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Assessing Grain Yield and Achieving Enhanced Quality in Organic Farming: Efficiency of Winter Wheat Mixtures System

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Abstract: Organic agriculture is gaining prominence nowadays; however, the quantity and quality of organic products are still a matter of discussion, and various methods are being tested to fill these gaps. A three-year field experiment was conducted to evaluate the response of winter wheat varieties and their mixtures in grain yield and quality. Four single croppings of winter wheat cultivars and eight mixed cropping systems sown with mixed or alternative rows of two cultivars were compared. There was no significant difference between different mixtures with regard to grain yield, but the protein content (PC) was increased in the mixture for a low-quality variety, such as Vanessa. The highest wet gluten (WG) (18%) and falling number (FN) (268 s) were obtained in Butterfly-Lorien and Butterfly-Vanessa, respectively. Water absorption (WA) in Butterfly solely and all of its combinations showed the best results. The Illusion-Vanessa mixture showed the highest stability (7.19 min). The difference in the sowing method in mixtures did not influence the grain yield and grain quality, except for the dough stability. The potential for grain quality improvement elucidated in this study may apply to further research; however, one needs to consider if it depends on greater wheat variety, input materials, and their interaction, simultaneously with the expected overall benefits from this approach.

Keywords: baking quality; intercropping; wheat grain yield; sustainable agriculture

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1. Introduction

Organic agriculture is gaining prominence nowadays because it can contribute to mitigating climate change by reducing greenhouse gas emissions via enhanced carbon storage in soil. Simultaneously, as a result of improving soil nutrition, storing more water, and being less prone to erosion, organic farming is said to be more resilient to adverse climate conditions than conventional agriculture [1].

Accompanying the development of organic agriculture in the world and Europe, the Czech Republic's agricultural policy is oriented toward organic farming, which provides environmental protection, as well as social and economic advantages. The Czech Republic's certified areas for organic common wheat and spelt wheat production in 2020 were 12,774 ha and 5184 ha, respectively, of the total 39,818 ha for grain cereals [2].

Organic farming is characterised by low soluble nitrogen availability, which reduces yields and grain quality [3,4]. As abiotic stresses are limiting factors for grain yield and quality, developing techniques to cope with them has always been challenging. Increasing grain yield and stability and improving the quality of winter wheat varieties are priorities in organic wheat production. Wheat grain quality depends not only on nutritional and meteorological conditions, but also on the genetic performance of varieties [3]. In different environmental conditions, it is necessary to cultivate different varieties; however, efforts in breeding and variety selection may improve grain yield and baking quality to only a certain extent. On the other hand, breeding improvements have been relatively slow integrating high grain yield and high grain protein due to their inverse relationship [5], which simultaneously take a long time and are expensive [4]. The key to fixing this issue is to draw attention to suitable farming methods, such as cropping with variety mixtures. Grain quality characteristics have been evaluated in wheat cultivar mixtures, which show promising, but inconsistent results in enhancing these characteristics.

Cultivar mixes contain cultivars that differ in a variety of ways, yet have enough traits to be cultivated together [6]. Each wheat variety has susceptibilities to several stresses that can cause fluctuations in the yield. Combining varieties with complementary traits can help yield stability where stresses occur unpredictably in any environment [7]. The superiority of cultivar blendings over pure-line cultivars has been observed in numerous crops by choosing cultivars that complement each other to derive important traits that meet specific production requirements [7]. The main advantages reported for mixtures are enhanced yield and yield stability [8–14]; better control of pests, diseases, and weeds [9,15–17]; reduced pathogen spread [18]; and obtaining an end product with desirable quality [19].

Protein content, gluten, flour yield, falling number, and test weight are attributes of varieties that have high baking quality [20]. One of the greatest ways to determine if flour samples will produce high-quality cakes has been proven to be test baking. The test takes a long time; thus, different approaches are required to determine the acceptability of flours for cakes in a shorter time. The quality of the completed goods is affected by the behaviour of the dough during mechanical handling; therefore, understanding the rheological qualities of dough is crucial for the effective production of a variety of bakery items. Information on the pasting capabilities of starch and the rheological characteristics of batter made from wheat flour might be valuable in determining the quality of items made from soft wheat. In order to examine and/or create alternative techniques for the prediction of baking quality, these qualities should be studied [21]. Mixolab (CHOPIN Technologies Mixolab 2, Villeneuve-la-Garenne, France) has the capability to measure physical dough properties, such as dough strength and stability, and also to measure the effect of the pasting properties of starch on the actual dough in real time as the torque produced by mixing the dough between two kneading arms during mixing and temperature constraint [21,22]. Thus, this is an alternate method that is efficient for testing the rheological properties of flour in baking production.

Studies on the mixed cultivation of the winter wheat varieties result in reduced to increased grain yield [8] and better control of weeds [16], pests, and disease [15,23]; however, there has not yet been much study of the stabilising grain yield and quality, especially in the study by Mixolab evaluating rheological properties, as well as the correlation quality and Mixolab parameters in organic farming. This study aims to test the above hypothesis. Three growing season experiments were conducted to examine a combination of winter wheat varieties to evaluate the wheat yield stability and grain quality in organic farming.

