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SUMMARY DISSERTATION THESIS

Wheat and legume mixtures in organic farming as a factor influencing grain quality

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Supervisor: doc. Ing. Petr Konvalina, Ph.D.

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Summary dissertation thesis

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Abstrakt

Ekologické zemědělství se postupně dostává do popředí zájmu; kvantita a kvalita bioproduktů jsou však stále předmětem diskuse. Proto se zkouší různé pěstitelské metody, jak tyto mezery vyplnit. Intercropping představuje alternativní zemědělskou metodu, která stabilizuje výnos plodin a efektivitu využití zdrojů, zejména v zemědělských systémech s nízkými vstupy. Může se jednat o využívání odrůdových směsí téže plodiny (1). Druhou možností je využívání směsí pšenice a leguminóz (2). Ve tříletém pokusu byly provedeny dva typy experimentů, zaměřené na pěstování pšenice seté a leguminóz a také odrůdových směsí pšenice s cílem posoudit výnos a kvalitu zrna pšenice seté v ekologickém zemědělství. Byla potvrzena kladná korelace mezi výnosem zrna pšenice a výnosovými prvky. Naopak negativní korelace byla potvrzena mezi výnosem zrna a jeho kvalitou. Zaznamenána byla kladná korelace mezi výnosem zrna, pekařskou jakostí a reologickými vlastnostmi testovanými na přístroji Mixolab. Významný vliv měl také průběh ročníku a faktor testované odrůdy.

V experimentu (1) s odrůdovými směsmi pšenice seté – ozimé formy byly použity čtyři odrůdy, které byly vysety buď v čistosevu (kontrola) nebo ve dvou variantách směsí – kdy došlo buď k setí tzv. ob řádek nebo bylo osivo smícháno před vlastním setím. Na základě výsledků nebyl zjištěn významný rozdíl mezi různými směsmi s ohledem na výnos zrna, ale pekařská jakost a reologické parametry byly u některých směsí zvýšeny. To ukazuje, že pozitivní efekt pěstování odrůdy s vyšším výnosem zrna (ale s nižší kvalitou) a nižším výnosem zrna (s vyšší kvalitou) se projevil ve stabilním výnosu zrna pšenice a zvýšené kvalitě zrna ve směsích. Přestože mezi odrůdovými směsmi pšenice a jednotlivými odrůdami pšenice nebyl statisticky významný rozdíl ve výnosu a kvalitě zrna, výsledky ukazují, že hodnoty výnosu zrna byly vyšší, zatímco hodnoty kvality zrna byly u jednotlivých odrůd pšenice nižší než u směsí. U méně jakostní odrůdy, jako je Vanessa, byl ve směsi zvýšen obsah bílkovin. Nejvyšší mokrý lepek (18 %) a číslo poklesu (268 s) byly zjištěny u kombinací Butterfly-Lorien a Butterfly-Vanessa, v daném pořadí. Absorpce vody u Butterfly a jejích kombinací vykazovala nejlepší výsledky. Směs Illusion-Vanessa vykazovala nejvyšší stabilitu těsta (7,19 min). Parametry zjištěné na přístroji Mixolab indikovaly, že odrůda Butterfly a její směsi vykazují lepší jakost zrna. Rozdíl ve způsobu setí ve směsích (ob řádek, smíchané osivo) neovlivnil výnos a kvalitu zrna. Potenciál pro zlepšení kvality zrna prezentovaný v disertační práci se může uplatnit v dalším výzkumu. Je ale zapotřebí dále analyzovat, zda efekt závisí spíše na odrůdě pšenice nebo interakcích s ostatními parametry. U směsí pšenice a luskovin (2) výsledky ukazují, že Butterfly vykazovala vyšší kvalitu, ale nižší výnos zrna než odrůda Lorien. Odrůda Butterfly měla pozitivní vliv na zvýšení obsahu bílkovin ve směsích pšenice seté a leguminóz ve srovnání s odrůdou Lorien. Směsi ozimé pšenice a různých druhů leguminóz významně ovlivnily výnos zrna pšenice seté. Směsi pšenice seté a leguminóz (přisеté na jaře) měly nižší výnos zrna než kontrolní varianta (čistosev pšenice seté) a směsi pšenice a leguminóz (seté na podzim). Nejvyšší obsah bílkovin byl zaznamenán u pšenice seté + jarního hrachu přisetého na jaře (10,06 %), následovaný pšenicí setou + jetelem červeným (9,86 %). Současně byl obsah

bílkovin také vyšší u pšenice seté a leguminóz zasetých na podzim než u pšenice seté. Reologické parametry vyhodnocené pomocí přístroje Mixolab. vykazovaly vyšší kvalitu pšenice ve směsích Butterfly a leguminóz, ale významné ovlivnění reologických parametrů nebylo zaznamenáno. Z výsledků disertační práce je patrné, že optimalizace výnosu pšenice seté a pekařské jakosti zrna prostřednictvím směsí pšenice seté a leguminóz vyžaduje volbu řešení založeného na různých agrotechnických postupech, odrůdách pšenice seté a druzích leguminóz.

