulation of sugar in the roots. In Season A, the total number of sunny hours from August to October was 556.4 h, while in Season B, it was higher, and totaling 615.6 h.

2.4. Harvest

The sugar beet harvest was performed manually at the end of each growing season. At harvest, samples were taken of all four hybrids from each fertilization variant (N_0, N_1, and N_2) in four repetitions. One repetition consists of plants taken from 5 m^2. Therefore, a total of 96 samples were included in the study.

Root yield (t ha^{-1}) and root quality (root sugar content, Na, K, and α-amino N) were determined after root extraction in the laboratory of the Sladorana d. d. Županja Sugar Factory (Županja). Standard methods were used to determine sucrose content and brei impurities (α-amino N, K, and Na) in the sugar beet root [55,56].

2.5. Statistical Analysis

After the chemical analysis of the sugar beet roots using Braunschweiger's formulas [57], extractable sugar (%) and white sugar yield (t ha^{-1}) were calculated using the following formulas:

Loss of sugar in molasses (LoM), [%]:

$$LoM = 0.12 \times (K + Na) + 0.24 \times \alpha - amino\ N + 0.48 \quad (1)$$

Extractable sugar (ES), [%]:

$$ES = sugar\ content - [0.12 \times (K + Na) + 0.24 \times \alpha - amino\ N + 1.08] \quad (2)$$

White sugar yield, [t ha^{-1}]:

$$Sugar\ beet\ root\ yield\ (netto)\ (\text{t ha}^{-1}) \times ES/100\ [\text{t ha}^{-1}] \quad (3)$$

The obtained data were statistically processed using the MS Office computer program Microsoft 365 Excel 2019 for Windows and the statistical program SAS 9.4 [58].

The difference between the investigated factors (fertilization and hybrid) was calculated using one-way analysis of variance at the significance level of $p \leq 0.05$. In addition, the interaction between the investigated treatments was calculated through the factorial analysis of variance.

In cases of a significant F test at the level of $p \leq 0.05$, individual LSD (least significant difference) tests were performed to compare the average values.

A correlation analysis was conducted to determine the interdependence of the obtained results. Depending on the correlation coefficient (r) value, the Roemer and Orphal correlation strength distribution table was used when interpreting the correlations [59].

3. Results

The influence of pre-sowing nitrogen fertilization and genotype was investigated by analyzing the achieved root yield and quality indicators, i.e., the sugar content in the roots and the content of molasses-forming elements (Na, K, and α-amino N). The following quality parameters were calculated based on the obtained data: loss of sugar in molasses, extractable sugar, and white sugar yield. The ANOVA analysis in Table 5 presents the different effects of N fertilizer use on sugar beet yield and quality parameters.

3.1. Sugar Beet Root Yield

The sugar beet root yield in Season A was approximately the same in both locations (91.6 t ha^{-1} at Županja and 95.1 t ha^{-1} at Vrbanja). The influence of pre-sowing N fertilization at the Županja location in Season A was not statistically significant for the average root yield (Figure 1a). On average, a very significant difference ($p \leq 0.01$) was determined between the hybrids, and Serenada exhibited the highest average root yield (98.8 t ha^{-1}), while Colonia showed the lowest average root yield (88.8 t ha^{-1}). In contrast to the Županja locality, in Season A, the influence of fertilization was more pronounced in Vrbanja, and the average yields differed very significantly ($p \leq 0.01$) only in relation to the control treatment (86.8 t ha^{-1}). Furthermore, the difference in average yields among hybrids was very significant ($p \leq 0.01$). The Serenada hybrid stood out, with the highest average root yield of 102.8 t ha^{-1}, while the Fred hybrid had the lowest yield (88.4 t ha^{-1}).

Table 5. Analysis of variance (ANOVA) of the pre-sowing nitrogen (N) and hybrids (H) effect on the sugar beet root yield and quality, along with their interactions.

Season/Location	Source of Variation	Root Yield	Sucrose Content	α- Amino N	K	Na	Loss of Sugar in Molasses	Extractable Sugar	White Sugar Yield
2014	N	ns	*	*	ns	ns	ns	**	ns
Županja	H	**	**	**	**	**	**	**	**
	N × H	ns	**	**	**	**	**	**	**
2014	N	**	**	**	**	**	**	**	**
Vrbanja	H	**	**	**	**	**	**	**	**
	N × H	ns	**	**	ns	**	**	**	**
2015	N	**	**	**	ns	**	*	**	**
Županja	H	**	**	**	**	**	**	**	**
	N × H	**	ns	**	**	ns	ns	ns	**
2015	N	**	ns	**	ns	*	**	ns	**
Vrbanja	H	**	**	**	**	**	**	**	**
	N × H	ns	ns	**	ns	**	**	**	**

ns: non-significant; * significant at $p < 0.05$; ** significant at $p < 0.01$.

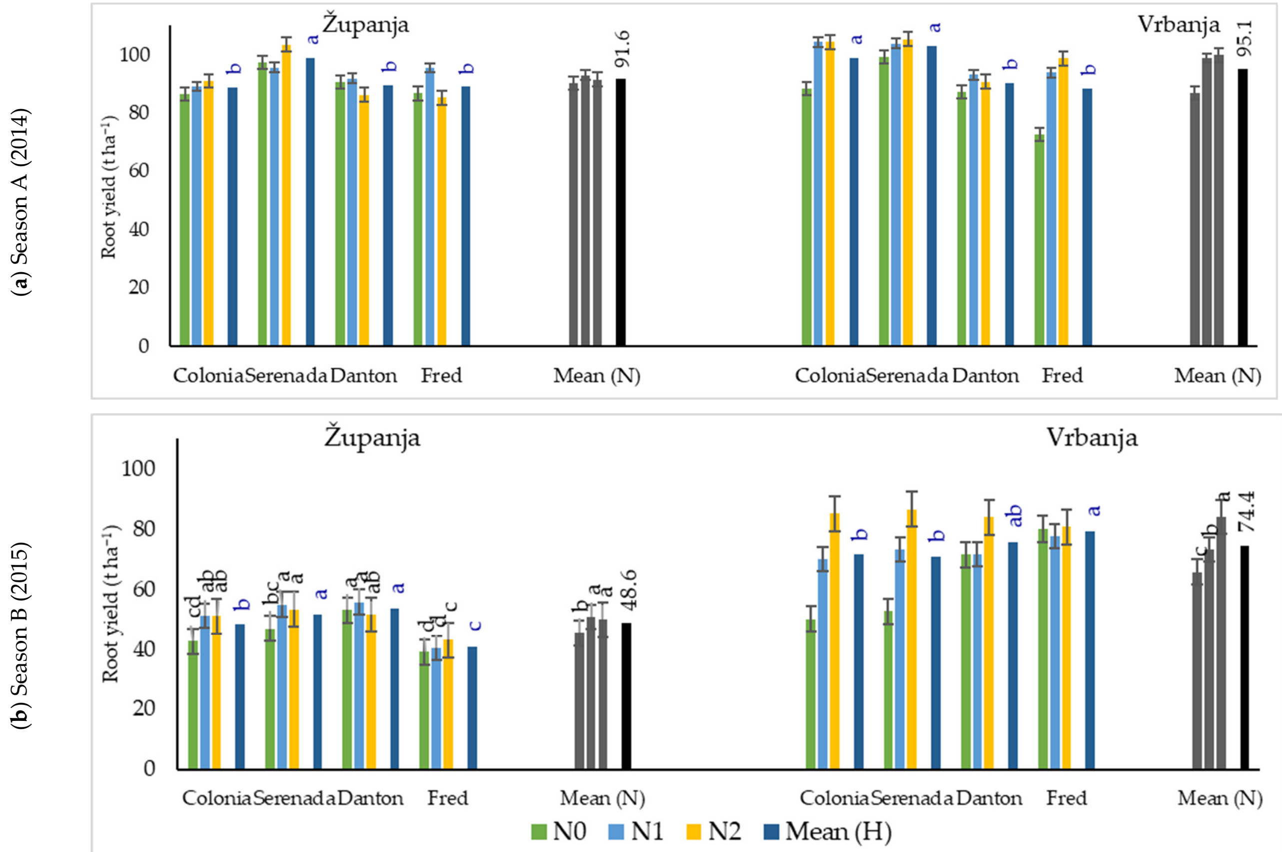

Figure 1. Sugar beet root yield (t ha^{-1}) resulting from nitrogen fertilization (vertical bars display the standard error of the mean for all values greater than 5%; different letters indicate statistically significant differences ($p \leq 0.05$).

