Article

Response of Wheat Yield and Protein-Related Quality on Late-Season Urea Application

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Abstract: Field experiments were conducted, aiming to evaluate whether the late-season foliar urea application (35 kg N ha⁻¹) is effective in improving grain yield and protein-related quality in ten common wheat cultivars during two consecutive growing seasons. On average, late urea application significantly (p < 0.05) increased grain yields, thousand kernel, and hectolitre weight. The total grain protein significantly (p < 0.05) increased by 17.5% under urea application, while dough mixing behaviour was improved by increasing flour water absorption and reducing the degree of softening. Considering dough elastic properties, the changes in dough energy and maximum resistance under urea application were strongly cultivar dependent and their values compared to control varying from −12.7% to +42.4% and −25.1% to +7.7%, respectively. The distribution of grain storage proteins was significantly influenced by late urea application, but to a lesser extent than the total grain proteins. The proportion of total gliadins, α- and γ-gliadins increased by 5.3%, 5.8%, and 6.5%, respectively, while total glutelins and high-molecular-weight glutelins decreased by 4.5% and 7.4%, respectively. In summary, the late-season application of urea has greatly improved the protein-related quality without considerable disruption of the storage proteins composition.

Keywords: wheat; cultivars; fertilization; foliar urea; quality traits; storage proteins

1. Introduction

Common wheat (Triticum aestivum L.), as an important source of human dietary proteins, is one of the most important cereal crops in the world. Wheat yield and bread-making quality are largely dependent on genotype, environmental factors, agronomic techniques, and nitrogen management [1]. Late-season nitrogen availability is particularly important for grain protein accumulation because the application of additional nitrogen (N) at heading or anthesis prolongs the grain-filling period and results in protein dominance over starch [2]. An additional advantage of N uptake from foliar spraying is less dependent on soil moisture [3–5]. Grain protein responses to urea spraying are relatively consistent and increases have been most commonly reported in the literature [2,6–8], while grain yield responses were more inconsistent, and depended more on several factors (soil moisture, basal N application, and timing) [7,9,10].

Compared to other cereals, wheat is the source of numerous bread-making and other foods products due to its unique storage protein characteristics [11]. Gluten proteins, as
the major storage proteins consisting of gliadins and glutenins, roughly account for 80% of total grain protein, and qualitatively and quantitatively are important determinants of the processing and nutritional quality of wheat [12]. Gliadins, as monomeric prolamin fractions, are responsible for dough extensibility, and according to their electrophoretic mobility, are divided into \( \alpha/\beta, \gamma, \) and \( \omega \)-gliadins. Polymeric glutenins are composed of high-molecular-weight and low-molecular-weight glutenin subunits linked with intermolecular disulphide bonds, and are responsible for dough elasticity [13,14]. Although the composition and number of gluten proteins are strongly genetically and environmentally determined, the cultivar-determined plant development time, N amount, N timing, and N availability significantly affect its number and size distribution [15–17]. Late-season N application is directly related to the increase in both total grain protein and the accumulation of all gliadin and glutenin components [17].

In Croatia, in the last 10 years, the average grain yield was 5.2 t ha\(^{-1}\) [18], and following Croatian regulations, the grain protein and hectolitre weight are the most important contributors in the market price formation. Since the premium quality of wheat (\( p > 15\% \)) in Croatia has a higher price, higher-protein cultivars without compromising yielding capacity are the most interesting to producers. In Croatia, a few studies considering late-season urea application on wheat agronomic, and some baking quality traits, have been conducted [7,19]. In general, only a small number of comprehensive studies have been conducted on the impact of late urea application on baking quality, with an emphasis on protein components. The main objectives of this study conducted during two consecutive crop seasons were to evaluate whether the late foliar application of urea effectively improves grain yield and protein-related quality, its impact on storage proteins distribution, and assessing the magnitude of cultivar response to foliar nitrogen treatments.

2. Materials and Methods

2.1. Plant Materials and Field Experiments

Ten winter wheat (\( Triticum aestivum \) L.) cultivars created at Agricultural Institute Osijek were tested over two consecutive years (2016/2017 and 2017/2018, hereafter indicated as 2017 and 2018, respectively) in Osijek, Croatia (45°33’20” N 18°41’40” E, 94 m altitude). All cultivars are classified as bread wheat and are currently represented in production in Croatia (Table 1). The cultivar list, pedigree and year of registration are listed in Table 1. Seeds were planted at the end and middle of October in 2016 and 2017, respectively, and harvested at the end of June in consecutive years. The harvested plot size was 7.56 m\(^2\), with a seeding rate of 500 kernels m\(^{-2}\). The field experiments were set up in a split-plot factorial design in three replications.