2. Materials and Methods

2.1. Experimental Site and Weather Conditions

Field experiments were conducted at a farmer's organic certified field (according to EU law, regularly monitored by KEZ, o.p.s. controlling organization) in Ceske Budejovice, in the southern region of the Czech Republic (48°58′26.4′′ N, 14°37′43.5′′ E), for the 3 consecutive growing seasons in 2019–2022. The conventional season of wheat cultivation starts from October to August of the next year. The soil in the experimental field was typical loamy soil (Orthic Luvisol, Loamy soil (medium)). The average air temperature in the period of our research was higher compared to the multi-year period (growing season 2019/20, 9.5 °C; growing season 2020/21, 8.4 °C; growing season 2021/22, 9.3 °C; and the multi-year period, 7.2 °C; Figure 1). The drought conditions in the 21 June 2020 growing season were higher than those in the 2019/20 and 2021/22 growing seasons. The total rainfall was 699 mm in the growing season 2019/20, 633 in the growing season 2020/21, 607 mm in 2021/22, and 633 mm in the multi-year period (Figure 1).

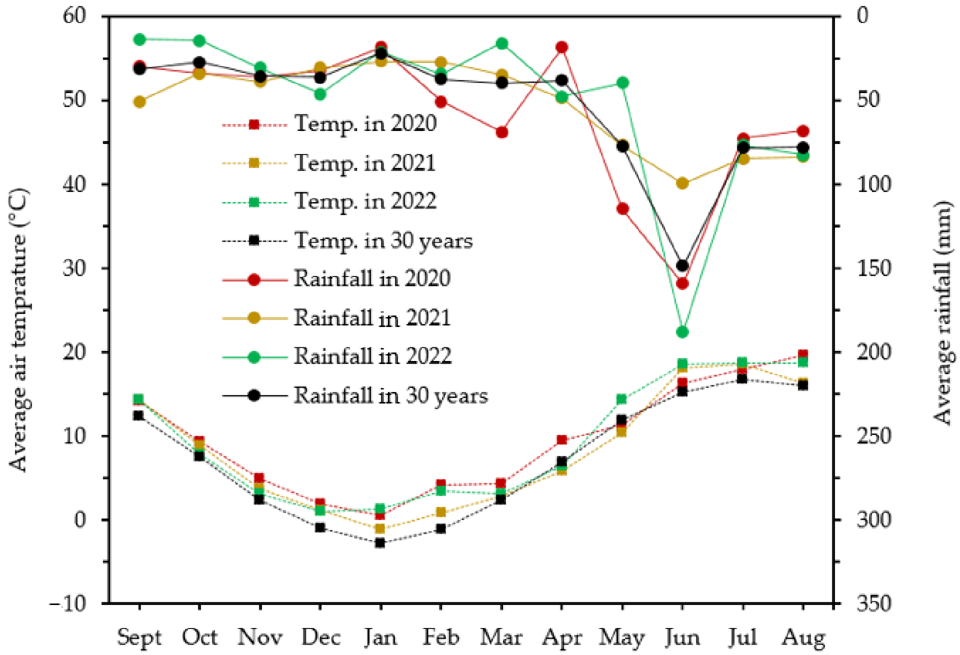

Figure 1. Average air temperature and precipitation, monthly, across 3 growing seasons and 30 years at the experiment location.

2.2. Wheat Cultivars

Four winter wheat varieties were used, which included the following. (1) Butterfly: medium grain yield, but highlighted with excellent quality (group A); medium tall to tall plants, with good resistance to lodging; good frost resistance and winter hardiness; good resistance to Fusarium head blight (FHB) and Septoria tritici blotch (STB) (*Mycosphaerella graminicola*); high resistance to brown rust (*Puccinia triticina)*; suitable for sowing after maize and cereals; excellent results in organic farming. (2) Illusion: stable yields and good baking parameters (group A: high protein, high specific weight, stable falling number), very good resistance to leaf diseases, increased resistance to stem base diseases (PCH1 gene), good management of nitrogen fertilization, very good stem rust resistance. (3) Lorien: suitable for dry and warm regions, with baking quality in group B (medium protein content, medium-specific weight); medium tall to tall plants, with good lodging resistance; medium to lower frost resistance; medium tillering capacity; good resistance to FHB. (4) Vanessa: high and stable yield, but lower in baking quality (group C: soft grain, low protein content); high resistance to triple rust (*Puccinia striiformis* f. sp. *tritici)*; high frost resistance and winter hardiness; short plants with good lodging resistance; successfully used in biscuit flour [24].

2.3. Experimental Design and Wheat Cultivation

The experiments examined the effects of the winter wheat variety on the stability of the wheat yield and the improvement of grain quality. Field trials were performed under organic farming using red clover as a preceding crop for the growing seasons. The soil was fertilized with composted sheep manure of 4 t ha^{-1} (8.9 kg N t^{-1}, 5.4 kg P_2O_5 t^{-1}, 17.7 kg K_2O t^{-1}) before ploughing. The sowing dates of winter wheat were 7 October, 8 October, and 5 October, and the harvest dates were 10 August, 10 August, and 3 August for the 2019/20, 2020/21, and 2021/22 growing seasons, respectively.

The small-plot experiment was arranged with the randomized complete-block experiment design, with three replicates. Every plot had a size of 15 m^2, without the use of pesticides and herbicides. Four single croppings of winter wheat cultivars and eight mixed croppings systems sown with mixed or alternative rows of two cultivars having 12.5 cm row spacing were applied (Table 1). In mixed intercropping, the winter wheat seeds were mixed before sowing. Alternate sowing of two winter wheat varieties in each separate row was used for row intercropping. Winter wheat was sown at a rate of 400 seeds m^{-2}.