The pre-sowing N fertilization in Season B at Županja had a very significant impact ($p \leq 0.01$) on the average yield, but these differences were determined only in relation to the control treatment (Figure 1b). Furthermore, the differences between the hybrids were highly significant ($p \leq 0.01$). Here, the Danton hybrid achieved the highest average yield (53.5 t ha^{-1}), while the Fred hybrid achieved the lowest average root yield (40.9 t ha^{-1}).

For Season B at the Vrbanja location (Figure 1b), N pre-sowing fertilization treatments had a very significant impact ($p \leq 0.01$) on average sugar beet root yield. The control treatment had the lowest root yield (65.8 t ha^{-1}), while increased fertilization (N_2 treatment) resulted in the highest average yield (84.1 t ha^{-1}). Among the hybrids, a significant difference ($p \leq 0.05$) was also found in the average root yield, with Fred having the highest yield (79.4 t ha^{-1}) while Serenada had the lowest root yield (70.9 t ha^{-1}).

3.2. Root Sucrose Content

Pre-sowing N fertilization had a significant influence ($p \leq 0.05$) on the achieved sugar content in Županja in Season A, and the highest sugar content was achieved for the control treatment (13.6%), and the lowest for the N_2 fertilization treatment (13.4%) (Figure 2a). On average, for all sowing intervals and fertilization treatments, the Colonia hybrid achieved the highest sugar content (14.0%), while Serenada exhibited the lowest average sugar content (13.1%). No significant differences were found between the sugar content of the Fred and Danton hybrids.

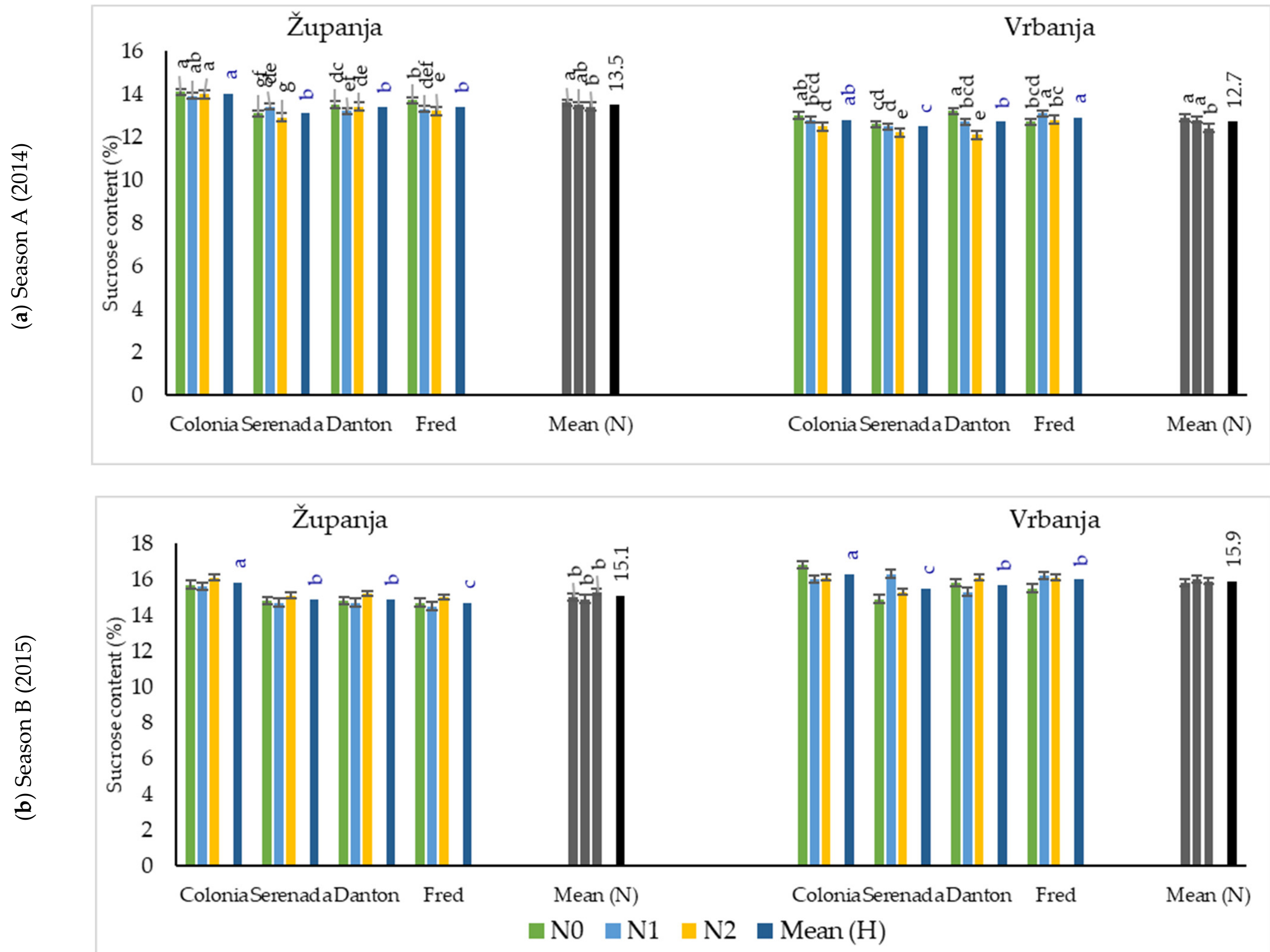

Figure 2. Sugar beet sucrose content (%) resulting from nitrogen fertilization (vertical bars display the standard error of the mean for all values greater than 5%; different letters indicate statistically significant differences ($p \leq 0.05$).

The influence of pre-sowing N fertilization on the achieved sugar content in Vrbanja for Season A was very significant ($p \leq 0.01$), and the highest sugar content was in the root for the control treatment (average 12.9%), while increased fertilization (N_2) resulted in a decrease in average sugar content in the root by 0.5% (Figure 2a). Regarding the sugar content, the hybrid Fred achieved the highest content, with an average of 12.9% sucrose, while the Serenada hybrid had the lowest sucrose content, with an average of 12.5%.

A very significant influence on the average sugar content ($p \leq 0.01$) was determined for N fertilization in Season B, where the highest sugar content was achieved with enhanced fertilization (N_2), totaling an average of 15.3% at the Županja location (Figure 2b). Significant differences ($p \leq 0.01$) were determined for the average sugar content between the hybrids. The highest sugar content in the root was found in the Colonia hybrid (15.8%), and the lowest was noted in the Fred hybrid (14.7%), while the Serenada and Danton hybrids exhibited the same average sugar content (14.9%).

The impact of different N fertilization treatments in Vrbanja in Season B was not statistically significant (Figure 2b). The determined differences in the average sugar content among the hybrids were very significant ($p \leq 0.01$), and the Colonia hybrid had the highest average root sugar content (16.3%), while the Serenada hybrid had the lowest average root sugar content (15.5%).

3.3. Brei Impurities: α-Amino N, K, Na

The average values of α-amino N determined in Season A (2014) at the Županja locality were 1.22 mmol per 100 g beet^{-1} (Figure 3a), while at another locality in Vrbanja, the average was 1.97 mmol per 100 g beet^{-1}. In Season B (2015), in Županja, the average α-amino N content was 1.58 mmol per 100 g beet^{-1}, and in Vrbanja, the beets showed an average of 1.39 mmol per 100 g beet^{-1} (Figure 3b).

The influence of the fertilization treatment was significant ($p \leq 0.05$) for the content of α-amino N in Season A, in which the highest amount of α-amino N was noted for the N_2 treatment (1.37 mmol per 100 g beet^{-1}) (Figure 3a). Serenada and Danton's hybrids had the highest average content of α-amino N (1.29 and 1.28 mmol per 100 g beet^{-1}, respectively), and compared to other hybrids, the differences were very significant at the $p \leq 0.01$ level.

At the Vrbanja location in Season A (Figure 3a), the highest content of α-amino N was determined for the N_2 treatment (average 2.17 mmol per 100 g beet^{-1}). Very significant differences ($p \leq 0.01$) were also found among the hybrids, with Serenada having, on average, the highest α-amino N (2.32 mmol per 100 g beet^{-1}) and Colonia the lowest (1.72 mmol per 100 g beet^{-1}) α-amino N in the roots.

The average values of α-amino N depending on the fertilization were statistically significantly different at the $p \leq 0.01$ level in relation to N fertilization in Season B in Županja (Figure 3b). The highest content was found in the beet roots of the control treatment (1.70 mmol per 100 g beet^{-1}). The influence of the hybrid on the achieved content of α-amino N was also very significant ($p \leq 0.01$), and according to the hybrid average, Serenada also had the highest α-amino nitrogen (1.97 mmol per 100 g beet^{-1}) at this location.

