

YIELDS AND QUALITY OF SPRING WHEAT GRAIN IN INTERCROPPING SYSTEM WITH DIFFERENT LEGUME SPECIES

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Mühlbachová, G., Hlásná Čepková, P., Capouchová, I., Konvalina, P., Vavera, R., and Janovská, D. (2022). Yields and quality of spring wheat grain in intercropping system with different legume species. *Agriculture (Poľnohospodárstvo)*, 68(4), 176–190.

Intercropping is a technique in which two or more crops are grown together that is reported to be soil and water friendly. Field trials were conducted at three different localities in the Czech Republic. Spring wheat was grown together with different legumes (Egyptian clover, crimson clover, red clover, white clover, common pea, dun pea, common vetch, bird's-foot-trefoil, common kidney vetch, and fenugreek) in an organic cropping system. Two basic systems were compared mixture and row-by-row cropping. The yield of grain was lower on average in row-by-row cropping than in the mixture. Lower yields were found in Prague (PR) compared to Uhřetěves (UH) and Zvíkov (ZV). The mixture system seemed to be more effective in grain production than cultivation in separate rows (PR 2.14 t/ha, UH 4.71 t/ha, ZV 3.00 t/ha) in terms of spring wheat grain yield in comparison with the mixture system (PR 2.45 t/ha, UH 5.44 t/ha, ZV 3.14 t/ha). Quality parameters such as crude protein, wet gluten, gluten index, Zeleny test of wheat grown with legumes were compared to the control. The mean quality parameters of both systems were the following: test weight 75.75%, crude protein 13.9%, wet gluten 31.5%, Zeleny test 56.2 mL, falling number 266.4 s. The combinations of spring wheat with Egyptian clover, white clover, common vetch, common kidney vetch, and bird's-foot-trefoil are recommended as the most promising according to the results obtained across all sites and considering all parameters.

Key words: intercropping, legumes, organic production system, spring wheat, yield, grain characteristics

Intercropping cereals with legumes is farming technique that is considered to be gentle on soils and aquatic ecosystem, because it reduces the risk of harm to human health from the inappropriate use of synthetic fertilisers. It can effectively control weed growth and produce higher yields (Eskandari 2011; Wasaya *et al.* 2013; Jensen *et al.* 2020 Dvořák *et al.* 2022). Growing crops in mixtures is not unknown as the production system has long

been used in South America and is also known in Europe. The legume-cereal intercropping system is the most common planting system (Lai *et al.* 2022). Recently, it has experienced a certain renaissance in Europe (especially in the western part), especially in view of climate change and the associated risks, but also the advantages of intercropping over monocropping in reducing fertilizer and pesticide use. Intercropping is the term used to describe the method

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of cultivation in which two or more crops are grown on a plot in separate rows, double rows, or strips (Glaze-Corcoran *et al.* 2020). Strip intercropping of maize with legumes is the most common form of intercropping worldwide (Li *et al.* 2020). Another intercropping option is to co-crop legumes and wheat. In cereals, intercropping has improved grain quality and increased yield, with plants using nitrogen (N) up to 30% more efficiently than in monoculture (Malézieux *et al.* 2008).

Grain legumes such as pea, field pea, and faba bean (Šarunaite *et al.* 2010; Tortorella *et al.* 2013; Chapagain 2014; Zang *et al.* 2015; Tsialtas *et al.* 2018; Jensen *et al.* 2020) and forage legumes (Vrignon-Brenas *et al.* 2018; Liu *et al.* 2022) are often studied in intercropping systems with cereals or perennial grasses. In organic farming in particular, the potential interaction between these species can improve and stabilize cereal yields. Wheat yields in organic farming show a large variability mainly due to limited N availability, the non-use of synthetic mineral N fertilisers, and weed competition (Poudel *et al.* 2002; Doltra *et al.* 2011; Vrignon-Brenas *et al.* 2018). Intercropping wheat with legumes can therefore eliminate or at least reduce the use of mineral fertilizers and improve soil fertility through biological nitrogen fixation (BNF) by legumes and its transfer to cereals (Liu *et al.* 2022). Legumes utilize N_2 from the atmosphere through BNF and this mechanism is considered a possible way to develop sustainable/regenerative agricultural systems (Yao *et al.* 2019). Legume crops fix nitrogen from the atmosphere through their symbiotic nitrogen fixation abilities, and fixed nitrogen can be transferred and utilized by cereal crops (Lai *et al.* 2022). Biological N fixation also depends on the crops used, as significant differences in N transfer can occur between legumes and cereals (Zang *et al.* 2015; Tsialtas *et al.* 2018). Grain legumes accumulate significant amounts of soil-derived nitrogen, suggesting that crop diversification through cereal-grain legume intercropping can be used to design cropping systems with higher nitrogen use efficiency (Haugaard-Nielsen *et al.* 2003, 2008; Jensen *et al.* 2020).

N deficiency and weed infestation are the key factors influencing yield and quality of production in winter wheat (*Triticum aestivum* L.) cultivated under organic conditions (Vrignon-Brenas 2018). Ce-

reals are generally less efficient at utilizing N sources than legumes. Published data from cereal-legume intercropping experiments have shown that cereals use a disproportionate share of soil N sources due to competitive interactions and complementary N acquisition in intercrops (Jensen *et al.* 2020). In addition, legume roots can stimulate microbial mineralization and increase the available N pool in the soil (Tortorella 2013). Recently, wheat genotypes suitable for intercropping have been screened to be registered in France (Moutier *et al.* 2022).

Here, preliminary results are presented on the suitability of the spring wheat variety ‘Registana’ intercropped with different legume species in two different cropping systems i.e. row-by-row and mixture in organic farming.

MATERIAL AND METHODS

Field trials

Field trials of spring wheat (*Triticum aestivum* L.), variety ‘Registana’, intercropped with legumes were conducted at three localities in the Czech Republic in 2019. The experimental fields were in Central Bohemia in Prague (PR), and Uhřetěves (UH), and South Bohemia in Zvíkov (ZV) with different soil and climatic conditions.

Ten legume species were used (Table 1) and the plants were grown in two systems: as a mixture (wheat homogeneously mixed with legumes) and row-by-row (alternating cereal and legume rows) with the spring wheat variety ‘Registana’. The trials were sown with a sowing machine, in the case of the row-by-row system wheat and legumes were sown separately. All field trials were conducted in plots of 12 m² (1.25 × 9.6 m) per replicate in treatment. The plots in the field were arranged in two blocks, one for mixture and the second for row-by-row; each of them was completely randomized as a block design in three replicates, with row spacing of 12.5 cm. The seeding density of spring wheat was 300 seeds per m². The control treatment was the cultivation of spring wheat variety ‘Registana’ without legumes with two seeding densities of 300 seeds per m² (SpW 3) – the same as it is in the case of cereal: legume mixtures – and 400 seeds per m² (SpW 4). The reason for using two densities was to

compare the density used in wheat field trials with legumes and the density currently most commonly used for spring wheat in organic farming. The seeding densities used are listed in Table 1.