Table 1. Winter wheat cultivars and their mixtures evaluated during 2019–2022 in organic management.

	Cultivars	**Seed Ratio**	**Sowing Method**	**Abbreviation**
1	Butterfly	Single	Control	Bu
2	Illusion	Single	Control	Illu
3	Lorien	Single	Control	Lo
4	Vanessa	Single	Control	Va
5	Butterfly+Lorien	1:1	Mixed	Bu+Lo-Mi
6	Butterfly+Vanessa	1:1	Mixed	Bu+Va-Mi
7	Illusion+Lorien	1:1	Mixed	Illu+Lo-Mi
8	Illusion+Vanessa	1:1	Mixed	Illu+Va-Mi
9	Butterfly+Lorien	1:1	Row-Row	Bu+Lo-Ro
10	Butterfly+Vanessa	1:1	Row-Row	Bu+Va-Ro
11	Illusion+Lorien	1:1	Row-Row	Illu+Lo-Ro
12	Illusion+Vanessa	1:1	Row-Row	Illu+Va-Ro

2.4. Plant Measurements and Quality Evaluations

The plant height and spike number were determined before harvest. After the plot harvest was grain cleaned and the mean samples were taken from each replication, the wheat grain yield was recorded and calculated at 14% moisture content. A thousand kernel weight (TKW) was also determined, and the hectoliter weight (HW) (kg hL^{-1}) was measured using the Dickey-john GAC500XT.

The PSY 20 (Mezos, Hradec Kralove, Czech Republic) and Quadrumat Junior machines (Brabender, Duisburg, Germany) were used to mill the wheat flour samples (1 kg). The Kjeldahl technique (Kjeltec 1002 System, Tecator AB, Hoganas, Sweden), based on N $*$ 5.7, was used to calculate the protein content (PC) (in dry matter). According to ICC Standard No. 137/1, wet gluten (WG) and the gluten index (GI) were tested using the Glutomatic 2200 and Centrifuge 2015 (Perten Instruments, Hägersten, Sweden). According to ICC standard No. 107/1 and AACC International method 56-81B, the falling number (FN) was measured on FN 1100 (Perten Inst., Sweden).

Mixolab was used to assess the rheological properties of wheat flour, including the consistency of the dough during mixing and the quality of the protein and starch, as well as the impact of enzymes, in accordance with the ICC standard method No. 173-ICC 2006. Amplitude is the measurement for the elasticity of the dough; the higher the value, the more elastic the flour. Stability is the measurement for the resistance to dough kneading; the longer the duration, the stronger the flour. Additional measurements were used as follows: C1—dough development; Torque C2—attenuation of protein due to mechanical work and temperature; Torque C3—gelatinization of starch; Torque C4—stability of hot gel; Torque C5—measured retrogradation of starch in the cooling phase; Slope α—attenuating rate of protein in warming; Slope β—starch gelatinization rate; Slope γ—enzymatic degradation rate [22].

2.5. Statistical Analysis

Statistical analyses were processed with the STATISTICA program (version 13.2, Stat-Soft, Inc., Tulsa, OK, USA). The data were statistically evaluated by an analysis of variance (ANOVA), and the results were subsequently tested for differences in means values by a Tukey's HSD (Honest Significant Difference) test. The principal component analysis (PCA) was applied for the multivariate statistical analysis of measured data between (1) grain yield and growth and grain components, and (2) grain yield and baking quality and grain quality evaluation by Mixolab.

3. Results

3.1. Wheat Grain Yield and Quality Parameters

Detailed results of the ANOVA analysis and mean comparison of wheat yield and grain quality affected by growing seasons, sowing methods (SM), and combination are presented in Table 2. Grain yield and yield components were significantly affected by the growing season. In general, the trend of season performance was 2019/20 > 2021/22 > 2020/21 for plant height, TKW, and the spike number. The HW and grain yield were lower in the 2020/21 growing season than in the 2019/20 and 2020/21 growing seasons. Grain quality, PC, and GI showed significantly higher values in the 2020/21 growing season, and WG ranged from 15.89 to 17.15% and had no effect in terms of the growing season. The falling number value was significantly higher in the 2019/20 growing season. Detailed results for individual years are presented as supplementary material in Table S1.

Table 2. Growth, yield, and quality of winter wheat under the effect of the harvest year, sowing method, and combination.