| Cultivars     | Year of Registration | Pedigree                   |
|---------------|----------------------|----------------------------|
| EL NINO       | 2016                 | Ficko/Felix                |
| FICKO         | 2007                 | Srpanjka/Rialto            |
| KATARINA      | 2006                 | Osk.5.B.4.1-94/Osk.5.140-22-91 |
| VULKAN        | 2009                 | Osk.3.343-1.97/Osk.15.291//KRH. 44-99 |
| SILVIJA       | 2010                 | Soissons/Hana             |
| KRALJICA      | 2010                 | Osk.5.698-4-99/Osk.4.21-7-99 |
| TIKA TAKA     | 2014                 | Osk. 15.294/Osk.4.503-5-98 |
| RENATA        | 2006                 | Žitarka//Osk.7.5-4-     |
| SRPANJKA      | 1989                 | 82/KBg.160/86/3/Srpanjka |
| OS OLMIPJA    | 2009                 | Osk.4.50-1/Zg. 2696       |

Total N fertilizer application in commercial wheat production in the eastern part of Croatia varies from 120–170 kg of N ha\(^{-1}\). In both growing seasons, before sowing, the basic fertilization consists of 74 kg N ha\(^{-1}\) by adding 400 kg ha\(^{-1}\) NPK (7:20:30) and 100 kg ha\(^{-1}\)
of urea (46% N). At beginning of the stem extension growth stage (BBCH 31/32), 150 and 130 kg ha$^{-1}$ KAN (27% N) were applied as top-dressing (Table 2). The late-season urea fertilization versus non-treatment (control) was applied as a split urea dose at booting (BBCH 45/49) and at the anthesis (BBCH 59/61) by spraying 250 L ha$^{-1}$ of 15% urea (46% N), which was an additional 35 kg N ha$^{-1}$ (Table 2). There was no precipitation within 24 h of foliar urea treatment. Agro-technical treatments were performed following a good agricultural practice.

Table 2. Soil N content in Osijek in 2017 and 2018.

| Location | Soil Type | Previous Crop | Season | Basic N Fertilization (kg N ha$^{-1}$) | N Top-Dressing (kg N ha$^{-1}$) | 15% Urea (46% N) | Total N (kg N ha$^{-1}$) |
|----------|-----------|---------------|--------|--------------------------------------|---------------------------------|------------------|-------------------------|
| Osijek   | Eutric cambisol | Soybean       | 2017   | 74                                   | 41                              | 0                | 35                      | 115                     | 150                     |
|          |           | Soybean       | 2018   | 74                                   | 35                              | 0                | 35                      | 109                     | 144                     |

1 $N_{CON}$-control without urea treatment; $N_{UREA}$-urea treatment.

All other cultural practices including the application of herbicides, insecticides, and fungicides to control major weeds, insects and foliar diseases were typical for commercial wheat production in Croatia. The investigation location is characterized by eutric cambisol soil type with slightly alkaline reaction ($pH_{KCL}$ of 7.17), 2.02% of organic matter content, 29.50 mg of $P_2O_5$ and 34.90 mg of $K_2O$ 100 g$^{-1}$ of soil with average precipitation sum of 694 mm, and an average annual temperature of 11.2 $^\circ$C (long term period, 1889–2019) (Table 3). In 2017, the total annual precipitation during the growing season was lower than in 2018 when additional intense rainfall occurred at the time of grain filling, while the temperatures during grain filling, as a key period for bread-making quality, were lower. Both experimental years with average annual temperatures, respectively, have been warmer than the long-term period (Table 3).

Table 3. Climatic data related to the growing seasons.

| KERRYPNX | 2016/2017 | 2017/2018 |
|----------|-----------|-----------|
| Sum rainfall (I-XII) mm | 571 | 718 |
| Sum rainfall in the growing season (X-VI) mm | 409 | 545 |
| Sum rainfall during grain filling (V-VI) 86 | 181 |
| LTP 1 sum rainfall (I-XII) mm | 694 |
| LTP sum rainfall in the growing season (X-VI) mm | 435 |
| LTP sum rainfall during grain filling (V-VI) 115 |
| Mean temperature (I-XII) $^\circ$C | 11.9 | 12.7 |
| Mean temperature in the growing season (X-VI) $^\circ$C | 8.3 | 9.7 |
| Mean temperature during grain filling (V-VI) 19.8 | 20.5 |
| LTP mean temperature (I-XII) $^\circ$C | 11.2 |
| LTP mean temperature in the growing season (X-VI) $^\circ$C | 6.9 |
| LTP Mean temperature during grain filling (V-VI) 18.9 |

1 LTP-Long term period (1889–2019).
2.2. Analysis

2.2.1. Grain Yield and Bread-Making Quality Parameters

Plots were harvested by a combine, and total grain yields are expressed as tons per hectare (t ha\(^{-1}\)) on a 13% moisture basis. The measurement of the 1000 kernel was done using a Marvin grain analyser (MARViTECH GmbH, Wittenburg, Germany). The grains harvested from the three replicates after cleaning were blended (50/50/50 w/w) and the resulting samples (approx. 1000 g) were used for bread-making quality and protein analysis. Grain protein content (N × 5.7, DM) and hectolitre weight were measured using an Infratec 1241 Grain Analyzer (FOSS, Hilleroed, Denmark), while dough rheology properties were analysed by Brabender (Brabender GmbH & Co. KG, Duisburg, Germany) farinograph and extensograph, according to ICC standard methods No 115/1 and ICC No 114/1, respectively.