Experimental sites

All three experimental localities are characterized by the following conditions: PR is located on the north-west side of Prague, Czech Republic (50.08N, 14.30E) with an elevation of 320 m, annual precipitation of 472 mm, mean annual air temperature of 8.4°C, and the soil is Chernozem (IUUS/ISTRIC/FAO, 2006) with a loamy clay texture. UH is in the eastern part of Prague (50.03N, 14.61E) with an elevation of 295 m, an annual precipitation of 575 mm, a mean annual air temperature of 8.3°C, and is a loamy Luvisol (IUUS/ISTRIC/FAO, 2006) with loamy texture. ZV is located in South Bohemia, (48.58N, 14.37E) with the highest altitude of all localities (490 m) and with the highest annual precipitation of 634 mm and the lowest mean annual air temperature of 7.2°C and it has loamy soil. The mean content of mineral nitrogen ($N_{\min}$) determined before sowing from the depth of 0–30 cm, mainly NO_3-N , was as follows: the lowest in PR – at 2.9 kg $N_{\min}$ /ha, 14.3 kg $N_{\min}$ /ha in UH and 37.5 kg $N_{\min}$ /ha in ZV. The trial was established on 21st March 2019

at PR and UH and on 4th April 2019 at ZV; the previous crop was Emmer wheat. Spring wheat and legumes were harvested on 18th July 2019 at PR and 5th August 2019 at UH and ZV.

Temperature conditions at the beginning of the growing season were similar at all sites, except for the ZV site, where the average temperature in March was 2°C higher than in PR and UH. Similarly, the mean temperature in May at ZV reached almost twice the value (21°C) than at the other localities (Figure 1). The summer months of June and July were warmer at the Central Bohemian sites (PR and UH) than in the South Bohemian region (ZV). In the first two months of the growing season, rain was more frequent at UH. On the other hand, there was significantly more precipitation in ZV from May to August 2019 (Figure 1).

Crop yields and laboratory analyses

Yields of spring wheat were determined after harvesting individual plots with a plot combine harvester and converted to hectares. The cereal and legume seeds were separated using sieves. The remaining impurities were removed manually. The wheat samples were prepared in laboratory grinder PSY MP 20 (Mezos, Hradec Kralove, Czech Republic) and with laboratory mill Quadrumat Junior

T a b l e 1
List of legume species and seeding rates used in the intercropping experiment

Legume species	Variety	Seeding rate [%]	Full seeding density [kg/ha]	Used seeding density [kg/ha]
Egyptian Clover (<i>Trifolium alexandrinum</i>)	–	50	15	7.5
Red clover (<i>Trifolium pratense</i>)	‘Bonus’	50	20	10
White (Dutch) clover (<i>Trifolium repens</i>)	‘Jura’	50	6	3
Crimson clover (<i>Trifolium incarnatum</i>)	‘Kardinál’	50	20	20
Common vetch (<i>Vicia sativa</i>)	‘Ebena’	30	90	27
Common pea (<i>Pisum sativum</i>)	‘Eso’	30	300	90
Field pea (<i>Pisum arvense</i>)	‘Arvika’	30	180	54
Bird’s-foot-trefoil (<i>Lotus corniculatus</i>)	‘Maleják’	50	15	7.5
Common kidney vetch (<i>Anthyllis vulneraria</i>)	‘Antyl’	50	15	7.5
Fenugreek (<i>Trigonella foenum graecum</i>)	–	30	25	7.5

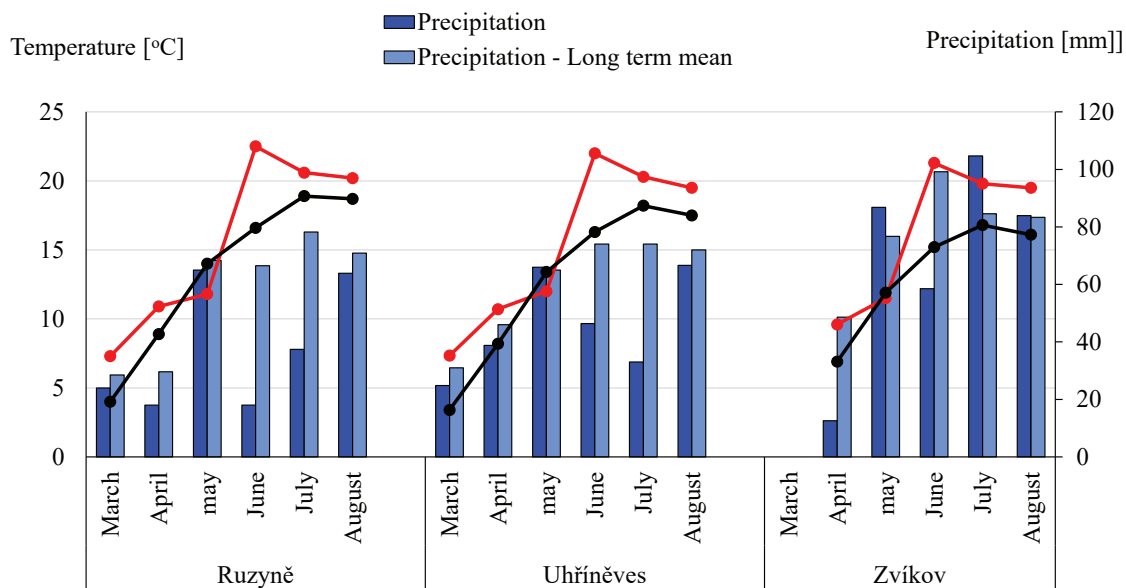


Figure 1. Weather conditions at experimental localities in 2019. The long-term mean temperatures and precipitation represent 20-year means for 1998–2018.

machine (Brabender, Duisburg, Germany). Prepared samples were stored in a cold (4°C) and dark place in plastic bags for future laboratory analyses. All samples were prepared in four replicates and results are presented as a mean of these replicates. For the dry matter content, 5 g of ground seed samples were dried in an electric hot-air drier at 135°C for 4 hours, according to the standard method. The baking quality of wheat samples were carried out according to the following methods: crude protein content was determined with a Kjeltec KT 200 (MILCOM servis a.s., Czech Republic) using the Kjeldahl method (ICC-Standard No. 105/2), whereby the N concentration was multiplied by 5.7 to convert to grain protein (in % in dry matter (DM)); Falling Number – according to Hagberg-Perten, (Falling Number 1400) (ICC-Standard 107/1), Sedimentation value according to Zeleny (ICC-Standard No. 116/1), wet gluten according to ČSN ISO 5531 (560515) (1993), and test weight EN ISO 7971-3:2019 (ISO 6971-3:2019).