Variable	Plant Height (cm)	Spike Number (No. m^{-2})	TKW (g)	Yield (t ha^{-1})	HW (kg hL^{-1})	Protein Content (%)	Wet Gluten (%)	Gluten Index (%)	Falling Number (s)
Growing season									
2019/20	92.06 ^{a}	414.11 ^{a}	50.38 ^{a}	5.75 ^{a}	72.26 ^{a}	9.22 ^{ab}	15.93	70.30 ^{b}	247.92 ^{a}
2020/21	67.57 ^{c}	290.28 ^{c}	41.34 ^{c}	2.52 ^{b}	67.90 ^{b}	9.49 ^{a}	15.89	88.73 ^{a}	240.31 ^{ab}
2021/22	84.72 ^{b}	354.27 ^{b}	43.30 ^{b}	5.69 ^{a}	72.95 ^{a}	9.03 ^{b}	17.15	74.05 ^{b}	227.53 ^{b}
p-Value	***	***	***	***	***	**	ns	***	**
Sowing method									
Control	80.84	330.83 ^{b}	45.62	4.84	71.30	9.15	16.22	79.77 ^{a}	242.14
Mixed	82.15	344.14 ^{b}	45.10	4.70	70.97	9.28	16.47	73.11 ^{b}	231.67
Row-Row	81.35	383.69 ^{a}	44.30	4.43	70.85	9.30	16.27	80.20 ^{a}	241.94
p-Value	ns	**	ns	ns	ns	ns	ns	**	ns
Combination									
Butterfly	82.54	304.47	45.89	4.37	71.79	9.60 ^{a}	18.12 ^{a}	81.15 ^{ab}	258.33 ^{a}
Illusion	79.87	320.63	45.97	4.94	73.92	9.47 ^{a}	17.86 ^{a}	72.27 ^{ab}	215.44 ^{c}
Lorien	86.57	311.84	48.61	4.78	69.99	9.22 ^{a}	16.65 ^{ab}	75.70 ^{ab}	239.67 ^{abc}
Vanessa	74.39	386.38	41.99	5.26	69.49	8.31 ^{b}	12.26 ^{b}	89.94 ^{a}	255.11 ^{ab}
Bu+Lo-Mi	86.00	282.02	47.12	4.45	71.29	9.48 ^{a}	18.03 ^{a}	68.02 ^{ab}	239.44 ^{abc}
Bu+Va-Mi	80.91	387.29	43.60	4.96	70.57	9.24 ^{a}	14.97 ^{ab}	75.88 ^{ab}	254.89 ^{ab}
Bu+Lo-Ro	85.78	345.82	46.03	3.85	70.73	9.52 ^{a}	15.93 ^{ab}	82.27 ^{b}	245.78 ^{abc}
Bu+Va-Ro	79.01	414.38	43.16	4.68	70.23	9.28 ^{a}	15.06 ^{ab}	85.09 ^{ab}	267.67 ^{a}
Illu+Lo-Mi	85.12	336.41	46.15	4.44	71.17	9.40 ^{a}	16.43 ^{ab}	75.16 ^{ab}	220.89 ^{bc}
Illu+Va-Mi	76.58	370.84	43.52	4.94	70.84	9.00 ^{ab}	16.46 ^{ab}	73.37 ^{ab}	211.44 ^{c}
Illu+Lo-Ro	84.59	356.77	45.08	4.30	71.06	9.22 ^{a}	17.57 ^{a}	75.47 ^{ab}	219.22 ^{c}
Illu+Va-Ro	76.01	417.77	42.94	4.88	71.38	9.18 ^{a}	16.51 ^{a}	77.97 ^{ab}	235.11 ^{abc}
p-Value	ns	ns	ns	ns	ns	***	**	*	***

TKW, thousand kernel weight; HW, hectoliter weight. Different letters within the column show a statistical difference at *p*-Value < 0.05, Tukey HSD test. ns (non-significant); $p \geq 0.1$; $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

SM was not significantly affected by wheat yield and grain quality, except for the spike number and GI. The spike number in Ro was higher than in the control and Mi. GI was significantly lower in Mi than in the control and Ro. Grain yield, PC, WG, and FN ranged from 4.43 to 4.84 t ha^{-1}, 9.15 to 9.30%, 16%, and 231.67 to 242.41 s, respectively.

In the combination of winter wheat varieties, as the same SM, the wheat yield and yield components were not significantly affected; however, the grain quality parameters were significantly affected. There was a lower PC in Vanessa sowing alone, but there were improvements in the combination (except Illu+Va-Mi). WG was lower in Vanessa than in the

Butterfly and Illusion varieties, and the combination of Illu+Va-Ro increased WG compared to individual Vanessa sown. GI in each individual variety did not significantly differ with their combination, and only Bu+Lo-Ro was lower than Vanessa. Illusion (215.44 s) and its combination (211.44, 220.89, and 219.22 s for Illu+Va-Mi, Illu+Lo-Mi, and Illu+Lo-Ro, respectively) were lower than in Butterfly (258.33 s) and Bu+Va combination both Mi (254.89 s) and Ro (267.67 s) in the FN parameter.

In the multivariate analysis of the grain yield and yield components, the grain yield was positively correlated with all yield parameters (r = 0.736, 0.676, 0.526, and 0.616 for plant height, the spike number, TKW, and HW, respectively) (Table 3 and Figure 2a). Grain yield was negatively correlated with baking quality parameters, except WG (r = 0.02) (Table 3 and Figure 2b). PC was positively correlated with WG, but negatively with falling number, and GI was negatively correlated with WG.

Table 3. Correlation between grain yield and yield components of winter wheat.

Variable	Plant Height (cm)	Spike Number (No. m^{-2})	Thousand Kernel Weight (g)	Hectoliter Weight (kg hL^{-1})	Yield (t ha^{-1})
Plant height	1	0.517	0.740	0.604	0.736
Number of spikes		1	0.337	0.304	0.676
Thousand kernel weight			1	0.362	0.526
Hectoliter weight				1	0.616

Correlations are significant at $p < 0.05$.