Klíčová slova: pšenice setá, ekologické zemědělství, směsi odrůd, směs pšenice seté a leguminóz, výnos zrna, pekařská jakost, reologický test, Mixolab, korelační analýza, analýza hlavních komponent.

Abstract

Organic agriculture is gaining prominence worldwide because of its benefits for humans and the environment. In organic wheat farming, enhancement in the quantity and quality of grain is still being debated, and various approaches are being explored to solve these issues. Intercropping is an alternative farming method that maximizes crop yield and resource usage effectiveness, especially in low-input agricultural systems, such as wheat/legume mixtures. Two experiments in a three-year field study were conducted on wheat/legume mixtures to assess grain yield and wheat quality in organic farming. Generally, there was a highly positive correlation between wheat grain yield and yield components, a negative correlation between grain yield and grain quality, and a high correlation between wheat grain yield, baking quality, and rheological properties tested by Mixolab. The weather conditions and cultivars significantly affected grain yield and grain quality.

In detail, (1) in the winter wheat mixtures experiment, four winter wheat cultivars and their mixtures sown with mixed or alternative rows were compared. Based on the results, winter wheat variety mixtures showed no significant difference in grain yield, but baking quality and rheological tested by Mixolab were enhanced in some mixtures. This shows that the positive effect of growing a higher grain yield-lower quality variety and a lower grain yield-higher quality was demonstrated in stable wheat grain yield and increased grain quality in the mixtures. Protein content (PC) in Vannessa was increased in the mixtures compared to Vanessa, which was sown alone. Butterfly-Lorien and Butterfly-Vanessa showed the highest wet gluten (WG) (18 %) and falling number (FN) (268 s). Butterfly and its component mixtures showed better results in the parameter of water absorption (WA). Mixolab parameter indicated that Butterfly and its component mixtures show a better quality variety. Sowing method did not effect wheat grain yield and grain quality. (2) In wheat and legume mixtures, the results show that Butterfly had higher grain quality but lower grain yield and yield components than Lorien. Butterfly cultivar positively affected increasing PC in wheat and legume species mixtures compared to Lorien cultivar. Mixtures of winter wheat and different legume species significantly affected wheat grain yield. Wheat and legume (sown in the springtime) mixtures had lower grain yield than wheat control (sown individual wheat) and wheat and legume (sown in the fall) mixtures. However, the highest PC in wheat + spring pea sown in springtime (Wheat+Sps, 10.06 %) and wheat + red clover (Wheat+Re, 9.86 %). At the same time, PC was higher in wheat + winter pea (Wheat+Wi) and wheat + faba bean (Wheat+Fa) than wheat was sown alone. Rheological parameters evaluated by Mixolab showed greater wheat quality in Butterfly-legume mixtures. Mixed and row-row intercropping of wheat and legume species did not significantly influence rheological properties. To conclude, customizing wheat yield and grain quality under the effect of winter wheat and legume mixtures requires considering the optimal solution based on different cultivates, wheat varieties and legume species to achieve the desired response.

Keywords: winter wheat, Mixolab test, intercropping, wheat/legume mixtures, grain yield, grain quality, correlation analysis, principal component analysis, organic agriculture.

Contents

Introduction

Bread wheat (*Triticum aestivum* L.) is one of the most important cereal crops grown worldwide. It is a staple food for over one-third of the world's population. Improving wheat production and quality in a healthy and environmentally responsible manner is a major agricultural concern because of shifting climatic conditions and environmental effects from agricultural activities. On the other hand, people are becoming more conscious of the safe and healthful food produced. Therefore, organic farming is interested in development. However, there are several obstacles to organic agricultural methods for producing wheat, such as low yield and reduced grain quality. These challenges result from several factors, such as the lack of nutrient availability, the pressure of pests and diseases, and the understanding of crop and soil management. Therefore, producing organic wheat necessitates comprehending alternative agriculture production systems and the effectiveness of agricultural inputs that maximize nutrients, and pest and disease protection management strategies to enhance healthy plants, yield, grain quality and a sustainable environment. In this context, the diversity of cropping systems in agriculture practices is becoming attractive for enhancing natural interactions and increasing agroecosystem resilience. Intercropping or mixing is a strategic solution to this issue. For example, each wheat variety is susceptible to several stresses that can cause fluctuations in yield. Improving crop genetic diversity through cultivating a mixture of cultivars can mitigate the combined effects of frost and heat in adverse environments, reduce the incidence of diseases and resilience to pest and disease pressure, inhibit crop lodging, and increase crop yield and stability.