Statistical analysis

Statistical analysis was performed using Statistica 14.0 software (TIBCO, USA). The same letters in the tables represent non-statistically significant differences according to Tukey's test (ANOVA) ($^+p < 0.05$ or $^{++}p < 0.01$).

RESULTS

Spring wheat grain yields

Not all variants were used for the statistical evaluation of yield. In the variants with Egyptian clover, crimson clover, red and white clover, kidney vetch, and bird's-foot-trefoil in UH site grown row-by-row, there were not enough legumes in all plots to cover the interrows. Therefore, only variants with common pea, field pea, common vetch, and fenu-greek, for which data from both row-by-row and mixtures were available, were used for the analysis. Nevertheless, samples for quality assessment were always taken from at least one plot.

However, statistical analyses were performed for all the variants used at PR and ZV (Table 2 and Table 3). Statistically significant differences in yield were found between PR and ZV (2.30 t/ha and 3.14 t/ha, respectively, at $p < 0.01$). The highest yield was observed in SpW4 (3.18 t/ha, $p < 0.05$) and the lowest in the variant with field pea (2.32 t/ha, $p < 0.05$). No statistically significant differences were found between the sowing systems and between sowing systems and variants. However, differences were found when evaluating sowing systems and sites. In general, mixtures had higher yields than the row-by-row system at both locations. Larger differences were found at PR. Differences were also found in the variant and site evaluation. At PR, a combination of

wheat and field pea had the lowest yield (1.84 t/ha, $p < 0.01$), while wheat grown with white clover had the highest yield (2.72 t/ha, $p < 0.01$). In contrast, in ZV, SpW 4 had the highest yield (3.87 t/ha, $p < 0.01$) and the lowest wheat with bird's-foot-trefoil (2.58 t/ha, $p < 0.01$). Table 4 shows the statistical analysis of all three sites. In general, the highest yields were found at the UH site and the lowest at the PR site. As shown in Table 3, yields were higher in mixtures than in row-by-row sowing system. The highest yields at UH were found in the SpW 4 treatment. The highest yield in wheat-legume combination was found in the variant with fenugreek (5.39 t/ha, $p < 0.01$). In contrast, the lowest yields were found in the variant with field pea (4.07 t/ha, $p < 0.01$), as in PR.

Quality of spring wheat grain

Grain test weight

The highest mean value of grain test weight was

observed at PR with 79.37 kg/hL, while the lowest was 72.73 kg/hL for grains harvested at UH. The test weight of grains was slightly higher in samples grown in a mixed cropping system (75.91 kg/hL) than in row-by-row (75.35 kg/hL) which was significantly different. Statistically significant differences were confirmed between all three localities as well as between cropping systems. At all sites, the trend was similar. The slightly lower grain test weight was observed in the row-by-row cropping system (Table 5).

The lowest test weight was found in the mixture with red clover and the highest in the mixture with bird's-foot-trefoil. These two variants were different from the others with balanced test weights. The lowest grain test weight in the row-by-row system was observed in combination with bird's-foot-trefoil, followed by red clover, and Egyptian clover.

T a b l e 2

Statistical analysis of yields of all variants in PR and ZV

Level		Yield
System	Mixture	$2.80 \pm 0.51^{a+}$
	Row-by-row	$2.64 \pm 0.66^{a+}$
Variant	SpW 3	$2.85 \pm 1.20^{ab+}$
	SpW 4	$3.18 \pm 0.79^{b+}$
	SpW + Egyptian clover	$2.79 \pm 0.39^{ab+}$
	SpW + Crimson clover	$2.64 \pm 0.55^{ab+}$
	SpW + Red clover	$2.59 \pm 0.79^{ab+}$
	SpW + White clover	$2.89 \pm 0.31^{ab+}$
	SpW + Common pea	$2.87 \pm 0.25^{ab+}$
	SpW + Field pea	$2.32 \pm 0.56^{a+}$
	SpW + Common vetch	$2.60 \pm 0.18^{ab+}$
	SpW + Bird's-foot-trefoil	$2.47 \pm 0.32^{ab+}$
	SpW + Common kidney vetch	$2.73 \pm 0.60^{ab+}$
	SpW + Fenugreek	$2.69 \pm 0.75^{ab+}$
Site	PR	$2.30 \pm 0.37^{a++}$
	ZV	$3.14 \pm 0.45^{b++}$

Letter indices indicate statistically significant differences and ⁺ at the $p < 0.05$ significance level and ⁺⁺ at the $p < 0.01$ significance level.

PR – Prague; ZV – Zvíkov; UH – Uhřetěves; SpW 3 – spring wheat Registana without legume species 300 seeds per m²; SpW 4 – spring wheat Registana without legume species 400 seeds per m².

T a b l e 3

Statistical analysis of differences between yields from all variants in PR and ZV sites