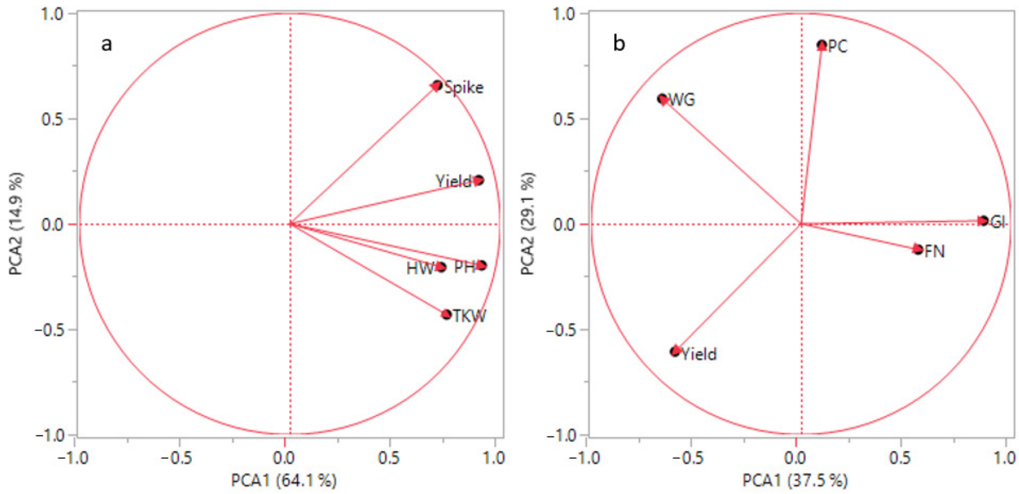

Figure 2. Principal component analysis (PCA) based upon various (**a**) grain yield and grain components and (**b**) grain yield with grain quality of wheat under the effect of winter wheat variety mixtures. PH, plant height; Spike, spike number; TKW, thousand kernel weight; HW, hectoliter weight; PC, protein content; WG, wet gluten; GI, gluten index; FN, falling number.

3.2. Mixolab Analysis

Mixolab advantages include the ability to measure cereal flour characteristics in one test as proteins, starch, and associated enzymes. Based on the results, approximately all of the Mixolab properties were affected by the growing seasons, except WA, time of C1, and amplitude (Table 4). WA, Time C1, and amplitude were in the range of 61.36–61.42%, 2.22–2.42 min, and 0.073–0.074, respectively. TC2 and TC5 were highest in the 2020/21 growing season, followed by the 2019/20 and 2020/21 growing seasons. TC3, TC4, and slope β were higher in the 2019/20 and 2020/21 growing seasons compared to the 2021/22 growing season. In contrast, slope α was higher in the 2021/22 growing season compared to the two other growing seasons. Slope γ was higher in 2019/20 than that in the 2020/21 and 2021/22 growing seasons. Greater stability was indicated in the 2020/21 growing season compared to the 2019/20 and 2021/22 growing seasons by 1.26 and 1.68 min, respectively. Detailed results for individual years are presented as supplementary material in Table S2.

Table 4. Rheological properties of dough by Mixolab of winter wheat under the effect of the harvest year, sowing method, and combination.

Variable	WA (%)	TimeC1 (min)	TC2 (Nm)	TC3 (Nm)	TC4 (Nm)	TC5 (Nm)	α	β	γ	Amp.	Stability (min)
Growing season											
2019/20	61.42	2.22	0.37 ^{ab}	1.67 ^{a}	0.91 ^{a}	1.57 ^{ab}	−0.081 ^{b}	0.513 ^{a}	−0.106 ^{b}	0.076	5.38 ^{b}
2020/21	61.36	2.25	0.38 ^{a}	1.63 ^{a}	0.94 ^{a}	1.72 ^{a}	−0.084 ^{b}	0.535 ^{a}	−0.091 ^{a}	0.074	6.64 ^{a}
2021/22	61.40	2.42	0.36 ^{b}	1.48 ^{b}	0.81 ^{b}	1.45 ^{b}	−0.075 ^{a}	0.429 ^{b}	−0.080 ^{a}	0.073	4.96 ^{b}
p-Value	ns	ns	**	***	**	**	***	***	***	ns	***
Sowing method											
Control	61.55	2.158	0.371	1.594	0.916	1.590	−0.080	0.492	−0.091	0.079	5.19 ^{b}
Mixed	61.25	2.305	0.363	1.584	0.850	1.525	−0.080	0.497	−0.095	0.071	5.74 ^{ab}
Row-Row	61.38	2.434	0.379	1.597	0.890	1.622	−0.080	0.487	−0.092	0.072	6.06 ^{a}
p-Value	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**
Combination											
Butterfly	66.49 ^{a}	2.15 ^{bcd}	0.38	1.38 ^{d}	0.70 ^{c}	1.13 ^{d}	−0.077 ^{ab}	0.405 ^{c}	−0.099	0.066 ^{cde}	5.77 ^{ab}
Illusion	60.61 ^{c}	1.62 ^{d}	0.38	1.57 ^{bc}	0.81 ^{ab}	1.45 ^{bcd}	−0.091 ^{c}	0.438 ^{bc}	−0.106	0.097 ^{a}	5.23 ^{b}
Lorien	59.81 ^{c}	1.92 ^{cd}	0.35	1.63 ^{abc}	0.96 ^{b}	1.70 ^{abc}	−0.081 ^{abc}	0.530 ^{abc}	−0.091	0.090 ^{ab}	4.66 ^{b}
Vanessa	59.29 ^{c}	2.95 ^{abc}	0.38	1.80 ^{a}	1.20 ^{a}	2.08 ^{a}	−0.070 ^{a}	0.595 ^{a}	−0.069	0.066 ^{cde}	5.09 ^{b}
Bu+Lo-Mi	62.64 ^{b}	2.14 ^{bcd}	0.36	1.50 ^{cd}	0.77 ^{ab}	1.35 ^{cd}	−0.080 ^{abc}	0.436 ^{bc}	−0.094	0.071 ^{bcde}	6.06 ^{ab}
Bu+Va-Mi	62.52 ^{b}	3.05 ^{ab}	0.37	1.63 ^{abc}	0.92 ^{b}	1.59 ^{bc}	−0.072 ^{a}	0.547 ^{ab}	−0.085	0.054 ^{e}	5.20 ^{b}
Bu+Lo-Ro	62.58 ^{b}	2.26 ^{bcd}	0.36	1.50 ^{cd}	0.81 ^{ab}	1.47 ^{bcd}	−0.075 ^{ab}	0.451 ^{bc}	−0.088	0.069 ^{cde}	5.74 ^{ab}
Bu+Va-Ro	62.67 ^{b}	3.51 ^{a}	0.40	1.61 ^{bc}	0.94 ^{b}	1.67 ^{bc}	−0.070 ^{a}	0.480 ^{abc}	−0.085	0.060 ^{de}	6.27 ^{ab}
Illu+Lo-Mi	59.91 ^{c}	1.93 ^{cd}	0.35	1.57 ^{bc}	0.85 ^{ab}	1.59 ^{bc}	−0.087 ^{bc}	0.471 ^{abc}	−0.094	0.083 ^{abc}	5.33 ^{b}
Illu+Va-Mi	59.93 ^{c}	2.10 ^{bcd}	0.36	1.64 ^{abc}	0.86 ^{ab}	1.57 ^{bc}	−0.079 ^{abc}	0.535 ^{abc}	−0.106	0.073 ^{bcde}	6.37 ^{ab}
Illu+Lo-Ro	60.04 ^{c}	1.88 ^{cd}	0.37	1.59 ^{bc}	0.86 ^{ab}	1.59 ^{bc}	−0.090 ^{c}	0.460 ^{bc}	−0.095	0.081 ^{abc}	5.04 ^{b}
Illu+Va-Ro	60.22 ^{c}	2.09 ^{bcd}	0.39	1.69 ^{ab}	0.95 ^{b}	1.76 ^{ab}	−0.082 ^{abc}	0.556 ^{ab}	−0.098	0.077 ^{bcd}	7.19 ^{a}
p-Value	***	***	ns	***	***	***	***	***	ns	***	***