2.2.2. Extraction and HPLC Analysis of Storage Proteins

The protein components of wheat were extracted sequentially according to Wieser and Seilmeier [6], with some modifications. First, albumins and globulin were extracted from 50 mg of wholemeal flour (Retsch centrifugal mill ZM1, Haan, Germany, 1 mm sieve) by 1 mL of extraction buffer (0.4 M NaCl) for 30 min at 25 °C. After centrifugation, the albumins and globulin supernatants were collected. The gliadins were then extracted from the remaining pellet with 1 mL of 50% 1-PrOH for 60 min at 25 °C, and after centrifugation, the gliadin supernatants were collected. At the end of the remaining pellet, the glutenins were extracted at 60 °C with 1 mL of a solution containing 50% (v/v) 1-PrOH, 2 M urea, 1% (w/v) dithioerythritol and 0.05 M Tris-HCl (pH 7.5) for 60 min. During the extraction procedure, all suspensions were vortexing every 10 min and centrifuged at 14,000 rpm for 15 min at 25 °C. The collected protein supernatants were stored at −20 °C and prior HPLC analysis was filtered through a PVDF 0.45 µm syringe filter.

The protein extracts were analysed according to the method of Wieser and Seilmeier [6] using reverse-phase-high-performance liquid chromatography (RP-HPLC) (Perkin Elmer Instruments, Waltham, MA, USA) equipped with Total-Chrom software and a photodiode array detector. Proteins elution was performed at 50 °C with a linear gradient of acetonitrile in 0.1% aqueous trifluoroacetic acid from 24 to 54% over 30 min at 1 mL min\(^{-1}\). Separations of proteins were done by the C18 column (5 µm 4.6 × 150 mm; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and their quantification was based on its peak area. The peak areas under albumins and globulins, gliadins, and glutenins chromatograms measuring 210 nm are used for calculations of their proportion (%) in total extractable proteins. For each sample, the extraction was done in duplicate and both extracts were analysed, with the results averaged.

2.3. Statistical Analyses

This research was based on a full factorial design, including the following independent factors: (i) genotypes—ten cultivars, (ii) growing seasons—two years, and (iii) nitrogen treatments—two levels. All traits’ determinations were carried out in two replications (N = 80). Descriptive statistics, an analysis of variance, and a principal component analysis were performed using Statistica ver. 14.0.0.15 (TIBCO Software Inc., Paolo Alto, CA, USA). The means differences were tested using Tukey’s HSD test at the levels of probability \( p < 0.05 \).

3. Results and Discussion

The analysis of variance (ANOVA) generally showed a significant effect of genotype (G), year (Y), and urea foliar treatment (N), as well as their interactions on the most traits considered (Tables 4 and 5). Significant differences between cultivars were found for agronomic and bread-making traits, indicating their different genetic potential. G was a dominant source of variation for thousand kernel weight, dough softening, and dough maximum resistance (Table 4). Cultivars Kraljica and Tika Taka resulted in the highest grain yield, followed by significantly different thousand kernel weight and hectolitre. A similar trend of different thousand kernel weight and hectolitre was also observed in cultivars OS Olimpija and El Nino, which obtained the lowest yields (Table 4).
Table 4. The mean values and the effects of the late-season urea application on yield and bread-making quality of wheat cultivars.