Level	System	Variant/site	Yield	Level	System	Site	Yield
System × variant	Mixture	SpW + Egyptian clover	2.85 ± 0.28 ⁺⁺	Variant × site	SpW 3	PR	1.88 ± 0.75 ^{at+}
		SpW + Crimson clover	2.90 ± 0.57 ^{at+}			ZV	3.82 ± 0.00 ^{bt++}
		SpW + Red clover	2.81 ± 1.05 ^{at+}		SpW 4	PR	2.50 ± 0.06 ^{abt+}
		SpW + White clover	3.09 ± 0.24 ^{at+}			ZV	3.87 ± 0.00 ^{bt++}
		SpW + Common pea	2.89 ± 0.40 ^{at+}		SpW + Egyptian clover	PR	2.48 ± 0.24 ^{abt+}
		SpW + Field pea	2.39 ± 0.75 ^{at+}			ZV	3.11 ± 0.08 ^{abt+}
		SpW + Common vetch	2.68 ± 0.26 ^{at+}		SpW + Crimson clover	PR	2.24 ± 0.37 ^{at+}
		SpW + Bird's-foot-trefoil	2.38 ± 0.18 ^{at+}			ZV	3.04 ± 0.37 ^{abt+}
	Row-by-row	SpW + Common kidney vetch	2.58 ± 0.10 ^{at+}		SpW + Red clover	PR	1.95 ± 0.16 ^{at+}
		SpW + Fenugreek	2.72 ± 0.37 ^{at+}			ZV	3.24 ± 0.45 ^{abt+}
		SpW + Egyptian clover	2.74 ± 0.60 ^{at+}		SpW + White clover	PR	2.72 ± 0.29 ^{abt+}
		SpW + Crimson clover	2.38 ± 0.57 ^{at+}			ZV	3.06 ± 0.28 ^{abt+}
		SpW + Red clover	2.38 ± 0.77 ^{at+}		SpW + Common pea	PR	2.67 ± 0.08 ^{abt+}
		SpW + White clover	2.69 ± 0.25 ^{at+}			ZV	3.08 ± 0.13 ^{abt+}
		SpW + Common pea	2.86 ± 0.19 ^{at+}		SpW + Field pea	PR	1.84 ± 0.04 ^{at+}
		SpW + Field pea	2.24 ± 0.61 ^{at+}			ZV	2.80 ± 0.18 ^{abt+}
System × site	Mixture	SpW + Common vetch	2.52 ± 0.08 ^{at+}		SpW + Common vetch	PR	2.48 ± 0.02 ^{abt+}
		SpW + Bird's-foot-trefoil	2.57 ± 0.48 ^{at+}			ZV	2.72 ± 0.21 ^{abt+}
		SpW + Common kidney vetch	2.88 ± 0.98 ^{at+}		SpW + Bird's-foot-trefoil	PR	2.37 ± 0.19 ^{abt+}
		SpW + Fenugreek	2.67 ± 1.25 ^{at+}			ZV	2.58 ± 0.47 ^{abt+}
		PR	2.45 ± 0.27 ^{at+}		SpW + Common kidney vetch	PR	2.35 ± 0.23 ^{abt+}
		ZV	3.14 ± 0.47 ^{bt+}			ZV	3.11 ± 0.65 ^{abt+}
		PR	2.14 ± 0.40 ^{at+}		SpW + Fenugreek	PR	2.12 ± 0.48 ^{at+}
		ZV	3.14 ± 0.45 ^{bt++}			ZV	3.27 ± 0.40 ^{abt+}

Letter indices indicate statistically significant differences and ⁺ at the $p < 0.05$ significance level and ⁺⁺ at the $p < 0.01$ significance level.PR – Prague; ZV – Zvíkov; UH – Uhřetěves; SpW 3 – spring wheat Registrana without legume species 300 seeds per m²; SpW 4 – spring wheat Registrana without legume species 400 seeds per m².

T a b l e 4

Statistical analysis of differences between yields from selected variants in PR, ZV and UH

Level	System	Variant/site	Yield	Level	System	Site	Yield
System × variant	Mixture	SpW + Common pea	3.60 ± 1.26 st	variant × site	SpW 3	PR	1.88 ± 0.75 st
		SpW + Field pea	2.98 ± 1.15 st			UH	5.38 ± 0.38 ^{efi}
		SpW + Common vetch	3.52 ± 1.47 st			ZV	3.82 ± 0.00 ^{bcd}
		SpW + Fenugreek	3.73 ± 1.76 st				
System × variant	Row-by-row	SpW + Common pea	3.36 ± 0.89 st	variant × site	SpW 4	PR	2.50 ± 0.06 ^{ab}
		SpW + Field pea	2.82 ± 1.09 st			UH	5.61 ± 0.57 st
		SpW + Common vetch	3.22 ± 1.22 st			ZV	3.87 ± 0.00 ^{bcd}
		SpW + Fenugreek	3.46 ± 1.63 st				
System × site	Mixture	PR	2.38 ± 0.26 ^{ab}	variant × site	SpW + Common pea	PR	2.67 ± 0.08 ^{ab}
		UH	5.30 ± 0.66 st			UH	4.70 ± 0.46 ^{def}
		ZV	3.27 ± 0.46 ^b			ZV	3.08 ± 0.13 ^{abc}
System × site	Row-by-row	PR	2.11 ± 0.54 st	variant × site	SpW + Field pea	PR	1.84 ± 0.04 st
		UH	4.72 ± 0.49 st			UH	4.07 ± 0.13 ^{bcd}
		ZV	3.25 ± 0.58 ^b			ZV	2.80 ± 0.18 ^{ab}
System × site	Row-by-row	PR	2.48 ± 0.02 ^{ab}	variant × site	SpW + Common vetch	PR	2.48 ± 0.02 ^{ab}
		UH	4.91 ± 0.41 ^{def}			UH	4.91 ± 0.41 ^{def}
		ZV	2.72 ± 0.21 ^{ab}			ZV	2.72 ± 0.21 ^{ab}
System × site	Row-by-row	PR	2.12 ± 0.48 st	variant × site	SpW + Fenugreek	PR	2.12 ± 0.48 st
		UH	5.39 ± 0.49 ^{efi}			UH	5.39 ± 0.49 ^{efi}
		ZV	3.27 ± 0.40 ^{abcd}			ZV	3.27 ± 0.40 ^{abcd}

Letter indices indicate statistically significant differences and ⁺ at the $p < 0.05$ significance level and ⁺⁺ at the $p < 0.01$ significance level.
 PR – Prague; ZV – Zvíkov; UH – Uhřetěves; SpW 3 – spring wheat Registrata without legume species 300 seeds per m²; SpW 4 – spring wheat Registrata without legume species 400 seeds per m².