WA, water absorption; TimeC1, time development of C1; TC2, torque C2; TC3, torque C3; TC4, torque C4; TC5, torque C5; Amp., amplitude. Different letters within the column show a statistical difference at *p*-Value < 0.05, Tukey HSD test. ns, non-significant; ** $p < 0.01$; *** $p < 0.001$.

Rheological properties evaluations by Mixolab were not significantly affected by SM, except stability. Stability showed higher significance in row–row (6.06 min) than that in mixed (5.74 min) and the control (5.19 min).

The combination significantly affected the rheological properties (except TC2 and slope γ); TC2 ranged from 0.35 to 0.40 Nm, and slope γ between −0.106 and −0.069. Butterfly and its mixtures were a higher WA than sole Lorien and Vanessa cultivars. Illusion and its combinations did not increase WA. TimeC1 was higher in Bu+Va-Ro and stability greater in Illu+Va-Ro compared to single wheat (except Vanessa for TimeC1 and Butterfly for stability). Vanessa was the highest TC3, TC4, TC5, slope α, and slope β. Only TC3 was lower in Bu+Va-Ro than that in the sole Vanessa cultivar. Butterfly and Illusion mixed with Vanessa significantly decreased TC4 and TC5, while their mixtures did not affect slope α and slope β compared to the sole Vanessa cultivar.

Grain yield was negatively correlated with TC2 and stability. PC was positively correlated with TC2 and WA, and negatively correlated with TC3, TC4, TC5, and slope alfa. At the same time, WG was positively correlated with WA and negatively correlated with TC3, TC4, TC5, slope alfa, and slope beta. On the contrary, GI was positive with TC2, TC4, TC5, beta, gamma, and stability. FN was positive with all rheological properties evaluated by Mixolab, except amplitude (Table 5 and Figure 3).

Table 5. Correlation coefficients between grain yield and quality indicators of winter wheat.

Variable	Yield ($t\ ha^{-1}$)	Protein Content (%)	Wet Gluten (%)	Gluten Index (%)	Falling Number (s)
Yield	1.00	−0.35 ***	0.02 ns	−0.47 ***	−0.12 ns
Water absorption	−0.07 ns	0.35 ***	0.23 *	−0.02 ns	0.28 **
Time of C1	0.02 ns	0.04 ns	−0.14 ns	0.10 ns	0.35 ***
Torque C2	−0.27 **	0.14 ns	0.01 ns	0.34 ***	0.52 ***
Torque C3	−0.08 ns	−0.20 *	−0.40 ***	0.21 ns	0.35 ***
Torque C4	−0.13 ns	−0.26 **	−0.45 ***	0.36 ***	0.57 ***
Torque C5	−0.19 *	−0.23 ns	−0.43 ***	0.39 ***	0.43 ***
A	0.26 **	−0.28 **	−0.21 *	0.09 ns	0.36 ***
B	−0.24 *	0.02 ns	−0.32 ***	0.26 **	0.34 ***
Γ	−0.05 ns	−0.17 ns	−0.19 ns	0.20 *	0.01 ns
Amplitude	−0.01 ns	−0.01 ns	0.15 ns	−0.16 ns	−0.35 ***
Stability (min)	−0.49 ***	0.32 ***	−0.04 ns	0.43 ***	0.25 **