| CULTIVARS       | GK *1 (t ha⁻¹) | TKW (g) | HL (kg hl⁻¹) | P (%) | WA (%) | DDT (min) | DS (FU) | E (EU) | R_MAX (EU) | R/EXT |
|-----------------|----------------|---------|--------------|-------|--------|-----------|---------|--------|------------|-------|
|                  | Genotype (G)   |         |              |       |        |           |         |        |            |       |
| EL NINO         | 6.0 b          | 37 b    | 80 b         | 12.4 c| 55.4 de| 3.2 d     | 73 d    | 114 f  | 347 a      | 1.9 a |
| FICKO           | 8.6 e          | 43 e    | 80 e         | 11.9 b| 56.4 de| 1.3 ab    | 71 d    | 76 b    | 401 b      | 2.1 ab|
| KATARINA        | 8.0 e          | 40 c    | 80 d         | 11.9 b| 55.8 c | 1.4 abc   | 72 d    | 74 b    | 597 b      | 2.8 ab|
| VULKAN          | 8.5 de         | 36 a    | 80 ab        | 12.1 c| 54.0 a | 1.5 bc    | 77 d    | 109 ef  | 537 g      | 2.2 bc|
| SILVIJA         | 8.0 e          | 46 g    | 81 e         | 12.7 f| 56.3 d | 5.5 f     | 51 b    | 74 b    | 414 b      | 2.3 b  |
| KRALJICA        | 8.4 f          | 42 d    | 81 f         | 12.4 e| 56.4 de| 1.5 abc   | 76 d    | 95 d    | 497 c      | 2.5 cd |
| TIFA TAKA       | 8.4 f          | 46 f    | 79 a         | 12.5 e| 56.7 e | 1.5 abc   | 76 d    | 95 d    | 497 c      | 2.5 cd |
| RENATA          | 7.7 c          | 45 f    | 81 a         | 12.3 d| 56.5 de| 1.5 abc   | 85 e    | 104 e   | 517 f      | 2.2 bc |
| SRPANJKA        | 8.0 d          | 37 b    | 81 a         | 11.6 a| 55.3 b | 1.3 a     | 109 f   | 87 c    | 470 d      | 2.5 cd |
| OS OLIMPIJA     | 6.4 a          | 43 e    | 82 f         | 13.7 f| 57.0 f | 4.1 e     | 38 a    | 129 g   | 597 h      | 2.2 ab |
| Mean            | 7.8            | 42      | 81           | 12.4 c| 56.0   | 2.3       | 73      | 93      | 482        | 2.3    |
| SD              | 0.9            | 4.0     | 1.8          | 1.6   | 1.4    | 1.9       | 28.7    | 29.2    | 112.5      | 0.6    |
| CV %            | 11.5           | 9.5     | 2.3          | 12.9  | 2.5    | 82.6      | 40.0    | 31.4    | 23.3       | 26.1   |
| Year (Y)        |                |         |              |       |        |           |         |        |            |       |
| 2017            | 7.3 a          | 43 b    | 82 b         | 11.5 a| 56.4 b | 2.1 a     | 81.9 b  | 76 a    | 443 a      | 2.6 b  |
| 2018            | 8.3 b          | 40 a    | 79 a         | 13.2 b| 55.6 a | 2.5 b     | 62.6 a  | 109 b   | 521 b      | 2.1 a  |
| DIFF 2018 vs. 2017 | +13.7         | -7.0   | -3.7         | +14.8 | -1.4   | +16.0     | -23.6   | +43.4   | +17.6      | -19.2 |
| NCON            | 7.8 a          | 41 a    | 80 a         | 11.4 a| 55.3 a | 1.6 a     | 81.6 b  | 92.5 a  | 507 b      | 2.6 b  |
| NUREA           | 7.9 b          | 42 b    | 81 b         | 13.4 b| 56.7 b | 2.9 b     | 62.9 a  | 93.2 b  | 457 a      | 2.0 a  |
| DIFF NUREA vs. NCON | +1.3          | +3.2   | +0.4         | +17.5 | +2.6   | +66.2     | -22.9   | +0.8    | -9.9       | -23.1 |
| Nitrogen (N)    |                |         |              |       |        |           |         |        |            |       |
| Means squares   |                |         |              |       |        |           |         |        |            |       |
| G               | 4 *            | 4.7 *   | 7.0 *        | 16.3 *| 33181.4 *| 3311.1 *| 55012 *| 55012 *| 55012 *|
| Y               | 20 *           | 95.3 *  | 95.4 *       | 69.6 *| 129 *   | 75665.5 *| 23367.7 *| 13670 *| 13670 *|
| N               | 0.2 *          | 34.3 *  | 1.7 *        | 94.6 *| 42.3 *  | 107362.7 *| 118.2 *| 43281 *| 43281 *|
| G × Y           | 1.3 *          | 5.5 *   | 14.1 *       | 2.2 * | 1.0 *   | 0.3 *    | 460.0 *| 255.3 *| 6110 *|
| G × N           | 0.0 ns         | 0.1 *   | 0.7 *        | 5.2 * | 12225.8 *| 43.7 *  | 1373 *| 1373 *|
| Y × N           | 0.0 ns         | 0.1     | 0.7          | 0.6 * | 4.9    | 10870.0 *| 675.3 *| 16694 *| 16694 *|

1 GY— thousand kernel weight g; TKW—total kernel weight g; HL—hundred kernel weight g; P—total grain protein; WA—water absorption; DDT—dough development time; DS—dough degree of softening; E—dough energy; R_MAX—dough maximum of resistance; R/EXT—resistance/extension ratio; NCON—control without urea treatment; NUREA—urea treatment. For each means within a column, values followed by different letters or * are significantly different at p < 0.05.
Table 5. The mean values and the effects of the late-season urea application on protein composition of wheat cultivars.