In the locality of Vrbanja in Season B, fertilization had a very significant influence ($p \leq 0.01$) on the content of α-amino N, and the highest content was found in the root for the N_2 treatment (average 1.75 mmol per 100 g beet^{-1}) (Figure 3b). According to the average of the hybrids, hybrid Fred stands out for the highest content of α-amino N (1.50 mmol per 100 g beet^{-1}), while Colonia had the lowest content (1.26 mmol per 100 g beet^{-1}). All established differences between hybrids were statistically significant at $p \leq 0.01$.

In general, the K content in the roots was lower in Season A compared to Season B (Figure 4a). The average K content in the roots in Županja in Season A was 2.77 mmol per 100 g beet^{-1}, while in Vrbanja, the average potassium content was 2.60 mmol per 100 g beet^{-1}. In Season B, the sugar beet root exhibited a bit higher K content, so at the Županja location, the average K content was 3.79 mmol per 100 g beet^{-1}, and in Vrbanja, it was an average of 3.88 mmol per 100 g beet^{-1} (Figure 4b).

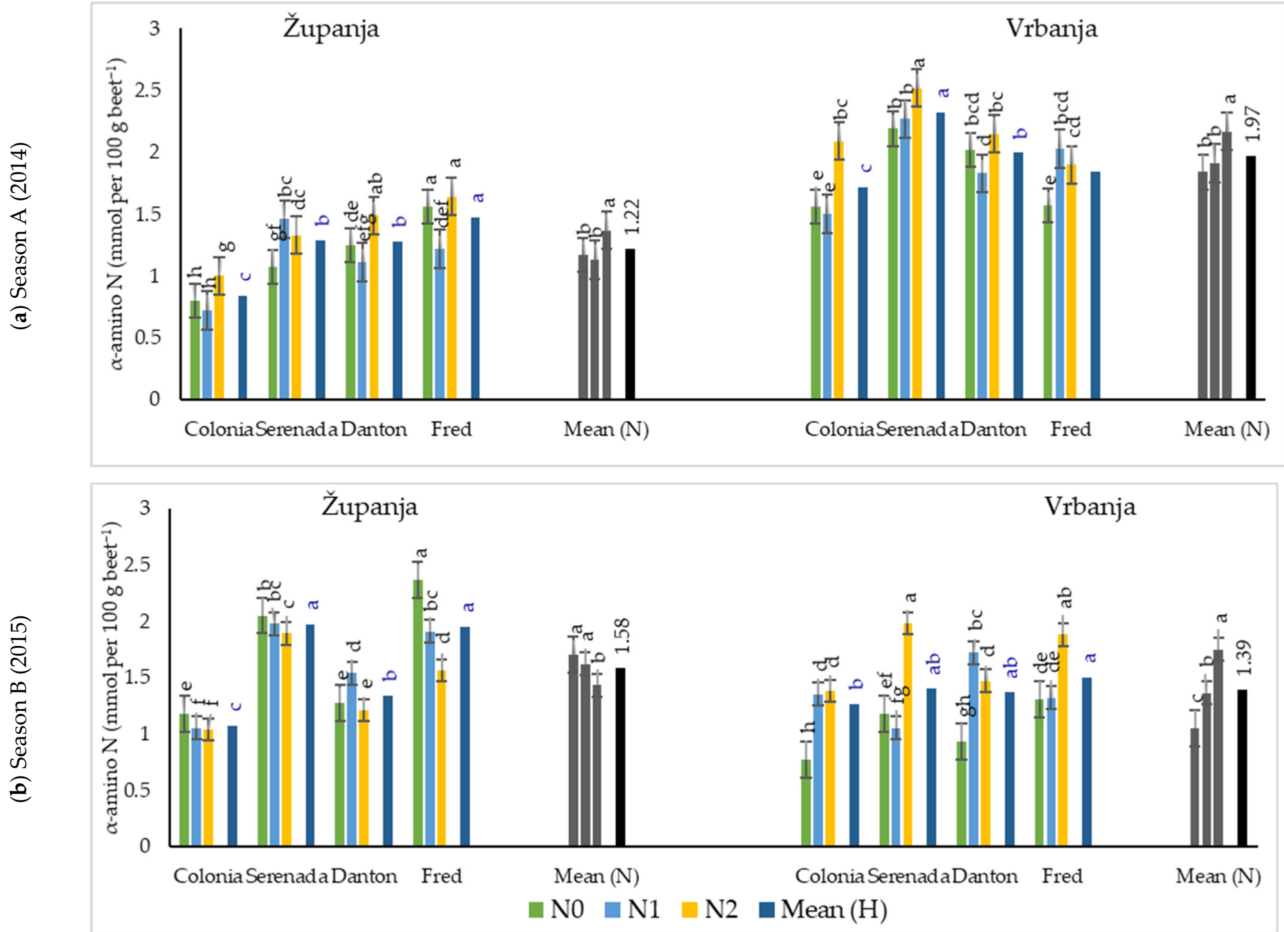

Figure 3. Content of α-amino N in sugar beet root (mmol per 100 g beet^{-1}) resulting from nitrogen fertilization (vertical bars display the standard error of the mean for all values greater than 5%; different letters indicate statistically significant differences ($p \leq 0.05$).

The influence of fertilization on K content in Županja in Season A was not statistically significant (Figure 4a). On other locations in Vrbanja, the N fertilization had a significant influence, and the highest average K content in the roots was found in the control treatment (2.66 mmol per 100 g beet^{-1}) and the lowest in the N_1 treatment (2.54 mmol per 100 g beet^{-1}). The hybrids differed from each other in the average K content at the $p \leq 0.05$ level, and the highest K content was found in the Serenada hybrid (2.74 mmol per 100 g beet^{-1}).

In Season B, at both locations (Figure 4b), depending on the pre-sowing N fertilization, the determined differences were not statistically significant. Regarding the average content of K, for the hybrids at the Županja location, Serenada and Danton did not differ significantly, and average values of 4.29 mmol per 100 g beet^{-1} and 4.20 mmol per 100 g beet^{-1}, respectively, were determined for them. Significant differences were found for the Colonia hybrid, which had the lowest amount of K in the root (average 2.70 mmol per 100 g beet^{-1}). At the Vrbanja location (Figure 4b), a higher content of K in the roots was found for the hybrids Serenada and Danton (on average 4.27 mmol per 100 g beet^{-1} and 4.23 mmol per 100 g beet^{-1}, respectively).

Based on analysis of the Na content in the roots, in Season A at the Županja location, the content was, on average, 0.59 mmol per 100 g beet^{-1} (Figure 5a), while in Vrbanja, it was higher, at 0.82 mmol per 100 g beet^{-1}. In Season B, the differences in the average Na content of the root were not as pronounced as in Season A, considering the locality.