ns (non-significant); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

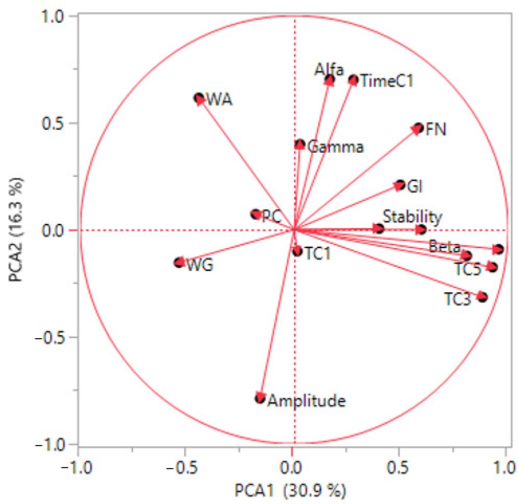

Figure 3. Principal component analysis (PCA) based upon various grain quality and Mixolab parameters of wheat under the effect of winter wheat variety mixtures. PC, protein content; WG, wet gluten; GI, gluten index; FN, falling number; WA: water absorption; TimeC1, time of C1; TC2, Torque C2; TC3, torque C3; TC4, torque C4; TC5, torque C5.

4. Discussion

Although the genetic properties of cultivars govern wheat grain yield, the role of each of these components in determining grain yield might vary depending on the growth conditions under the various conditions and agronomic factors [25]. Konvalina et al. (2009) [20] reported that differences in variety and growing conditions influenced wheat yield. In agreement with their findings, the effect of the growing season influenced grain yield; however, the difference in cultivars and their mixtures did not significantly affect grain yield in this study (Table 2). Harasim et al. (2016) [26] reported that the yield components are the factors that influence winter wheat crop production. The number of spikes per unit area and TKW are often regarded as critical yield components [27,28], and the increase of the spike number increased the grain yield of wheat [29]. Many authors, however, found that increasing the number of spikes per unit area did not result in increased yield [30] and that a reduction of one yield component can be compensated for by a more favourable effect of another trait, resulting in a minimal change in grain yield [31]. A substantial year effect was observed for the yield and yield components in this study. The plant height, spike number, TKW, and yield were considerably higher in the 2019/20 growing season than those in the other seasons (Table 2). The lowest spike number in the 2020/21 growing season (Table 2) was observed because of the lower number of seeds germinated. Heavy rain in the period before and after sowing affected the erosion of the topsoil, reducing nutrients in the soil and affecting the germination of seeds (low seed germination rate). In addition, the lower prolonged temperatures during January and February had a great influence on soil nutrients (nitrogen), which greatly affected growth (slower plant growth, a smaller number of spikes per m^2 and TKW) and seed yield formation (lower grain yield). A positive correlation was found between yield components and grain yield (Table 3 and Figure 2a), which indicated that the yield components significantly affected the grain yield, greater plant height, and spike number, and TKW increased the grain yield. On the other hand, the drought that began in spring greatly reduced the number of grains per spike because that drought during flowering led to poor seed setting and, consequently, a lower grain number per spike [30].

Weather factors impact not just growth, development, and grain yield, but also grain quality. Some previous studies suggested that the growth conditions might have a considerable influence on gluten composition, quality, and total kernel protein composition. These consequences are related to the effect of high temperatures on lowering the time of dry matter buildup, shortening the grain-filling stage, and, ultimately, reducing the kernel weight [32]. A higher temperature was seasonal in the 2019/20 and 2021/22 growing seasons compared to that in the 2020/21 growing seasons (Figure 1). Showing similar results with previous studies indicating a negative correlation between yield and grain quality [3], our results reveal that the grain yield was higher, but PC was lower (Table 2 and Figure 2b). A negative correlation was also found between the grain yield and PC and GI; the grain yield and GI was a low correlation and there was no significant difference ($r = 0.02$, $p > 0.05$) (Table 5). The grain yield was lowest and TKW lower, but the grain quality, such as PC, was highest in the 2020/21 growing season compared to the 2019/20 and 2021/22 growing seasons (Table 2). There was no effect of the growing season on WG; however, GI was higher in the 2020/21 growing season, followed by the 2021/22 and 2019/20 growing seasons (Table 2).

Applying cultivar mixtures appear as an affordable strategy for farmers, as they tend to buffer the impact of fluctuating environmental conditions on crop performance. Combinations were created to guarantee complementarity and synergy between component cultivars. Wheat combinations should be able to generate outcomes equivalent to or better than the finest available sole varieties. Wheat cultivar mixes have been extensively researched for their potential to reduce the impact of airborne disease outbreaks. In field studies under several situations, cultivar mixes outperformed single cultivar stands [33,34]. Moreover, combinations have been demonstrated to stabilize yield over time [35,36]. In fact, however, wheat cultivar mixture experiments did not always demonstrate a positive mixture effect, in some experiments, only a few of the tested mixtures were successful [37], and a few were unsuccessful simultaneously for yield, grain quality and coping to disease reduction as study of Dai et al. (2012) [38]. In this study, the mixtures of winter wheat variety did not negatively affect grain yield. Consistent with previous studies, in some cases, the mixtures improved grain protein content and bread-making quality [34]. This is reconfirmed in this study, e.g., Vanessa has a low baking quality but itself mixtures improved in this study (Table 2, 10.6% and 8.5% PC was higher in mixtures Bu+Va and Illu+Va than Vanessa grown alone).