| CULTIVARS | AG (%) | GLI (%) | GLU (%) |
|-----------|--------|---------|---------|
|           | T GLI | ω-GLI  | α-GLI   | γ-GLI  | T GLU | HMW | LMW | GLI/GLU |
|           |       |         |         |        |       |     |     |         |
| Genotype  | (G)   |         |         |        |       |     |     |         |
| EL NINO   | 19.1  | 45.7    | 4.6    | 26.1   | 15.0  | 35.2 | 10.0 | 25.2    | 1.32 |
| FICKO     | 22.1  | 46.7    | 7.8    | 24.1   | 14.8  | 31.2 | 11.3 | 19.8    | 1.56 |
| KATARINA | 19.5  | 47.2    | 7.7    | 22.9   | 16.6  | 33.3 | 12.5 | 20.8    | 1.43 |
| VULKAN    | 16.1  | 45.0    | 3.7    | 24.2   | 17.1  | 38.9 | 10.5 | 28.4    | 1.20 |
| SILVIJA   | 17.4  | 46.5    | 4.7    | 23.9   | 17.9  | 36.1 | 10.7 | 25.4    | 1.32 |
| KRALJICA  | 21.6  | 46.1    | 7.5    | 24.6   | 13.9  | 32.3 | 11.5 | 20.9    | 1.44 |
| TAKA      | 16.6  | 42.9    | 4.7    | 27.1   | 17.0  | 34.2 | 8.6  | 25.6    | 1.44 |
| RENATA    | 16.5  | 45.3    | 4.8    | 25.5   | 14.9  | 38.2 | 11.5 | 26.7    | 1.19 |
| CONFICKO  | 18.1  | 45.7    | 4.9    | 25.3   | 15.5  | 36.2 | 10.3 | 25.9    | 1.27 |
| OSILOMPIJA| 12.9  | 50.7    | 4.9    | 29.0   | 16.8  | 36.4 | 10.3 | 26.1    | 1.40 |

Mean of square

| Year (Y) |        |        |        |        |        |        |        |
|----------|--------|--------|--------|--------|--------|--------|--------|
|          | Mean   | SD     | CV %   | 2017   | 2018   | DIFF 2018/2017 | DIFF NCON/NUREA | DIFF NCON/NUREA |
|          | 18.0   | 3.5    | 19.4   | 17.2   | 18.8   | +9.3   | −2.0    | +5.3    |
|          | 46.8   | 3.5    | 7.5    | 45.0   | 48.6   | +8.0   | +5.3    | +1.1    | +5.8    | +6.5    | −4.5    | −7.4    | −2.9    | +11.0  |

Mean of square

| G        | 56.6  | 42.2  | 17.8  | 38.3  | 12.2  | 50.6  | 8.0   | 66.3  | 0.1    |
| Y        | 64.6  | 209.0 | 1.9   | 182.3 | 0.0   | 506.1 | 54.0  | 229.6 | 1.6    |
| N        | 3.6   | 132.7 | 0.0   | 44.3  | 20.7  | 92.4  | 16.8  | 30.3  | 0.4    |
| G × Y    | 25.2  | 17.8  | 0.3   | 7.6   | 6.3   | 14.9  | 2.4   | 11.0  | 0.1    |
| G × N    | 4.7   | 12.5  | 0.8   | 4.1   | 4.8   | 16.8  | 3.1   | 9.5   | 0.0    |
| Y × N    | 7.4   | 24.9  | 0.2   | 10.8  | 0.8   | 5.2   | 5.8   | 0.0   | 0.0    |
| G × Y × N| 8.3   | 12.2  | 1.4   | 1.8   | 1.9   | 21.7  | 3.9   | 10.3  | 0.1    |

AG 1—albumins and globulins; GLI—total gliadins; GLU—total glutenins; HMW—high-molecular-weight glutenins; LMW—low-molecular-weight glutenins; GLI/GLU—gliadins/glutenins ratio; NCON—control without urea treatment; NUREA—urea treatment. For each means within a column, values followed by different letters or * are significantly different at p < 0.05.

On average, cultivars Silvia and OS Olimpija had the highest grain protein content. Cultivars Kraljica and OS Olimpija with water absorption above 56% and the lowest degree of softening showed the best dough mixing behaviour, while cultivars Silvia, Renata, and OS Olimpija with dough energy above 100 cm² and a well-balanced resistance/extensibility ratio showed the best dough elasticity performance (Table 4). Nadew [20], in his review study, states that a better understanding of the effects of both climatic conditions and agronomic treatments on wheat quality traits is becoming a crucial issue.

The two growing seasons showed different climatic trends in rainfall distribution and temperatures (Table 3) causing a significant variability of considered traits over the years (Table 4). Y was dominant for grain yield, hectolitre, and dough energy (Table 4). On average, a significant increase in grain yield and a decrease in thousand kernel weight and hectolitre were noticed in 2018 compared to in 2017 (Table 4). Considering grain protein and dough rheological properties, grain protein, dough development time, dough energy, and dough maximum resistance were higher in 2018, while flour water absorption, dough degree of softening, and resistance to extension ratio were lower compared to in 2017 (Table 4). The present work showed that the grain yield and bread-making quality
properties of wheat were significantly influenced by the genetic and environmental factors, in accordance with others [21–27].