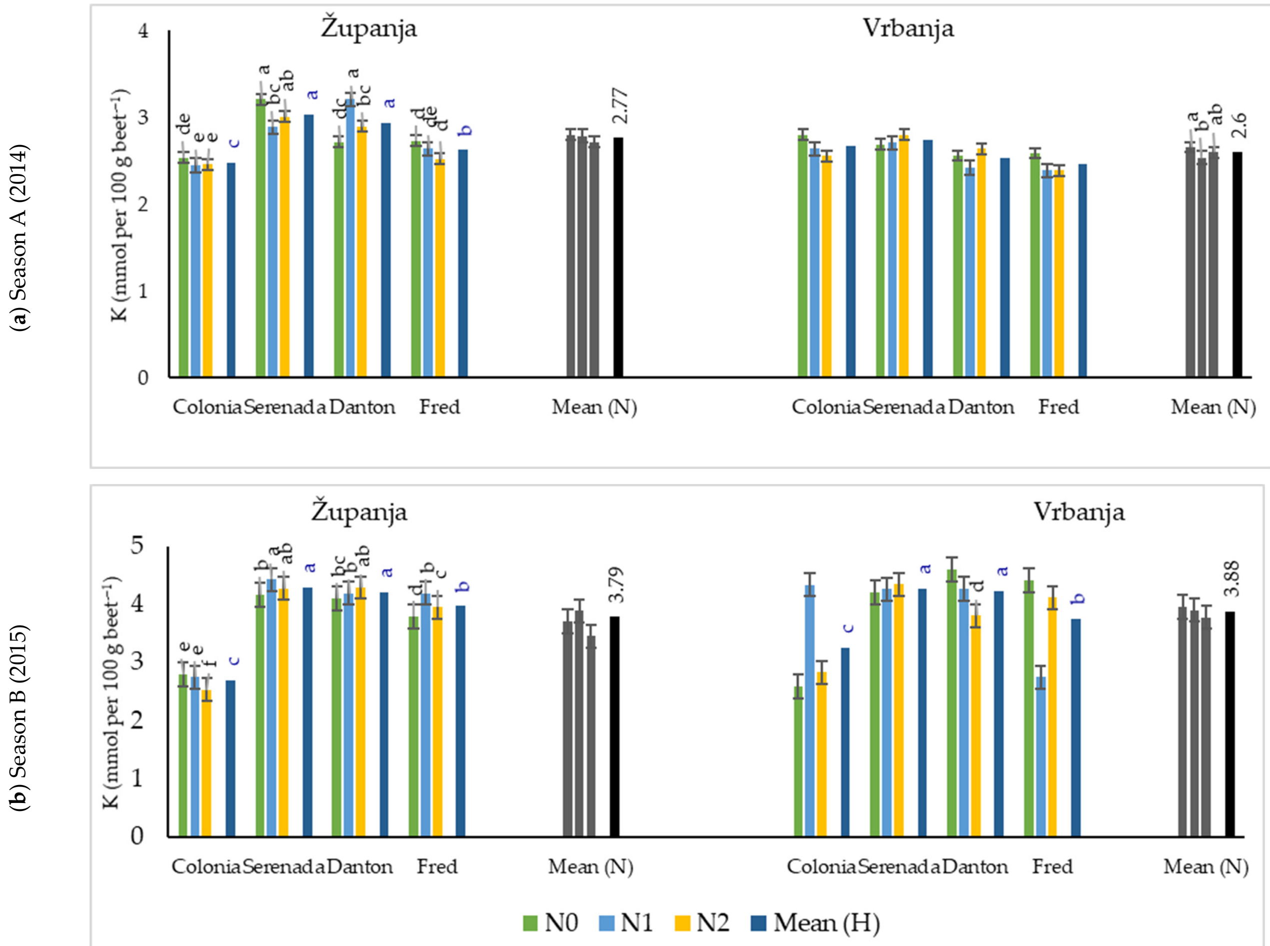

Figure 4. Content of K in sugar beet root (mmol per 100 g beet^{-1}) resulting from nitrogen fertilization (vertical bars display the standard error of the mean for all values greater than 5%; different letters indicate statistically significant differences ($p \leq 0.05$).

In Season B, the average Na content in the roots in Županja was 0.32 mmol per 100 g beet^{-1} (Figure 5b), while in Vrbanja, it was 0.30 mmol per 100 g beet^{-1} (Figure 5b).

For Season A at the location Županja (Figure 5a), the influence of the genotype was very significant ($p \leq 0.01$), and the Serenada hybrid had the highest average Na content in the root (0.71 mmol per 100 g beet^{-1}), while at the same time, the Fred hybrid had the lowest Na content (0.50 mmol per 100 g beet^{-1}).

In Season A, in Vrbanja, a very significant influence of fertilization ($p \leq 0.01$) on the Na content in the roots was determined (Figure 5a), in which the sugar beet root of the control treatment had, on average, the highest Na content in the root (0.87 mmol per 100 g beet^{-1}). According to the average, the hybrids also differed very significantly ($p \leq 0.01$) in regards to Na content. The Serenada hybrid had, on average, the highest sodium content (0.98 mmol per 100 g beet^{-1}), while Colonia showed the lowest sodium content in the extractions (0.63 mmol per 100 g beet^{-1}).

Concerning N fertilization, a statistically very significant ($p \leq 0.01$) difference in Na content at the Županja location in Season B (Figure 5b) was only found for the increased N pre-sowing fertilization treatment (N_2). Hybrid Seredana showed the highest Na content on average (0.42 mmol per 100 g beet^{-1}), while Danton had the lowest Na content, on average (0.24 mmol per 100 g beet^{-1}), with very significant differences ($p \leq 0.01$) between them.

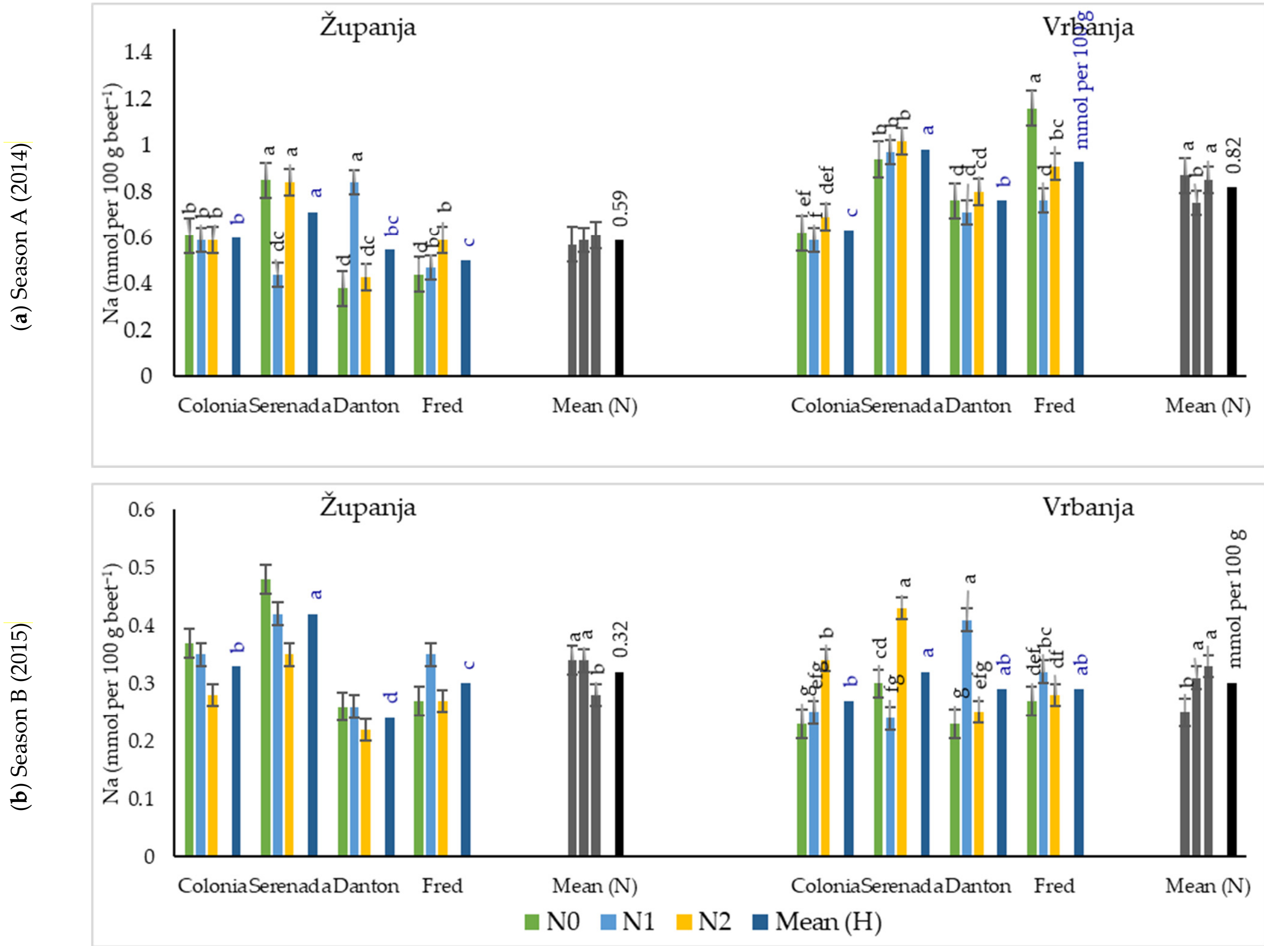

Figure 5. Content of Na in sugar beet root (mmol per 100 g beet^{-1}) resulting from nitrogen fertilization (vertical bars display the standard error of the mean for all values greater than 5%; different letters indicate statistically significant differences ($p \leq 0.05$).

The determined differences in Na regarding N fertilization were statistically significant ($p \leq 0.05$) for the Vrbanja location in Season B (Figure 5b). The roots of the control treatment had the lowest amount of Na (average 0.25 mmol per 100 g beet^{-1}), while the roots of the N_2 treatment had the highest Na content (average 0.33 mmol per 100 g beet^{-1}). The Serenade hybrid had the highest average Na content in Vrbanja in Season B (0.32 mmol per 100 g beet^{-1}), while the other hybrids did not differ in Na content (average 0.29 mmol per 100 g beet^{-1}).

3.4. Loss of Sugar in Molasses

According to this study, the average loss of sugar in molasses in Season A in the Županja locality was 1.45% (Figure 6a), while in Vrbanja, it was 1.64%. In Season B, the average loss of sugar in molasses was approximately the same at both locations, totaling 1.68% at the Županja site (Figure 6b) and 1.65% at the Vrbanja site.