T a b l e 5
Characteristics of spring wheat grain quality

	Level	Test weight [kg/hL]	Crude protein [%]	Wet gluten [%]	Gluten index [%]	Zeleny test [mL]	Falling number [s]
Variant	SpW + Common pea	76.03 ± 2.55 ^{d++}	13.97 ± 1.30 ^{e++}	31.86 ± 6.11 ^{e++}	91.83 ± 8.94 ^{a++}	56.83 ± 9.10 ^{bcd++}	262.25 ± 43.49 ^{ab++}
	SpW + Egyptian clover	75.48 ± 2.97 ^{ab++}	13.93 ± 1.57 ^{e++}	32.10 ± 6.32 ^{cd++}	92.17 ± 10.33 ^{a++}	56.50 ± 11.66 ^{bcd++}	270.08 ± 44.13 ^{b++}
	SpW + Red clover	75.27 ± 2.85 ^{a++}	13.81 ± 1.96 ^{bce++}	30.76 ± 6.62 ^{b++}	94.67 ± 5.00 ^{bce++}	54.83 ± 12.66 ^{ab++}	264.50 ± 49.41 ^{ab++}
	SpW + Crimson clover	75.51 ± 3.22 ^{abce++}	13.64 ± 1.89 ^{ab++}	31.27 ± 7.25 ^{b++}	93.67 ± 8.05 ^{abce++}	53.83 ± 12.36 ^{a++}	261.50 ± 50.48 ^{ab++}
	SpW + White clover	75.71 ± 3.06 ^{bcd++}	13.80 ± 1.94 ^{bce++}	30.90 ± 6.80 ^{b++}	93.50 ± 7.90 ^{ab++}	55.00 ± 11.31 ^{abce++}	262.25 ± 44.53 ^{ab++}
	SpW + Field pea	75.70 ± 3.19 ^{abced++}	14.39 ± 1.34 ^{e++}	32.78 ± 4.79 ^{e++}	93.00 ± 7.71 ^{ab++}	57.33 ± 9.36 ^{d++}	262.33 ± 44.57 ^{ab++}
	SpW + Fenugreek	75.73 ± 2.99 ^{b++}	13.58 ± 1.55 ^{a++}	29.54 ± 5.03 ^{a++}	93.67 ± 6.96 ^{abce++}	54.83 ± 11.31 ^{ab++}	256.50 ± 57.23 ^{a++}
	SpW + Bird's-foot-trefoil	75.50 ± 2.79 ^{abce++}	13.98 ± 1.60 ^{e++}	31.95 ± 5.58 ^{e++}	95.00 ± 4.94 ^{bce++}	58.33 ± 10.73 ^{d++}	269.17 ± 45.17 ^{b++}
	SpW + Common kidney vetch	75.48 ± 2.92 ^{ab++}	13.82 ± 1.82 ^{bce++}	31.85 ± 7.78 ^{e++}	95.67 ± 3.28 ^{e++}	57.75 ± 11.38 ^{d++}	269.50 ± 50.89 ^{b++}
	SpW + Common vetch	75.93 ± 3.36 ^{cd++}	14.18 ± 1.63 ^{d++}	32.63 ± 6.95 ^{de++}	93.17 ± 9.28 ^{ab++}	57.17 ± 11.65 ^{cd++}	266.58 ± 47.76 ^{b++}
System	Mixture	75.91 ± 2.87 ^{b++}	13.79 ± 1.74 ^{a++}	31.42 ± 6.63 ^{a++}	93.57 ± 7.47 ^{a+}	55.80 ± 11.41 ^{a++}	261.57 ± 40.97 ^{a++}
	Row-by-row	75.35 ± 2.91 ^{a++}	14.02 ± 1.51 ^{b++}	31.70 ± 5.82 ^{b++}	93.70 ± 7.27 ^{a+}	56.68 ± 10.37 ^{b++}	267.37 ± 51.23 ^{b++}
Site	PR	79.37 ± 0.84 ^{c++}	11.76 ± 0.58 ^{a++}	24.43 ± 1.94 ^{a++}	98.15 ± 1.53 ^{b++}	41.60 ± 4.02 ^{a++}	266.45 ± 17.05 ^{b++}
	ZV	74.80 ± 0.34 ^{b++}	15.14 ± 0.45 ^{c++}	38.61 ± 2.44 ^{c++}	84.45 ± 5.54 ^{a++}	63.05 ± 2.73 ^{b++}	210.68 ± 15.61 ^{a++}
	UH	72.73 ± 0.97 ^{a++}	14.83 ± 0.59 ^{b++}	31.66 ± 2.20 ^{b++}	98.30 ± 1.22 ^{b++}	64.08 ± 2.54 ⁺⁺	316.28 ± 16.62 ^{c++}

Table 5 – continue

Variant × system	SpW + Common pea	Mixture	76.33±2.97 ^{hi++}	13.97±1.60 ^{defg++}	32.48±7.69 ^{hij++}	92.00±10.12 ^{abcd++}	56.00±11.03 ^{abc++}	254.33±23.75 ^{abcd++}
		Row-by-row	75.72±2.28 ^{bcdefgh++}	13.98±1.07 ^{efg++}	31.24±4.69 ^{defg++}	91.67±8.57 ^{abc++}	57.67±7.69 ^{bcd++}	270.17±58.70 ^{ef++}
	SpW + Egyptian clover	Mixture	75.83±3.19 ^{defgh++}	13.84±1.46 ^{bcdef++}	32.09±7.03 ^{ghi++}	93.33±8.80 ^{abcde++}	56.67±10.71 ^{abc++}	270.67±39.08 ^{ef++}
		Row-by-row	75.12±2.98 ^{ab++}	14.01±1.81 ^{fg++}	32.10±6.19 ^{ghi++}	91.00±12.41 ^{a++}	56.33±13.57 ^{abc++}	269.50±52.50 ^{def++}
	SpW + Red clover	Mixture	75.47±2.60 ^{bcdef++}	13.69±2.24 ^{bcde++}	30.64±7.88 ^{bcd++}	93.33±6.62 ^{abcde++}	55.00±12.44 ^{abc++}	262.00±50.10 ^{abcde++}
		Row-by-row	75.07±3.31 ^{ab++}	13.94±1.85 ^{cdefg++}	30.89±5.84 ^{bcd++}	96.00±2.61 ^{e++}	54.67±14.05 ^{ab++}	267.00±53.35 ^{cdef++}
	SpW + Crimson clover	Mixture	75.87±3.43 ^{defgh++}	13.46±2.20 ⁺⁺	30.89±8.84 ^{bcd++}	92.00±10.18 ^{qbcde++}	53.67±14.75 ^{a++}	272.00±46.13 ^{ef++}
		Row-by-row	75.15±3.28 ^{abc++}	13.82±1.72 ^{bcdef++}	31.65±6.08 ^{efgh++}	95.33±5.68 ^{e++}	54.00±10.90 ^{a++}	251.00±56.68 ^{ab++}
	SpW + White clover	Mixture	76.12±2.93 ^{fghi++}	13.54±2.08 ^{ab++}	30.98±7.62 ^{bcd++}	93.00±9.32 ^{abcde++}	53.67±12.23 ^{a++}	253.83±35.20 ^{abc++}
		Row-by-row	75.30±3.42 ^{bcd++}	14.05±1.95 ^{fghi++}	30.81±6.61 ^{bcd++}	94.00±7.07 ^{abcde++}	56.33±11.29 ^{abc++}	270.67±54.35 ^{ef++}
	SpW + Field pea	Mixture	75.61±3.53 ^{bcdefg++}	14.34±1.39 ^{hij++}	33.64±4.56 ⁺⁺	95.00±3.85 ^{de++}	58.33±8.87 ^{cd++}	258.67±35.53 ^{abcde++}
		Row-by-row	75.80±3.15 ^{cdefgh++}	14.44±1.42 ⁺⁺	31.92±5.28 ^{fghi++}	91.00±10.32 ^{a++}	56.33±10.58 ^{abc++}	266.00±55.46 ^{bcd++}
	SpW + Fenugreek	Mixture	76.02±3.52 ^{efghi++}	13.60±1.86 ^{ab++}	30.19±6.16 ⁺⁺	94.33±5.72 ^{bcd++}	55.00±13.31 ^{abc++}	250.00±53.60 ^{a++}
		Row-by-row	75.43±2.65 ^{bcd++}	13.55±1.36 ^{ab++}	28.88±4.10 ⁺⁺	93.00±8.53 ^{abcde++}	54.67±10.21 ^{ab++}	263.00±65.05 ^{abcde++}
	SpW + Bird's-foot-trefoil	Mixture	76.45±2.65 ⁺⁺	13.64±1.87 ^{abc++}	31.11±6.37 ^{cdef++}	95.33±4.23 ^{e++}	56.33±13.57 ^{abc++}	269.00±38.60 ^{cdef++}
		Row-by-row	74.55±2.82 ⁺⁺	14.32±1.36 ^{hij++}	32.79±5.11 ^{ijk++}	94.67±5.96 ^{cde++}	60.33±7.71 ^{d++}	269.33±54.76 ^{def++}
	SpW + Common kidney vetch	Mixture	75.83±3.06 ^{defgh++}	13.67±1.85 ^{bcde++}	30.23±7.13 ^{bc++}	96.00±3.22 ^{e++}	57.00±12.46 ^{abcd++}	260.00±56.32 ^{abcde++}
		Row-by-row	75.13±3.01 ^{abc++}	13.96±1.94 ^{cdefg++}	33.47±8.73 ⁺⁺	95.33±3.61 ^{e++}	58.50±11.33 ^{cd++}	279.00±48.06 ^{f++}
	SpW + Common vetch	Mixture	75.62±2.98 ^{bcd++}	14.18±1.88 ^{ghi++}	32.01±6.91 ^{ghi++}	91.33±11.91 ^{ab++}	56.33±12.68 ^{abc++}	265.17±49.21 ^{abcde++}
		Row-by-row	76.25±3.97 ^{ghij++}	14.17±1.52 ^{ghi++}	33.24±7.60 ^{ijk++}	95.00±6.29 ^{de++}	58.00±11.66 ^{bcd++}	268.00±50.91 ^{cdef++}