The late urea application significantly ($p < 0.05$) increased, on average, grain yield, thousand kernel weight, and hectolitre (Table 4). The extent of grain yield changes under urea foliar application was cultivar-dependent and varied from $-1.35\%$ (Silvija) to $+2.1\%$ and $+2.4\%$ (Srpanjka and OS Olimpija, respectively) (Table 6). Pushman and Bingham [28] reported occasional grain yield increases under the late foliar urea application, while Dick et al. [29] did not find any significant change in the same trait. Varga and Svečnjak [7] reported increased grain yield and hectolitre under urea spraying, but only at low basal N rate, while thousand kernel weight increased at both low and high basal N rates. The observed non-significant cultivar × urea interaction for grain yield (Table 4) indicated that all cultivars showed similar responses to late-season urea application, despite their determined differences in grain yield in accordance with Varga and Svečnjak [7]. In comparison with agronomic traits, the response of bread-making quality parameters to late-season applications of urea was great. According to the ANOVA (Table 4), foliar N treatment had a great effect on grain protein, water absorption, dough development time, and resistance/extensibility ratio, while cultivar × urea interaction was significant for all quality traits (Table 4). The urea application increased protein content on average by 17.5\% (Table 4), which is consistent with previous research [2,7,8,10] that also reported consistent protein increases with foliar urea application at anthesis or flowering stage. Cultivars Ficko, Silvija, Kraljica, and OS Olimpija with grain protein increases by 22.3\%, 16.1\%, 19.5\%, and 36.2\%, respectively, in accordance with Croatian regulations, were of higher quality, which is substantial for profitable production (Table 6). Under urea application, the water absorption, on average, increased from 55.3\% to 56.7\%, and dough development time was delayed from 1.6 min to 2.9 min, while the degree of softening, dough maximum resistance, and resistance/extensibility ratio were decreased (Table 4). The changes of dough energy and dough resistance maximum as very important indicators of gluten strength were strongly cultivar-dependent and their values under urea application changed from $-12.7\%$ (Kraljica) to $+42.4\%$ (Srpanjka) and $-25.1\%$ (OS Olimpija) to $+7.7\%$ (Vulkan), respectively (Table 6). Many authors also confirm the improvement of the wheat rheological parameters under late foliar urea application, so Varga et al. [19] reported that the foliar nitrogen application at flowering improved dough water absorption and dough development time by 2.5\% and 28.4\%, respectively. Blandino et al. [30] found that the foliar N fertilizer applied at anthesis over two growing seasons at the silt loam soil increased dough strength and decreased at the sandy loam soil, while at the sandy loam soil, a significant increase in extensibility was achieved in the 2008–2009 growing season. The same authors state that the foliar N fertilizer applied at anthesis affects the farinograph water absorption and degree of softening at the silt loam soil site and dough development time at the sandy loam soil site, both in the 2009–2010 growing season. Tea et al. [31] were simultaneously applied foliar N and S at the anthesis stage of winter wheat during two growing seasons, and noted an increase in dough strength and extensibility. Rekowski et al. [32] emphasized the higher protein content, water absorption, and specific baking volume under 180 kg N ha$^{-1}$ of urea split into two applications (90 kg N ha$^{-1}$ each at tillering and stem elongation growth stage).
Table 6. Effect of the genotype x nitrogen interaction on agronomic and bread-making quality traits and protein components in wheat cultivars (means over 2017–2018).