In Županja in Season A (Figure 6a), N pre-sowing fertilization had no statistically significant influence on the loss of sugar in molasses. On the other hand, the determined differences between the hybrids were very significant ($p \leq 0.01$). Serenada showed the highest average sugar loss in molasses (1.54%), while Colonia had the lowest (1.30%).

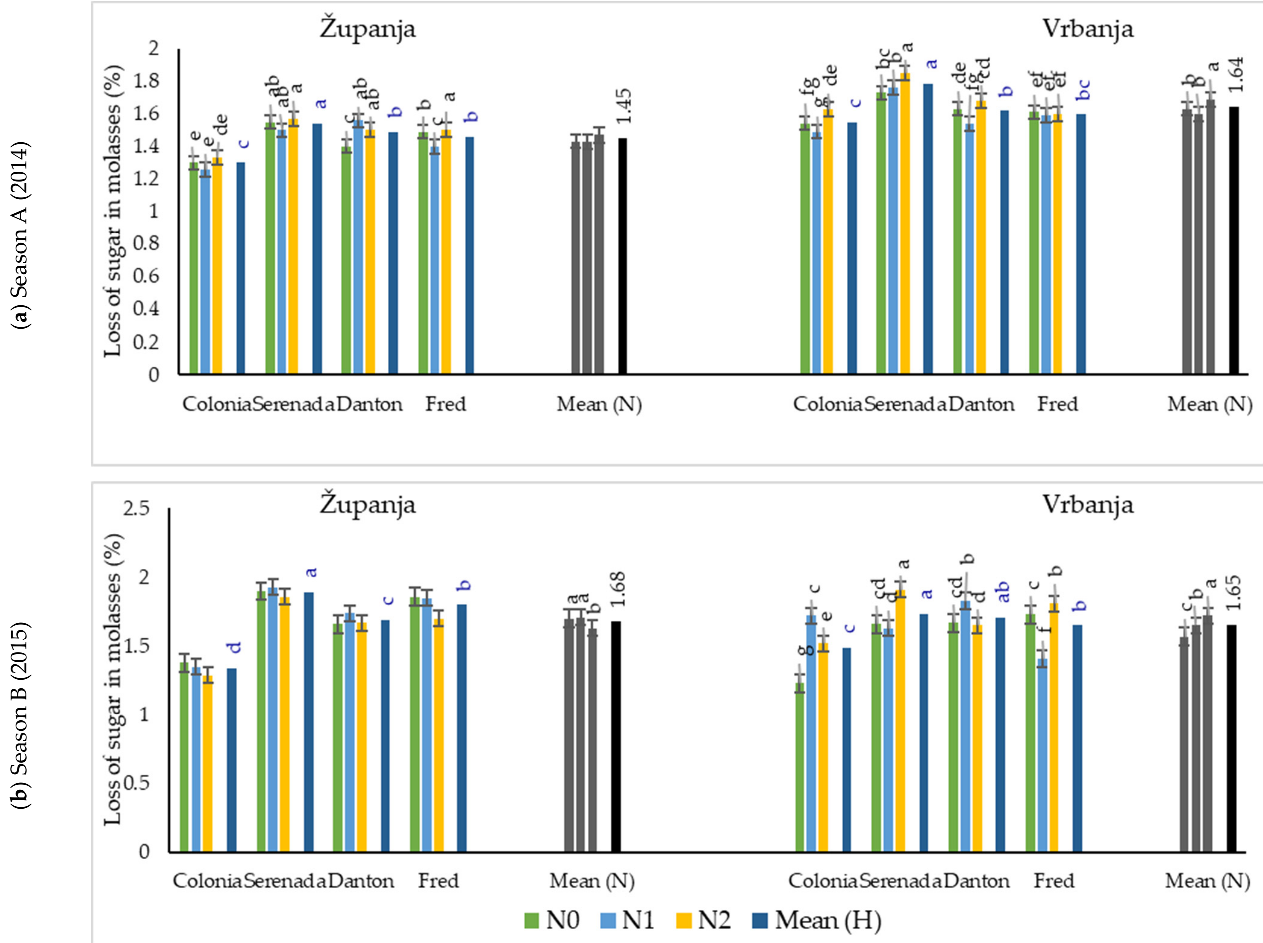

Figure 6. Loss of sugar in molasses (%) resulting from nitrogen fertilization (vertical bars display the standard error of the mean for all values greater than 5%; different letters indicate statistically significant differences ($p \leq 0.05$).

Depending on the N pre-sowing fertilization at the Vrbanja site in Season A, the highest determined loss of sugar in molasses was in the N_2 fertilization treatment (1.69%, on average). Among the hybrids, Serenada exhibited, on average, the highest sugar in molasses content (1.78%), while the Colonia hybrid had the lowest sugar content in molasses (1.55%).

In Season B at the Županja site, the influence of N pre-sowing fertilization was significant ($p \leq 0.05$). The lowest sugar loss in molasses was obtained from the beets of the N_2 fertilization treatment (1.63% on average), which was higher by 1.70% compared to that of the control. The hybrids differed significantly ($p \leq 0.05$) in terms of sugar loss in molasses, with Serenada having, on average, the highest (1.89%) and Colonia the lowest (1.34%) determined sugar loss in molasses (Figure 5b). At the Vrbanja site, the influence of fertilization was also very significant ($p \leq 0.01$), and the plants of the control treatment had, on average, the lowest sugar loss in molasses (1.57%), while the beet in the emphasized fertilization (N_2) treatment of the highest sugar loss in molasses (1.72%). According to the average values of the hybrids, Serenada had the highest average sugar in molasses (1.73%), while Colonia had the lowest (average 1.49%), in both the Vrbanja location as well as in Županja.

3.5. Extractable Sugar

In Season A, extractable sugar was, on average, 11.7% in the Županja site and 10.7% in Vrbanja (Figure 7a). In Season B, the extractable sugar was higher than in Season A, so in Županja, it averaged 13.1%, and in Vrbanja, it averaged 14.0% (Figure 7b).