Letter indices indicate statistically significant differences ⁺ at the $p < 0.05$ significance level and ⁺⁺ at the $p < 0.01$ significance level.

PR – Prague; ZV – Zvíkov; UH – Uhřetěves; SpW – spring wheat.

Crude protein

In content of crude protein statistically significant differences were found between the three localities (Table 5) and between the two cropping systems. The row-by-row cropping system resulted in a slightly higher crude protein content (14.02%) than the mixture (13.79%). The samples harvested in ZV had the highest crude protein content (15.14%), in contrast to the mean value of all treatments in PR (11.76%).

At PR, the higher crude protein content in the grains was confirmed in the combination of wheat with common vetch (12.0%), common pea (12.35%), field pea (12.6%), and bird's-foot-trefoil (11.96%). The lowest crude protein content was found in the combination of wheat with crimson clover (11.19%), red clover (11.25%), and white clover (11.28%). Control samples of spring wheat without a legume had 11.45% protein at SpW 3 and SpW 4.

The crude protein content of spring wheat grain on average was higher at UH compared to PR and ZV. Growing spring wheat in a mixture resulted in mean values of crude protein content across all sites of 13.79% and 14.02% in the row-by-row cropping system. In UH, crude protein content higher than 15% was found in common vetch, red clover, field pea and white clover. In contrast, both control treatments without legumes SpW 3 (14.44%) and SpW 4 (14.04%) had lower values.

The crude protein content at ZV averaged at 15.45% in the mixture and 14.83% in the row-by-row system. The highest crude protein content was found in the control treatments SpW 3 (15.98%) and SpW 4 (15.50%), respectively. The crude protein content of the other treatments in the mixed system ranged from 15.12 to 15.76%. Crude protein content in the row-by-row system showed greater differences between treatments and ranged from 14.16 to 15.64%. Crude protein content in the treatments with vetch was higher than 15.0% in both systems with higher values in mixed cropping; the lowest values were for Egyptian clover in mixed cropping and fenugreek in the case of row-by-row system (14.16%).

Wet gluten

Statistically significant differences were found in wet gluten content between locations. In PR, sam-

ples had the lowest mean value (24.43%), in contrast with results from UH (31.66%) and ZV (38.61%), where significantly higher means of wet gluten content were found. In addition, significant differences in wet gluten content were found between growing systems, where slightly higher values were found in row-by-row system (Table 5).

At PR, the highest wet gluten content in the mixture system was found in the combination of wheat and field pea (27.99%), followed by Egyptian clover (25.70%) and common pea (25.11%). Similarly, the highest content of wet gluten in the row-by-row system was recorded in the treatment with field pea (27.90%), followed by common pea (27.32%), and bird's-foot-trefoil (26.45%).

The highest content of wet gluten in the locality UH was found in the mixture system with field pea (35.03%), followed by common vetch (32.61%), red clover (31.59%), and crimson clover (31.01%). The lowest value of wet gluten content (28.60%) was observed in variant with fenugreek and common kidney vetch (28.66%). The content of wet gluten in row-by-row cropping was more balanced within the treatments. The highest content of wet gluten in the row-by-row system was observed in the treatment with red clover (34.92%), followed by bird's-foot-trefoil (34.38%), and white clover (34.26%).

In general, the highest values for wet gluten content were obtained at the ZV site. At this site, a higher mean value was found for the mixture system (39.44%) than for the row-by-row cropping system (37.77%). The highest content of wet gluten in the mixture system was found in the common pea (41.92%), Egyptian clover (40.87%) and crimson clover (40.71%). The lowest content of wet gluten was observed in the treatment with fenugreek (37.73%). The observed values of the content of wet gluten in the row-by-row system were less uniform compared to the mixture. However, the highest content of wet gluten across all legume species and localities was observed in ZV in a row-by-row system combining wheat with common kidney vetch (42.98%) and common vetch (42.14%). The lowest value of wet gluten content was found for fenugreek (33.31%).

Gluten index

The mean value of gluten index (GI) was 93.86%

for all variants and localities. Similar mean values of GI were observed in samples from the UH and PR sites i.e. 98.30% and 98.15%, respectively. The lowest mean value was observed in samples from the locality ZV (84.45%). Only minor differences were found between the two cropping systems. The mean value of GI for the mixture cropping system was 93.57% and 93.70% for the row-by-row cropping system. In general, only minor differences (95–99%) were observed at the PR and UH localities, while much greater variability between treatments was observed at ZV (75–93%).