| CULTIVARS | AG (%) | GLI (%) | GLI/GLU (%) |
|-----------|--------|---------|-------------|
| GY 1 (tha⁻¹) | TKW (g) | HL (kg hl⁻¹) | P (%) | WA (%) | DDT (min) | DS (FU) | E (cm²) | RMAX (FU) | R/EXT |
| EL NINO | 18.5 f | 19.6 h | 45.3 cd | 46.1 de | 4.4 bc | 4.7 edf | 26.2 b | 26.1 df | 14.8 bcd | 15.2 fed | 36.1 h | 34.3 def | 11.4 ef | 8.6 a | 24.7 f | 25.6 edf | 1.28 e | 1.35 fgh |
| FICKO | 23.6 c | 20.6 f | 46.3 de | 47.2 ef | 7.7 hi | 7.9 i | 23.4 bc | 24.7 def | 15.1 fed | 14.5 bc | 30.1 a | 32.2 bc | 11.5 de | 11.1 b | 18.5 a | 21.1 b | 1.55 b | 1.58 m |
| KATARINA | 19.6 h | 19.3 h | 47.2 ef | 47.2 ef | 8.3 ii | 7.1 gh | 22.4 ab | 23.3 bc | 16.5 bcd | 16.8 gh | 33.2 cde | 33.5 cde | 12.3 gh | 12.7 b | 20.9 b | 20.7 b | 1.43 h | 1.42 h |
| VULKAN | 15.7 c | 16.5 c | 40.9 a | 49.0 e | 3.8 ab | 3.5 a | 21.8 a | 26.5 ab | 15.3 fed | 19.0 e | 43.4 cde | 44.9 edf | 12.2 gh | 8.8 a | 31.2 b | 25.6 edf | 0.97 | 1.43 q |
| SILVIA | 16.7 c | 18.1 c | 46.0 cd | 47.0 ef | 4.6 cdef | 4.7 cdef | 23.7 cd | 24.1 cef | 17.7 hi | 18.1 ik | 37.3 c | 34.8 fh | 11.3 * | 10.2 a | 26.1 ef | 24.7 c | 1.24 c | 1.40 b |
| KRALJICA | 22.0 c | 21.1 c | 44.8 c | 47.3 ef | 8.0 i | 7.9 f | 24.4 cef | 24.8 b | 12.4 a | 15.5 edf | 33.1 cde | 31.6 ab | 12.0 f | 10.9 edf | 21.1 b | 20.7 b | 1.37 df | 1.50 ki |
| TAKA | 16.2 c | 17.0 c | 46.2 ab | 50.2 h | 4.6 cd | 4.8 cdef | 27.0 h | 27.3 i | 16.6 b | 17.3 h | 35.6 gh | 32.4 b | 8.9 a | 8.3 a | 26.7 f | 24.6 c | 1.36 bdf | 1.53 lm |
| TAKA | 16.2 c | 15.0 c | 42.6 b | 48.0 h | 4.5 cd | 5.1 ef | 23.2 gh | 27.2 i | 14.2 f | 15.6 edf | 39.4 h | 37.1 f | 11.4 ef | 11.5 ef | 27.9 h | 25.5 cde | 1.09 b | 1.30 edf |
| SRPANJKA | 18.3 c | 17.9 c | 44.8 c | 46.6 c | 5.0 df | 4.7 cd | 24.7 df | 25.7 f | 14.9 b | 16.1 ef | 36.9 h | 35.6 gh | 10.4 b | 10.2 a | 26.5 f | 25.4 c | 1.23 c | 1.31 def |
| OS OLIMPIJA | 13.3 a | 12.5 a | 50.5 b | 50.8 b | 4.5 cdef | 5.4 i | 28.3 c | 29.7 c | 17.7 b | 15.9 g | 36.1 d | 36.7 h | 10.1 a | 10.6 b | 26.1 df | 26.1 def | 1.41 b | 1.39 b |

GY 1—grain yield; TKW—thousand kernel weight g; HL—hectolitre weight; P—protein content; WA—water absorption; DDT—dough development time; DS—degree of softening; E—dough energy; RMAX—maximum of resistance; R/EXT—resistance/extensibility ratio. AG—albumins and globulins; T GLI—total gliadins; T GLU—total glutenins; HMW—high-molecular-weight glutenins; LMW-GS—low-molecular-weight glutenins; GLI/GLU—gladins/glutenins ratio; NCON—control without urea treatment; NUREA—urea treatment. Different letters in same rows and columns indicate significant differences between cultivars according to Tukey’s test at p < 0.05.
RP-HPLC was used to compare the protein components distribution in the grain wheat by a quantitative comparison of elution profiles. The ANOVA performed on the storage proteins composition showed a general significant effect of the Y, G, and N, and their interactions (Table 5). The influence of Y was dominant for most protein components, while G and late urea application contributed more variance to the ω- and γ-gliadins, respectively (Table 5). As we have already mentioned, the total protein content was higher in 2018 than in 2017, but in terms of protein composition, different trends were recorded. A significant increase in albumins and globulins, total gliadins, ω-gliadins, α-gliadins, γ-gliadins, and gliadins/glutenins ratio was observed in 2018 compared to 2017, while total glutenins and high-molecular-weight and low-molecular-weight glutenin subunits were decreased (Table 5).

The use of late-season urea resulted in higher total and α-and γ-gliadins, while the accumulation of albumins and globulins, total glutenins, their high-molecular-weight and low-molecular-weight components were reduced, which means that, compared to the total protein content (increased by 17.4%), the grain protein composition was significantly influenced by late urea application, but to a lesser extent (Table 5). Tea et al. [33] provided evidence that N and S fertilizers applied by foliar spraying at anthesis, simultaneously, play an important role in controlling the storage protein synthesis and the degree of polymerization, which in turn affects dough mixing properties. Xue et al. [2] noticed that the distribution of an additional N rate at a late booting stage significantly increased the relative abundance of gliadins and the x-type of high-molecular-weight glutenins. Ferrari et al. [34] stated that the higher N foliar doses of urea mainly improved the grain protein content and both high- and low-molecular-weight glutenins. In contrast, Blandino et al. [8] studied the impact of different late-season N fertilizer forms at the same rate and timing application over the course of 3 years, and found a weak effect impact of post-anthesis N acquisition on the protein composition in high protein common wheat. The significant impact of G on protein composition indicated the diversity of cultivars used in this experiment (Table 5).