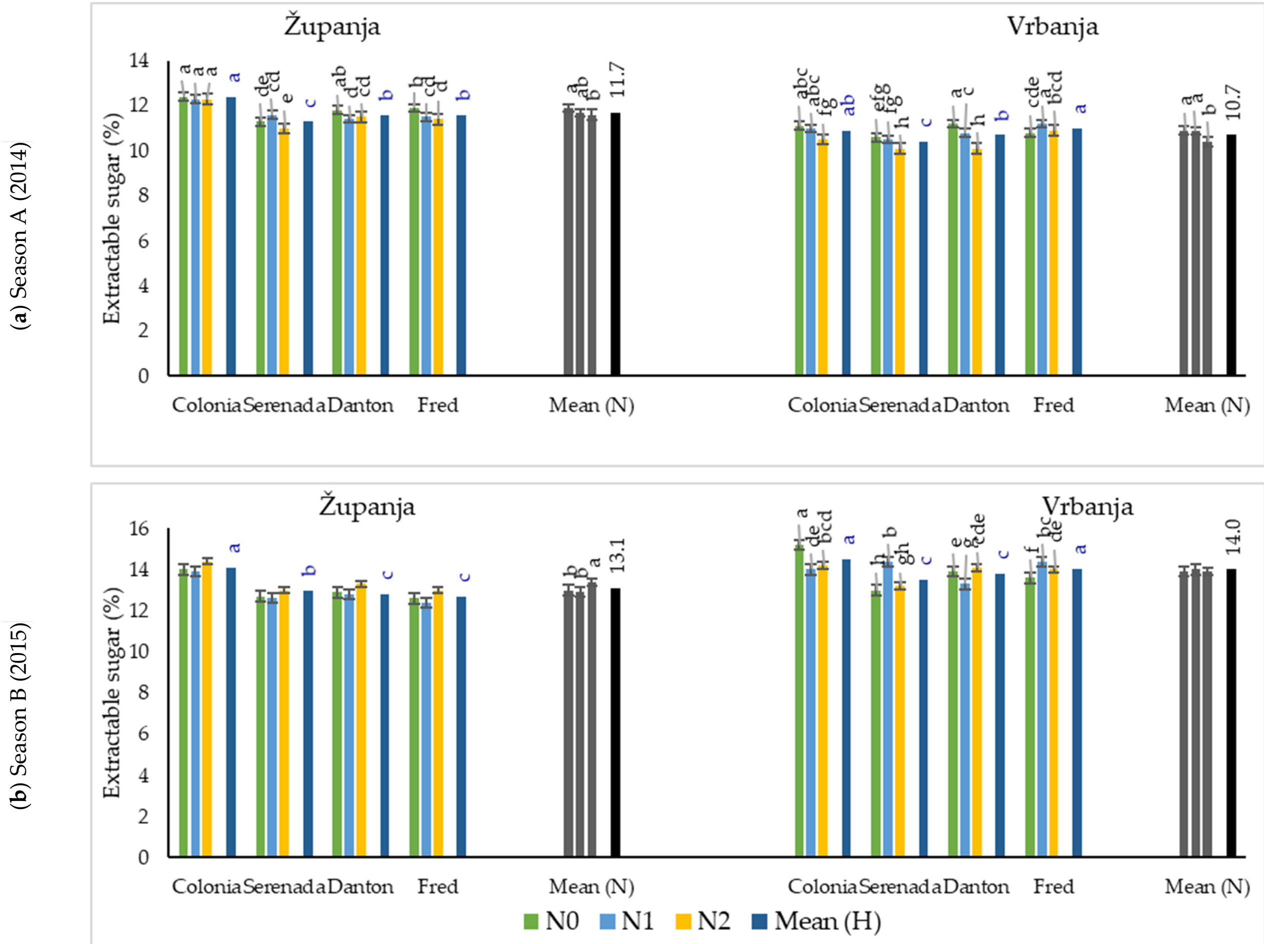

Figure 7. Extractable sugar (%) resulting from nitrogen fertilization (vertical bars display the standard error of the mean for all values greater than 5%; different letters indicate statistically significant differences ($p \leq 0.05$).

For Season B, the influence of N pre-sowing fertilization was also significant ($p \leq 0.05$), as it was in Season A. Thus, the highest extractable sugar at the Županja site (Figure 7b) was obtained from the treatment with increased fertilization (N_2), with an average of 13.4%. The hybrids differed significantly ($p \leq 0.05$) from each other, in regards to the extractable sugar. The highest extractable sugar was found in Colonia (14.1% on average), where the average extractable sugar was higher than in the other experimental areas, while Fred had the lowest determined extractable sugar (12.7% on average). The average extractable sugar for the Vrbanja site (Figure 7b) was higher than that of the other experimental sites. The influence of pre-sowing N fertilization on extractable sugar was not statistically significant. On the contrary, the influence of genotype was also very significant in this case ($p \leq 0.01$), with Colonia in the locality of Vrbanja expressing the highest average extractable sugar (14.5%), and Serenada showing the lowest (average 13.5%).

All researched treatments had a very significant ($p \leq 0.01$) impact on extractable sugar at the Županja locality in Season A. Depending on the pre-sowing N fertilization, the sugar beets from the control treatment exhibited the highest extractable sugar (11.9% on average). Hybrid Colonia stands out with the highest extractable sugar (12.4% on average), while

Serenada had the lowest (11.3% on average). At the other location in Vrbanja (Figure 6a), increased fertilization (N_2) resulted in a significant decrease in the extractable sugar of the beet root compared to the samples from the reduced nitrogen fertilization (N_0 and N_1). The hybrid Fred had the highest average extractable sugar (11.0%), and Serenada had the lowest (10.4%).

3.6. White Sugar Yield

In Season A (2014), the white sugar yield at both locations was approximately similar (10.7 t ha^{-1} in Županja and 10.2 t ha^{-1} in Vrbanja (Figure 8a). In Season B, the differences between the locations were more significant; thus, the white sugar yield in Županja was lower (6.4 t ha^{-1}) compared to that in Vrbanja (10.4 t ha^{-1}) (Figure 8b).

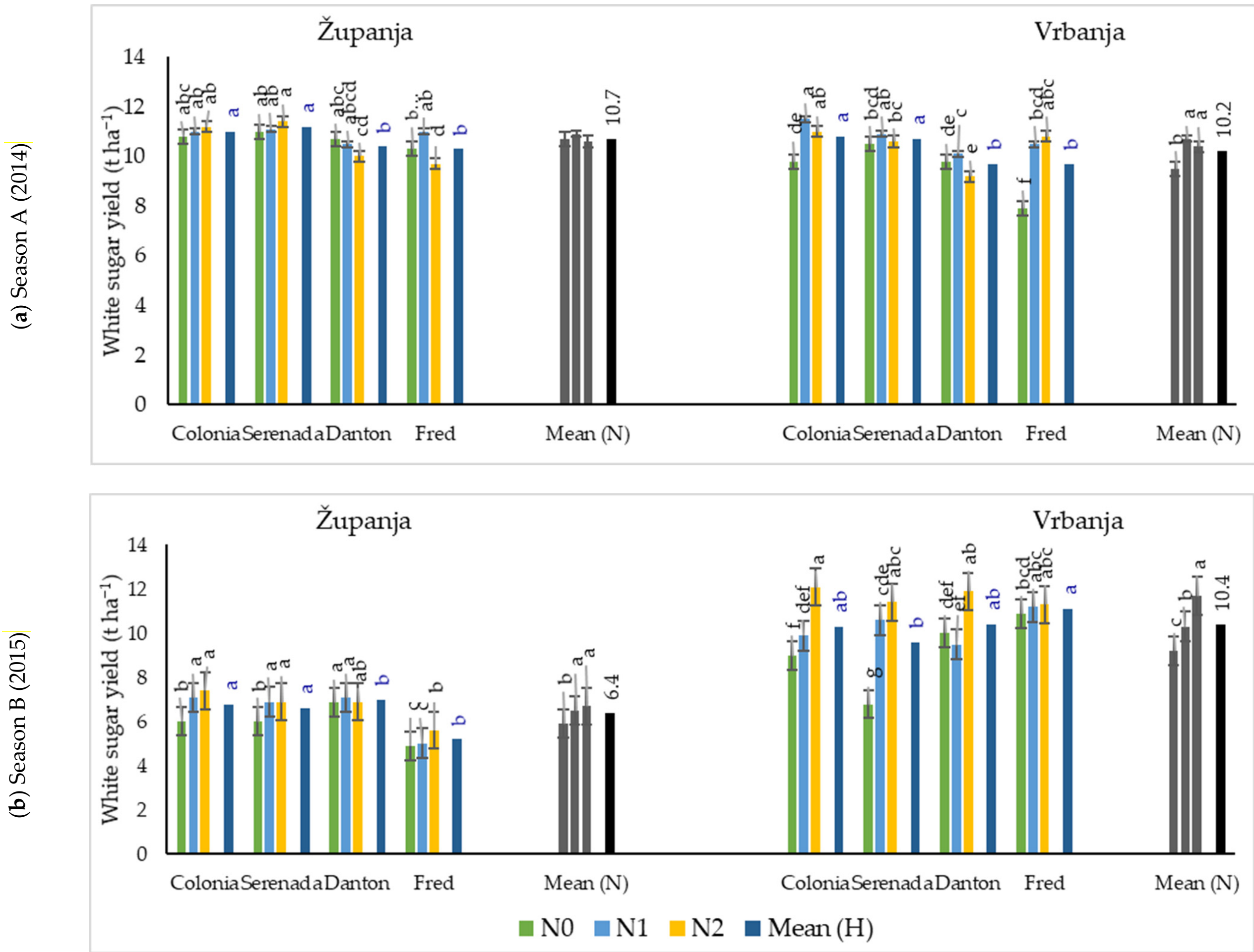

Figure 8. White sugar yield (t ha^{-1}) resulting from nitrogen fertilization (vertical bars display the standard error of the mean for all values greater than 5%; different letters indicate statistically significant differences ($p \leq 0.05$).

For Season A, nitrogen pre-sowing fertilization did not have a significant influence on the white sugar yield at the Županja site (Figure 8a), while the hybrids differed significantly in regards to white sugar yield, with Serenada achieving the highest average white sugar yield (11.2 t ha^{-1}), while Fred had the lowest (10.3 t ha^{-1}). At the site in Vrbanja, pre-sowing N fertilization had a very significant ($p \leq 0.01$) effect on the white sugar yield, with the highest yield for the N_1 treatment (average 10.7 t ha^{-1}). The Colonia hybrid also exhibited the highest average white sugar yield (10.8 t ha^{-1}).

Increasing the amount of N fertilization positively affected the average white sugar yield in Season B at the Županja site (Figure 8b). The differences between the hybrids were very significant ($p \leq 0.01$), with Danton having the highest average (7.0 t ha^{-1}) and Fred having the lowest average (5.2 t ha^{-1}) white sugar yield. At the Vrbanja site, N pre-sowing fertilization had a positive and very significant effect ($p \leq 0.01$) on increasing the white sugar yield, and the highest yield was obtained for treatment (N_2) (11.7 t ha^{-1}). The influence of the hybrid was also very significant ($p \leq 0.01$), and the highest white sugar yield was achieved by the hybrid Fred (11.1 t ha^{-1}).