The mixture of varieties helps to increase biodiversity and reduce pests and diseases. However, our findings show that mixtures of wheat varieties are not different compared to pure varieties in pests and diseases. The incidence of common wheat diseases was low. It was instead a weak incidence of powdery mildew (*Blumeria graminis*). We also noticed a weak occurrence of non-specific spotting in the stands (*Stagonospora nodorum*, *Septoria tritici*, *Drechslera tritici-repentis*). The attack of rust on plants was sporadic (*Puccinia recondita* and *striiformis*), and it was instead an exceptional occurrence of isolated clusters of rust on wheat leaves. In several plots, cockroach feeding (*Oulema melanopus*) was observed on wheat leaves. The level of disease and pest infestation was such that it did not cause an economically significant reduction in yield.

The assessment of the rheological parameters of wheat flour dough during mechanical handling is critical because hence affects the effective manufacture of the bakery and the quality of the finished products. There was a significantly affected in almost all of the rheological properties evaluated by Mixolab in this site under the effect of growing season and combination (Table 4). Similarly to baking quality, mixtures of winter wheat variety improved rheological properties (higher in the value of WA, stability, TC2 while lower the value of TC3, TC4, TC5 and amplitude in mixture compared to a single variety, especially Vanessa), this also indicated by a high correlation between baking quality and rheological properties (Table 5, Figure 3).

Correlation coefficients among grain yield, grain quality, and rheological parameters analysed by Mixolab under study were determined (Table 5). Hoang et al. (2022) [39] reported that grain yield was a high positively correlated with rheological quality evaluated by Mixolab, however, grain yield was a negative correlation with Mixolab properties in our results.

The dough mixing parameters such as stability and WA can be assessed in the first stage of the Mixolab test, an increase in the torque is observed until a maximum is reached and the dough can resist the deformation for some time, the higher the index, the more the quality of the flour. The value of stability normally ranges from 4.96 to 11.42 min. Table 4 shows the stability assessed by Mixolab for winter wheat varieties and mixtures in between optimal values. This shows that different cultivation conditions affected the resistance to dough kneading of wheat flour. In this study, under the effect of the sowing method, row–row was higher stable than the control and mixed as well as mixture slightly improved stability. TC2 (protein weakening) and slope alpha (protein network weakening speed) metrics were acquired during the second phase. The TC2 value of strong wheat flour is more than 0.4 Nm. If this value is between 0.5 and 0.6 Nm, it implies better protein quality, greater gluten resistance to heating, and a stronger gluten network [40]. In this study, the value of TC2 ($\leq$4) was lower than the optimal value for baking quality. Although there was no significant difference in correlation between protein and gluten quality with TC2 in our study, a highly positive correlation was found between PC with WA and stability. This indicates the potential in the mixtures of cultivars. This is also evident in the next steps, the starch gelatinization depicted in the three-stage when the temperature rises, starch granules absorb water, and amylose molecules leak out, resulting in an increase in viscosity. The amylolytic activity revealed in the fourth stage reduces the consistency. The degree of decline is determined by amylase activity; the greater the index, the lower the amylase activity. At the fifth stage, the temperature reduction produces an increase in consistency due to gel formation; a greater TC5 value indicates a higher amount of starch retrogradation; and it appears able to assess the texture of the cakes. PC and WG were a negative correlation with TC3, TC4, and TC5 (Table 5), this is in agreement with the previous study by Hoang et al. (2022) [39]. The parameters of baking quality and rheological properties are lower than the optimal value for baking but the mixtures of cultivars have a potential method to improve grain quality in organic farming. Grain yield was not increased in the mixtures compared to a single growing, expect better quality as one positive effect. Increasing the number of varieties in each ingredient, also add more material like organic fertilizer, and intercropping with leguminous can be considered for a better overall effect.

5. Conclusions

In order to evaluate the effect of the use of the mixed cultivation of varieties on the yield and grain quality of wheat, this research study was carried out over three years. The findings showed that the grain yield was significantly different among the growing seasons. The grain quality increased in a few variants, such as Butterfly+Vanessa and Illusion+Vanessa, increased the protein content to 10.6% and 8.5%, and was higher than the Vanessa variety sown singly. In our study, applying combinations of winter wheat varieties did guarantee stable grain production. It increased baking quality and rheological properties measured by Mixolab in a few mixtures, such as Butterfly, and improved rheological properties in its mixtures compared to Lorien and Vanessa grown singly. The Illusion variety did not increase the rheological properties of the mixtures. The positive effect of growing a more profitable variety (but with lower quality) and a less good variety (with higher quality) was thus demonstrated when a certain averaging occurred. The yield stabilized, but was lower, while the bakery quality improved. To achieve maximum efficiency in mixture cultivation, it is necessary to consider different wheat varieties, input materials, their interaction, and the overall benefits of this approach in future studies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13050937/s1, Table S1: Growth, yield, and quality of winter wheat under the effect of the harvest year, sowing method, and combination; Table S2: Rheological properties of dough by Mixolab of winter wheat under the effect of the harvest year, sowing method, and combination.

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