It is well established that albumins and globulins, which constitute 15–20% of total grain protein, mainly have structural and metabolic functions, with a limited effect on wheat baking quality [11]. In our study, the proportion of albumins and globulins on average ranged from 12.9% (OS Olimpija) to 21.6% (Kraljica) (Table 5), which is similar to our previous findings [35]. Considering the wheat baking quality, monomeric gliadins (40–50% of total grain protein) and polymeric glutenins (30–40% of total grain protein) are the most important, and predominantly define dough elasticity and extensibility [36]. The lowest proportion of total gliadins (45.0%) had the cultivar Vulkan, while cultivar OS-Olimpija had the highest (50.7%). The average proportion of glutenins ranged from 31.2% (Ficko) to 38.9% (Vulkan) (Table 5). Even though high-molecular-weight glutenins constitute only 10% of total grain protein, their impact on wheat baking quality is very important [37], and in our study, this component varied from 8.6% (Tika-Taka) to 12.5% (Katarina) (Table 5).

The ratio of monomeric and polymeric gluten proteins (gliadins/glutenins) is considered a good indicator of dough strength/extensibility balance. A higher gliadins/glutenins ratio has been associated with impaired dough rheology properties [38,39]. In our study, the gliadins/glutenins ratio was increased by 11.0% under urea treatment (Table 5), and its variations were strongly cultivar dependent, so, in cultivar Katarina, the gliadins/glutenins ratio was decreased by 0.7%, while in cultivar Vulkan, it increased by 47% (Table 5), which is in accordance with the study of Wieser and Seilmeier [6], and Blandino et al. [8]. De Santiset al. [40] reported that the higher gluten strength observed in modern genotypes correlated with the presence of superior high-molecular-weight glutenins and low-molecular-weight glutenins alleles, the differential expression of specific storage proteins, and an increased glutenins/gliadins ratio. In our recent study [31], the old cultivars have also shown a lower proportion of high- and low-molecular-weight glutenins and higher gliadins/glutenins ratio compared to modern ones. The obtained data were analysed by principal component analysis which transforms data into linear, uncorrelated, and meaningful principal components (PCs). The first (PC1) and second factors (PC2) explain 33.6% and
27.7% of the total variation, respectively (Figure 1). The PC1 was highly positively related with gliadins/glutenins ratio and negatively with total glutenins, low-molecular-weight glutenins, and resistance/extensibility. PC2 was highly and positively connected to dough energy and α-gliadins, and negatively to albumins and globulins (Figure 1).

Figure 1. Biplot relative to the principal component analysis performed on grain yield, breadmaking quality and proteins traits (GY—grain yield; TKW—thousand kernel weight; HL—hectolitre weight; P—total grain protein; WA—water absorption; DDT—dough development time; DS—dough degree of softening; E—dough energy; RMAX—dough maximum of resistance; R/EXT—resistance/extensibility ratio. AG—albumins and globulins; GLI—total gliadins; GLU—total glutenins; HMW—high-molecular-weight glutenins; LMW-GS—low-molecular-weight glutenins; GLI/GLU—gliadins/glutenins ratio. ELN—El Nino; FIC—Ficko; KAT—Katarina; VUL—Vulkan; SIL—Silvija; KRA—Kraljica; TIK—Tika Taka; REN—Renata; SRP—Srpanjka; OLI—OS Olimpja).

Cultivars (Renata, Tika Taka, Silvija and El Nino) were clearly distinguished along the PC1 axis across control and urea treatments. On average, the urea treatment group is characterized by higher total protein and higher values of protein-related parameters (water absorption, dough development time, thousand kernel weight, total gliadins, α- and γ-gliadins, and gliadins/glutenins), while the control group is positively related to R/EXT, DS, and high-molecular-weight glutenins. Cultivars Katarina, Kraljica, and Ficko under both treatments form the second group characterized by a higher GY, AG, and ω-gliadins proportion. Cultivar OS Olimpja, as a Croatian gluten-strengthening improver, was separated from others under both treatments. This cultivar is characterized by the highest hectolitre, total protein, and excellent dough rheological properties, accompanied by the lowest albumins and globulins, the highest total and α-gliadins and well-balanced gliadins/glutenins ratio. The largest PCs distance between the two nitrogen treatments was found for the cultivar Vulkan (Figure 1).

4. Conclusions

Despite different climate conditions over two growing seasons, the late-season urea treatments notably increased total protein content and greatly improved the protein-related quality traits without considerable disruption of the storage proteins composition.
the cultivar choice is a crucial factor in achieving high processing quality, the obtained results could further contribute to the development of specific N fertilisation strategies in order to enhance the processing quality of common wheat.

**Author Contributions:** Conceptualisation, D.H. and K.D.; methodology, G.D. and K.D.; validation, M.T. and D.M.; formal analysis, D.H.; investigation, K.D. and D.H.; visualisation, D.N. and M.T.; writing—original draft preparation, D.H. and K.D.; writing—review and editing, D.M., G.D., D.N. and L.A.; Supervision, G.D. All authors have read and agreed to the published version of the manuscript.

**Funding:** This activity was supported by a program of continuous scientific work on the creation of new wheat cultivars at the Agricultural Institute Osijek.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

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