3.7. Correlation Analysis

The correlation analysis of the achieved root yield and the quality indicators showed a similar trend in both study years in all localities. In general, in all locations, highly statistically significant complete correlations in the positive direction were determined for the relationship between root yield and white sugar yield, followed by between sugar content and extractable sugar (Table 6). On the contrary, a completely negative significant correlation was found between the sucrose content and the Na content of the beet root (r = −0.777). Furthermore, in general, brei impurities (α-amino N, K, Na) and loss of sugar in molasses were, in most cases, negatively correlated with the sugar content in the roots. A complete and positive, very highly significant correlation was established between sugar content and extractable sugar (r = 993***) and between sugar beet root yield and white sugar yield (r = 0.905***).

Table 6. Pearson's correlation coefficient for the sugar beet yield and quality parameters in two seasons for the Županja and Vrbanja locations (RY—root yield; SC—sucrose content; K—potassium; Na—natrium; N—nitrogen; LSM—loss of sugar in molasses; ES—extractable sugar; WSY—white sugar yield).

	RY	**SC**	**K**	**Na**	**Amino N**	**LSM**	**ES**	**WSY**
RY	1	−0.543 <0.0001	−0.481 <0.0001	0.528 <0.0001	0.078 0.0293	−0.196 <0.0001	−0.516 <0.0001	0.905 <0.0001
SC		1	0.486 <0.0001	−0.777 <0.0001	−0.373 <0.0001	−0.044 0.2270	0.993 <0.0001	−0.148 <0.0001
K			1	−0.488 <0.0001	0.041 0.2571	0.692 <0.0001	0.413 <0.0001	−0.337 <0.0001
Na				1	0.308 <0.0001	0.062 0.0837	0.775 <0.0001	0.229 <0.0001
amino N					1	0.717 <0.0001	0.462 <0.0001	−0.130 0.0003
LSM						1	−0.156 <0.0001	−0.285 <0.0001
ES							1	−0.114 0.0015
WSY								1

4. Discussion

Due to favorable conditions for nitrogen mineralization in the soil, in Season A, the sugar beet root yield was satisfactory, even in the control treatment (Figure 1a), which was less than the average yield for the N_1 and N_2 treatments (95.8 t ha^{-1}) for an average of 7.2 t ha^{-1}. Fertilization had a more pronounced impact on the achieved root yield in Season B (Figure 1b), where, according, to the average of both locations, the best yield was achieved with CAN supplementation (67.0 t ha^{-1}), which was 11.4 t ha^{-1} more compared to the average yield of the control treatment. Different results were obtained by Kristek et al. [60], based on research conducted in a dry (2009) and wet (2010) year. The authors point out that 200 kg ha^{-1} of CAN in a dry year increased root yield from an average of 48.20 t ha^{-1}

to 55.89 t ha^{-1}, while in a wet year, the yield decreased from 74.5 t ha^{-1} at 70.7 t ha^{-1} compared to that of the control treatment (without fertilization in spring). According to the investigation of the influence of nitrogen fertilization (0, 90, 179, 269, and 358 kg ha^{-1} N) of eight genotypes in the United States of America (Wyoming), Stevens et al. [61] state that in a year with favorable weather conditions for nitrogen mineralization (2003), the yield in the control treatment was 63.5 t ha^{-1}, while in years with unfavorable weather conditions for mineralization (2004 and 2005), the root yield was 41.9 t ha^{-1} and 46.6 t ha^{-1}.

In Season A, the sugar content in the roots was low and amounted to an average of 13.1% for both localities, while in Season B, it was 2.4% higher on average and amounted to an average of 15.5% (Figure 2a,b). In general, the achieved sugar content in Season A was largely influenced by the higher intensity of the fungus *Cercospora beticola* Sacc attack. The weather conditions were favourable for the development of the disease, so that despite the timely use of fungicides carried out on four occasions, a greater number of leaves were damaged after August, which certainly had a negative impact on the quality because the stored sugar was used up in the development of new leaves. According to a three-year study (2010–2012), Kristek et al. [62] emphasize the importance of the repeated application of fungicides for protection against the fungus *Cercospora beticola* Sacc. in our agroecological conditions, which is explained by the differences in root yield and root sugar content between one (69.91 t ha^{-1} with 15.19%) and three treatments (77.13 t ha^{-1} with 15.53%). Furthermore, Kristek et al. [63] emphasize the selection of fungicide active substances due to the possible appearance of resistant strains of the fungus *Cercospora beticola* Sacc. Pavlů and Benešová [64] reported that cercosporin leaf spot is the most serious disease of the sugar beet leaf apparatus and that with a moderate attack, there can be losses in regards to the sugar beet root yield of 10–30%, and sugar content can reduce by 0.2–2.5%. Pidgeon et al. [65] state that *Cercospora beticola* Sacc. in Europe occurs more often in drier and warmer areas, such as Austria and Germany, while in Western and Northern Europe, it occurs less often. In addition to the amount of precipitation, in this research, the year 2014 had a total of about 60 fewer sunny hours during August, September, and October, which, along with the attack of the fungus *Cercospora beticola* Sacc., could negatively affect the accumulation of sugar in the roots.

Increased fertilization (N_2), which included top dressing with CAN (with 300 kg of NPK 15:15:15 pre-sowing), in Season A had a negative effect on the achieved sugar content, which was the lowest in that treatment at both locations (13.4% in Županja and 12.4% in Vrbanja). Probably, due to the favorable conditions for leaf re-growth, a higher amount of nitrogen in the soil favored a slightly higher development of leaves, as a result of which the stored sugar was consumed to restore the aboveground mass. In Season A, the conditions were favorable for the mineralization of nitrogen in the soil, and the higher supply of nitrogen further supported canopy re-growth. In the third part of the sugar beet vegetation, when the accumulation of sugar in the roots intensifies, the beet has the least need for nitrogen. Pospišil [66] points out that in years with more precipitation and slightly higher nitrogen fertilization, the technological maturity of sugar beet occurs later. Draycott and Chirstenson [67] state that nitrogen deficiency 4 to 6 weeks before harvest has a positive effect on sugar accumulation, but at the same time, it can also affect the reduction of root mass if the weather conditions are favorable for the growth of the aboveground mass. This can decrease leaf re-growth and root dry matter, since the plant takes storage nutrients for young leaf development and hypocotyl elongation. According to Wang et al., 2021 [68] excessive N in the soil improves the vegetative growth of the sugar beet, but at the same time, inhibits the translocation of the dry matter from the vegetative organs to the tap root; thus, the final root yield is reduced, but the sugar content is also decreased, along with the beet quality.

In Season B, fertilization mostly had a positive effect on increasing the sugar content. The best variant was found in the Županja locality under increased fertilization (N_2), where the average sugar content was 15.3%. In contrast, in the Vrbanja locality, the best result was 16.0%, achieved in the pre-sowing fertilization treatment (N_1), although the differences between the fertilization treatments were not statistically significant. Similar results for the reduction of sugar content in the roots due to higher nitrogen supply were reported by Stevens et al. [69]. Based on a two-year study, the authors point out that the increase in nitrogen fertilization (78 kg ha^{-1} N to 212 kg ha^{-1} N) reduced the sugar content in the roots from 18.3% to 17.5%, i.e., by an average of 0.8%, while root yield increased from 52.2 t ha^{-1} at a lower level of fertilization to 58.9 t ha^{-1} at a higher level of fertilization. On the other hand, Malnou et al. [70] obtained the best results with sugar beet fertilization, as was the case, for example, in Season B of this study. According to a three-year study (2000–2002) in Great Britain (Broom's Barn) on sandy soil with favorable water-air conditions, the authors concluded that pre-sowing fertilization with 40 kg ha^{-1} N, with top dressing with the same amount of N in the 2–4 leaf stage, resulted in the highest sugar content in the roots of 17.7%, while higher amounts of N in the fertilizer (120 kg ha^{-1} nitrogen) reduced the sugar content by 0.7%. Based on the achieved yields and quality of sugar beet as early as 1950 in Germany, Lüdecke [71] states that fertilization with 120 kg ha^{-1} of nitrogen and with an NPK nutrient ratio of 1:0.8:1.2 is economically justified.