Zeleny sedimentation test

The Zeleny sedimentation test determines the quality of wheat and the baking strength of the flour. Samples from localities ZV and UH had similar mean values of Zeleny test at 63.05 mL and 64.08 mL, respectively. In contrast, samples from PR had significantly lower Zeleny test values (41.60 mL). The Zeleny test mean values were almost the same in both cropping systems at 55.80 mL for the mixture and 56.68 mL for row-by-row.

At PR, the Zeleny sedimentation test value was 40.20 mL for the mixture cultivation system and 43.00 mL for the row-by-row system. The lowest value of the test (35 mL) was for the variant with crimson clover in mixed system. Low values were observed in the variants with white clover (38 mL) and fenugreek (38 mL) in the mixture and for red clover in the row-by-row system (37 mL). On the other hand, the highest values of the Zeleny test were obtained for combinations of wheat and bird's-foot-trefoil (51 mL), common pea (48 mL), common kidney vetch (44 mL), field pea, and vetch (both 43 mL) in row-by-row system.

The Zeleny test at UH showed higher mean values compared to PR, where the mean values were 62.70 mL in the mixture cropping system and 65.45 mL in the row-by-row system (Table 5). Lower values of Zeleny were observed in the mixture system. The highest values were found for the combination with common kidney vetch (66 mL), bird's-foot-trefoil, and common vetch (both 65 mL). However, even higher values of the Zeleny test were obtained for the combination of wheat with bird's-foot-trefoil (68 mL), Egyptian and red clover (67 mL) and kidney vetch (66.5 mL), common vetch (66 mL) in the row-by-row system.

The values of the Zeleny test in the samples from ZV were very similar to those observed in UH, but here the values from the mixed cropping system showed higher mean values (64.50 mL) of the Zeleny test than in the row-by-row cropping system (61.60 mL). The highest value was found for the spring wheat grown with crimson clover and the control treatment SpW 3 (66 mL). Four treatments reached 65 mL (combination with Egyptian clover, common pea, field pea, bird's-foot-trefoil) in the mixture cropping system. The value of the Zeleny test in the row-by-row system was lowest in the treatments with crimson clover (56 mL), fenugreek (58 mL), and red clover (60 mL). The highest value of the Zeleny test in the row-by-row system was found in treatments with common vetch, common kidney vetch (both 65 mL), and common pea (64 mL).

Falling number

The falling number (FN) is the most widely used method for determining the potential of sprouting damage and was determined by AACC method 56-81B. Its international standards are given by ICC 107/1, and ISO 3093-2004. The falling number test can be used to detect so-called weather or sprouting damaged wheat or rye that affects bread quality. The mean value of FN for all localities and all variants was 265.51 s. The lowest mean values were obtained for samples from ZV (210.68 s), followed by PR (266.45 s) and UH (316.28 s). A statistically significant difference between FN and the two cropping systems were confirmed (Table 5).

Lower values of FN in the mixture system were found in treatments with common kidney vetch (230 s), red clover (242 s), and white clover (243 s) at PR. The highest FN was found in the treatment with Egyptian clover (301 s), common vetch (275 s) and fenugreek (269 s). In the row-by-row system, FN was lowest in the treatment red clover (256 s), whereas all others were higher than 271 s. The highest FN values were observed in combination with common vetch (284 s).

In samples harvested at UH, the highest value of FN was observed. The mean values for the mixture cropping system were 308.15 s and the system row-by-row 324.40 s. Lower values in the mixture system were found in the treatments with common

pea (283 s), and Egyptian clover (290 s). In contrast, the highest value of FN was found in the treatment with common kidney vetch (332 s) and crimson clover (329 s). The FN in the row-by-row system was lower in treatments with crimson clover (301 s) and common vetch (315 s). The highest FN was found in treatments with white and red clover, and common kidney vetch (all 331 s).

Significantly lower values of FN were observed in samples harvested at ZV compared to those harvested at UH and PR. FN in samples from the mixture cropping system was 217.80 s and 203.55 s for the row-by-row system. The lowest value of the falling number in the mixture system was found in the variant with fenugreek (183 s). On the other hand, the highest FN was found in the treatments with common pea (232 s), bird's-foot-trefoil (230 s), and crimson clover (229 s). FN in the row-by-row system was lower, especially in the variants with crimson clover (180 s), fenugreek (184 s), common pea (200.50 s), and field pea (202 s). A higher falling number was found in the treatment with common kidney vetch (224 s) and red clover (214 s).

DISCUSSION

Yields of spring wheat grain

Spring wheat was intercropped with different legumes in an organic production environment at three sites in the Czech Republic. Winter bean-wheat intercropping led to higher yields under low-input management than sole crop equivalents and high intercrop yields were associated with high wheat component yields (Nelson *et al.* 2021). Organically grown crops may show greater variability in yields due to limited N availability and greater weed competition (Poudel *et al.* 2002; Doltra *et al.* 2011; Vrignon-Brenas *et al.* 2018). The $N_{\min}$ content showed differences between the studied sites. The lowest $N_{\min}$ content was found at the site PR, where 2.9 kg $N_{\min}$ /ha was found at the depth of 0–30 cm. The low $N_{\min}$ content could have affected the yields at PR, which were the lowest among all the sites. Grain yields at UH were highest on average, although $N_{\min}$ content was lower (14.3 kg N/ha at 0–30 cm) compared to ZV (37.5 kg N/ha at 0–30 cm).

The effects of legumes on spring wheat yields

varied from site to site. Intercropping can increase yields whereby cereals can use nitrogen more efficiently than in monoculture (Malézieux *et al.* 2008; Dvořák *et al.* 2022). Differences in yield between treatments were observed at all experimental sites. Intercropping with legumes in a mixture system (PR) increased wheat yields about 102.07%–121.16% of the yield of wheat grains sown without legumes (SpW 3), except for red clover and field pea, which achieved only 85.48% and 77.18% of the wheat grown without legumes. The row-by-row system showed greater differences between treatments and mostly lower yields compared to the mixture, probably due to the greater spacing between rows and the greater competition between wheat plants within the rows, which ended up with lower yields. However, white clover and common pea increased wheat yields by up to 104.15% and 112.86% in this system as well.

The UH locality had slightly lower mean values for yields in the mixture system, ranging from 77.32% to 106.69% compared with the variant without legumes (SpW 3). Similar low wheat yields of 73.79–93.68% (of the maximum yield of the control samples) were obtained in the row-by-row cropping system at the UH site. In comparison to SpW 3, lower yields in the intercropping system (58.90–92.93% of spring wheat grain yield) were also observed at ZV. Wheat grain yields might be related to $N_{\min}$ content, which was highest at the ZV and UH localities. The very low $N_{\min}$ content at PR might result in a greater benefit from biological nitrogen fixation (BNF) by legumes and its transfer to cereals (Liu *et al.* 2022). Similarly Tripathi *et al.* (2021) concluded that intercropping with legumes could save a significant quantity of N, which in turn reduces the cost of cultivation and can enhance soil health parameters.

Grain quality of spring wheat

Wheat quality depends on genetic factors, environmental conditions, growing location, and agronomic practices at different stages of wheat growth (Mäder 2007).

The test weight wheat grains should be at least 76 kg/hL according to the Norm of the Czech Republic ČSN 46 1100-2 (461100). The variety 'Registana' is of bread quality and should reach a mini-

mum test weight of 76 kg/hL as claimed by breeder. The wheat samples from PR met this requirement in both the mixture and row-by-row cropping systems. While the test weight in the mixture system was similar within treatments, the row-by-row cropping system showed greater variation. The test weight at the other two experimental sites did not reach the required level of test weight for baking-quality criteria. It cannot be excluded that the lower yields in the samples from PR improved this parameter compared to UH and ZV, where relatively higher yields were obtained.

Crude protein

According to the Norm ČSN 46 1100-2, the crude protein content to reach baking quality should be at least 11.5%. The content of crude protein at PR on average reached 11.5%. Egyptian clover and grain legumes (common pea, field pea, and common vetch), in particular, increases crude protein content to more than 11.5%, which met the wheat quality category A (baking quality) requirements. In general, crude protein content was higher in samples grown intercropped row-by-row with grain legumes increasing this quality parameter. In fact, intercropping can increase wheat grain protein content in organic and low-input production systems (Pellegrini *et al.* 2021). However, a low amount of N fertilisation in intercropping systems can increase the overall production and N yield of intercrops (Neugschwandtner *et al.* 2021). Unfortunately, here intercropping has not been shown to have a positive effect on increasing protein content as in the case wheat with faba bean as reported by Sammama *et al.* (2022).

UH and ZV had higher $N_{\min}$ contents in soils and, in combination with legumes, can improve BNF and its transfer to cereals (Liu *et al.* 2022). This trend resulted in significantly higher crude protein content in the samples from UH and ZV than in PR. In addition, biological N fixation depends on the crops used, as significant differences in N transfer from legumes to cereals can occur. In this case, grain legumes seem to improve crude protein better than forage legumes (Zang *et al.* 2015; Tsialtas *et al.* 2018).

Wet gluten

Wet gluten is an indicator of baking quality and

should be at least 25% (ČSN 46 1100-2). The lower mean value of wet gluten in samples from PR compared to other sites is due to the lower $N_{\min}$ content in the soil, which may affect the crude protein content of the wheat samples. Similar to crude protein content, intercropping with grain legumes (mainly pea) increased wet gluten content. The obtained data indicate that the initial $N_{\min}$ content at each site plays an important role in the wet gluten content of the harvested samples. In fact, $N_{\min}$ content in the arable layer of 0–30 cm was only 2.9 kg N/ha in PR, 14.3 kg N/ha in UH, and 37.5 kg N/ha in ZV. In addition, some legumes also increased the crude protein content, such as Egyptian clover or field pea. The mean wet gluten content also increased under the row-by-row cropping system at PR and UH localities, which could be due to the lower yields under this cropping system as well as better nitrogen uptake, which could benefit from biological nitrogen fixation and its concentration in wheat grains (Liu *et al.* 2022).

Zeleny sedimentation test

The Zeleny sedimentation test is one of the parameters used for the baking quality of wheat according to the ČSN standard EN ISO 5529 (461022) and predicts the baking strength of the flour. According to this standard, minimum sedimentation should be 30 mL. All our wheat samples exceeded 30 mL but, similar to crude protein or wet gluten, lower sedimentation test values were observed in PR than at the other two sites (UH and ZV), where values exceeded 60 mL. In PR, legumes increased sedimentation test values in both cropping systems i.e., in the mixture and row-by-row. At each site, different legumes increased the value of the Zeleny test across both systems, but it was found that red clover, crimson clover and fenugreek in PR and common pea in UH had lower values of Zeleny sedimentation test in comparison with SpW 3. The limiting N availability in organic systems affected many parameters at all sites and the legumes of both types – grain (common pea, field pea, common vetch) and the forage legumes (Egyptian and crimson clover) confirmed the results of Šarunaite *et al.* (2010); Tortorella *et al.* (2013); Chapagain (2014); Zang *et al.* (2015); Tsialtas *et al.* (2018); Vrignon-Brenas *et al.* (2018); Jensen *et al.* (2020); and Liu *et al.* (2022) as

well as possibly improved the values of the Zeleny sedimentation test.

Falling number

The falling number is internationally standardized (ICC 107/1, ISO 3093-2004, AACC 56-81B) and the most widely used method for determining the activity of alpha-amylase; it should not be less than 220 s. The PR and UH sites in all treatments a falling number achieved higher values than 220 s, with higher values under the row-by-row system compared to the mixture. In PR, a higher falling number in comparison with SpW 3 was observed in both systems. Higher values were observed in the row-by-row system. In UH, crimson and red clover together with common kidney vetch showed higher values of falling number compared to the other treatments, including SpW 3. In ZV, on the other hand, the falling number was relatively low and often did not even reach 220 s. In addition, the falling number in the row-by-row system decreased even more. The reasons for the different falling numbers at the individual sites are not clear.

CONCLUSIONS

Intercropping of the spring wheat variety 'Registana' with different types of legumes was evaluated at three localities in the Czech Republic. Preliminary results showed differences in grain yields and quality between the three sites (PR, UH, and ZV). Lower yields were observed in PR compared to samples from UH and ZV. However, legumes in PR increased both yields and quality of grain production. Intercropping in UH and ZV slightly reduced yields due to the competition of legumes with wheat, but the quality of production was mostly higher thanks to the interaction with legumes and N uptake in interaction with legumes. The mixture system appeared to be more effective in grain production than cultivation in separate rows, where greater differences were observed between treatments in yields and quality assessment. Based on the comprehensive evaluation of various sites and multiple parameters, the most favourable and promising combinations involve spring wheat paired with

Egyptian clover, white clover, common vetch, common kidney vetch, and bird's-foot-trefoil.

Acknowledgement: This work was funded by the project NAZV QK1910046 of the National Agency for Agricultural Research of the Ministry of Agriculture of the Czech Republic and partially funded by the Ministry of Agriculture of the Czech Republic (No. RO0423).

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Received: December 22, 2022

Accepted: April 6, 2023