In this study, fertilization had a different effect on extractable sugar, which can largely be attributed to different weather conditions in the years of the study (Figure 7a,b). In Season A, increased fertilization (N_2) had a negative effect on the extractable sugar in the beets, which in Season A was reduced by 0.4% compared to that of the control. On the contrary, according to the average of Season B, increased fertilization (N_2) resulted in an increase in the sugar utilization of beets by an average of 0.2% compared to that of the control. Such a result is closely related to the sugar content in the roots, which was reduced in Season A and increased in Season B in the N_2 treatment (Figure 2). In addition, in Season A, along with the lowest sugar content (12.9%) in the roots, the beets in the N_2 treatment had, on average, the highest content of Na and α-amino nitrogen (0.73 mmol per 100 g $beet^{-1}$, i.e., 1.77 mmol per 100 g of $beet^{-1}$) compared to other fertilizing treatments, which had a negative effect on extractable sugar. On the other hand, in Season B, the highest sugar content (15.6%) was found in the increased fertilization treatment (N_2), and of the molasses-forming elements, only the α-amino nitrogen content was found to have a higher concentration compared to the other fertilization treatments (on average, 1.54 mmol per 100 g $beet^{-1}$).

According to the research of different doses of NPK fertilizer in two years with a lack of precipitation, Jaćimović et al. [72] in the dry year (2002) obtained the highest beet sugar utilization of 15.0% on the variant with the least amount of nitrogen (50 kg ha^{-1} nitrogen, 100 kg ha^{-1} P and 100 kg ha^{-1} K), while in an extremely dry year (2003) they determined the highest utilization of sugar on beet of 12.5% on the variant without potassium fertilization (100 kg ha^{-1} N and 100 kg ha^{-1} P).

Although higher concentrations of sodium and α-amino were determined in this study in the year with more precipitation, Mäck and Hoffmann [73] state that in dry conditions, the concentration of amino nitrogen and betaine increased in the beet roots.

Hoffmann [74] states that in Germany, through breeding work in the period from 1975 to 2005, the sugar content in the roots increased by 0.02% per year, while the content of K, Na, and α-amino N decreased by 0.77, 0.13, and 0.38 mmol per kg^{-1} beets per year, respectively. According to a four-year study (2009–2012) of 10 sugar beet hybrids in Croatia, Kristek et al. [75] determined that the average content of molasses elements in the roots was 3.23 mmol per 100 g $beet^{-1}$ K, 1.54 mmol per 100 g $beet^{-1}$ Na, and 3.53 mmol per 100 g $beet^{-1}$ α-amino N.

In this study, increased N fertilization resulted in the lowest K content in beet roots in both years of the study (average 3.14 mmol per 100 g $beet^{-1}$) (Figure 7). At the same time, increased pre-sowing N fertilization resulted in the highest average content of α-amino N in the roots (average 1.68 mmol per 100 g $beet^{-1}$), which, according to the average of the experiments, increased by 14% compared to that of the control treatment (average 1.45 mmol per 100 g $beet^{-1}$). As for the Na content, according to the average of both years of research, the highest sodium content in the roots was achieved with increased nitrogen fertilization (0.52 mmol per 100 g beet^{-1}). Hoffmann [76] states that the increased treatment of sugar beet with N increases the concentration of sodium in the roots, which is explained by the intake of sodium as an ion that maintains the balance between cations and anions in the cell when N is in nitrate form, while, on the contrary, the intake of K available nitrogen does not have such a large influence.

Furthermore, in this research, based on the correlation analysis, a negative correlation was established between the content of molasses-forming elements (K, Na, α-amino N) and the sugar content in the roots, followed by the extractable sugar and sucrose content. Abbasi and Rezaei [77], analyzing 25 sugar beet genotypes, also point out that the content of molasses-forming elements is negatively correlated with the sugar content in the roots and the yield of crystalline sugar. Based on the analysis of 17 genotypes, Bosemark [78] points out that negative correlations were found between the root mass and sugar content and the content of molasses-forming elements (K, Na, α-amino N) and sugar content.

In this study, the highest sugar yield was not achieved with the same fertilization in both years of the study. In Season A, the highest sugar yields were achieved with pre-sowing fertilization (N_1), and on average, for both localities, the yield of biological sugar increased by an average of 0.8 t ha^{-1}, and the yield of pure sugar increased by an average of 0.7 t ha^{-1}, when compared to control. In Season B, on average, for both research locations, the treatment with enhanced nitrogen fertilization (N_2) resulted in an increase in the yield of biological sugar by an average of 1.9 t ha^{-1} and the yield of pure sugar by an average of 1.6 t ha^{-1}, when compared to the control treatment. Under our agroecological conditions, in 2007, which exhibited high summer temperatures and a lack of precipitation, Kristek et al. [61] obtained the best results for the pre-sowing nitrogen fertilization treatment, while increasing the amount of nitrogen through top dressing in the 2–4 leaf phase had a negative effect on the achieved results. According to the analysis of sixteen sugar beet hybrids, the authors point out that the best root yield (average 74.17 t ha^{-1}), sugar content (16.35%) and pure sugar yield (10.71 t ha^{-1}) were achieved with pre-sowing fertilization (54 kg ha^{-1}), while the increase in nitrogen fertilization (108 kg ha^{-1} nitrogen) led to an increase in soluble non-sugars, especially AmN, and ultimately, to a decrease in sugar yield. Kristek et al. [62] also emphasize the influence of weather conditions on sugar beet production results. Namely, the authors point out that the best sugar yield in the dry year (2009) was obtained with top dressing with 160 kg ha^{-1} of CAN (8.00 t ha^{-1}), while on the other hand, in the year with more precipitation (2010), the best yield of pure sugar was achieved without nitrogen fertilization in spring (9.70 t ha^{-1}).

Hoffmann [75] states that root yields and root sugar content are negatively correlated, and that this is one of the criteria for dividing beets into sugary Z types, with a high sugar content, and yielding E types, with a high root yield. NZ-type hybrids are less common in our country today, while Z-type hybrids are more common in production. Kristek et al. [60] emphasize that due to the shorter growing season, producers more often decide on sugary types of hybrids that have the advantage of earlier extraction periods.

According to the sugar content in the root, Colonia generally achieved the highest average sugar content of 14.7% in both years of the study, while Serenada had the lowest average sugar content of 14.0% (Figure 5). Although the hybrids differed significantly in sugar content in this study, if the average sugar content of both years of the study is observed, the Z types of hybrids did not have a higher sugar content. Kristek et al. [74] found statistically significant differences in the sugar content under our agroecological conditions, depending on the production characteristics of the hybrid. The authors concluded that the sugar content of eight Z-type hybrids was, on average, 0.57% higher than that of eight normal and N-type hybrids.

According to their study of six hybrids from the selection company KWS in three localities in Croatia (Nijemci, Meretine, and Međimurje), Jurišić and Kristek [79] highlight the Colonia hybrid, according to the achieved root yield and sugar yield. The authors state that on average the highest sugar beet root yield, had hybrid Colonia (74.78 t ha^{-1}), sugar content hybrid Severina (16.08%), while the highest sugar yield, was achieved by the

Colonia hybrid (10.81 t ha^{-1}). According to research in Croatia, Kristek et al. [61], based on a four-year study (2009–2012) of 10 sugar beet hybrids, obtained an average root yield of 82 t ha^{-1}, with a root sugar content of 14.97%, with the authors emphasizing that the Colonia hybrid achieved the highest average sugar content (15.40%).

Hoffmann [74] states that no interaction between genotype and nitrogen fertilization has been established, and that genotypes with a high concentration of amino nitrogen or betaine have a genetically higher concentration, regardless of the supply of nitrogen in the soil and the weather conditions. By analyzing sugar beet roots at 22 locations in Germany over two years of research, Hoffmann and Märländer [80] identified 20 to 80 mmol kg^{-1} beet of total nitrogen in the roots, of which 5 to 25 mmol kg^{-1} beet is α-amino N, with a very high correlation (r^2 = 0.90), while the correlations between betaine and nitrate content in the roots were not significant. In a later study, Hoffmann and Märländer [81] analyzed 57 genotypes from 22 locations in Germany (mainly luvisol and chernozem), and pointed out that sugar beet roots contain from 25 to 65 mmol per kg^{-1} beet of total N, of which 6 to 24 mmol per kg^{-1} beet is α-amino N.

5. Conclusions

For this study, the field experiment was set up with three different nitrogen fertilization rates, which were chosen based on the usual fertilizations used by farmers in Eastern Croatia. in the season with more rainfall, sugar beet root yield was higher as compared to the season with the lack of rainfall. According to this study, the highest sugar beet root yield was achieved for the Serenada hybrid, by applying nitrogen pre-sowing and in topdressing (105.4 t ha^{-1}), in the year with more rainfall, whereas the lowest root yield was achieved for hybrid Fred (39.0 t ha^{-1}) in the dryer year. Even though there were different influences on sucrose content, brei impurities, and extractable sugar among N treatments of the study for both locations and seasons, generally, the highest white sugar yield was achieved with N_2 treatment, which included N pre-sowing, with the topdressing application.

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