On the other hand, crop diversification via intercropping increases the activity of microorganisms, which is advantageous in the soil, can regulate the biogeochemical soil cycles, and improve soil fertility, e.g., intercropping with legume crops. Cereal-legume intercropping is a practical and valuable agronomic wheat-growing strategy because a high amount of fixed N supplied into the system through the N-fixation of legume could increase grain quality.

Furthermore, intercropping supports biodiversity by producing a more diverse habitat for the population. Mixing crops provides a more varied and balanced feed for these organisms, improving the area's ecological balance. Ecological intensification with tools like intercrops will be necessary to enhance potential crop protection, resilience to climate change, and lower agriculture's carbon footprint.

Through an analysis of the wheat grain yield and grain quality effects of several winter wheat/legume mixtures, our goal was to shed light on the possibilities of various approaches towards achieving high-quality food production and sustainable organic farming. The study's findings will contribute to developing potential strategies, enabling farmers and policymakers to make informed decisions that promote organic wheat production's sustainable agriculture.

1 Literature Review

Wheat is a staple crop that feeds millions worldwide (Erenstein et al., 2022). It provides one of the main sources of vegetable protein and carbohydrates for humans (Iqbal et al., 2022). In 2022, the wheat area harvested worldwide was 219 million ha, grain yield was 3.7 t ha^{-1}, and production was 808 million tonnes (Faostat, 2022). In Czech Republic, the most important cereal grown is winter wheat, which covers over a fourth of the total area under crops (28.9 %), more than 854 thousand hectares and grain yield reached 6.1 t ha^{-1} in 2022 (Harvest Estimates, 2021).

Organic farming is more sustainable than conventional farming because it has fewer negative environmental and health impacts (Gamage et al., 2023). Despite its many advantages, organic winter wheat farming is characterised by low soluble nitrogen availability, reducing yields and grain quality (Ceseviciene et al., 2009; Konvalina et al., 2007). Nitrogen is one of the crucial macroelements, as it notably influences wheat yield and baking quality (Lacko-Bartosova et al., 2021). Mineral N fertilizers are used in split applications during the growing season to meet crop N requirements while reducing the risk of N loss. However, this practice is not allowed in organic cereal farming. Splitting the rate of organic fertilizers is often not feasible in organic wheat cultivation, as most organic fertilizers need to be broadcast all at once before sowing, resulting in an insufficient N supply to wheat, which can be detrimental to plant growth, yield, and grain quality (Guiducci et al., 2018). Even in the case of top-dress application, the amount of N availability from organic fertilizers could be low because of slow-decomposition processing during the winter time, causing a limited grain yield (Tosti et al., 2016) and the grain protein content to be low due to lower winter N accumulation in vegetative organs (Giuliani et al., 2015).

On the other hand, pests such as rodents and insects can destroy crops without control. However, many chemicals are not allowed in organic farming because they are not of natural origin and damage the environment to protect crops. Moreover, organic farming requires much knowledge because it uses a systems approach to control fertility, weeds, and pests rather than relying on off-farm inputs. Despite excellent returns, organic grain production lags (Gamage et al., 2023).

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 (Ceseviciene et al., 2009). 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 in integrating high grain yield and high grain protein due to their inverse relationship (Chen et al., 2020; Iqbal et al., 2016), which simultaneously take a long time and are expensive (Hoang et al., 2022; Konvalina et al., 2007). The key to fixing this issue is drawing attention to suitable farming methods, such as cropping with various wheat and legume mixtures. Grain quality characteristics have been evaluated in wheat cultivar mixtures, showing promising but inconsistent results in enhancing these characteristics (Faraji, 2011).

Intercropping is a multiple-cropping practice that involves two or more crops simultaneously on the same field and is one of the simplest ways to diversify agriculture (Huss et al., 2022). There are some approaches to intercropping, for example, mixed intercropping, row intercropping (planting plants in alternating rows with the main crop, strip intercrops, and relay intercropping (Glaze-Corcoran et al., 2020; Huss et al., 2022). These various types of complementary intercrops can efficiently utilize space, water, and nutrients (Xu et al., 2020), smother weeds (Verret et al., 2017), improve natural suppression of pests (Daryanto et al., 2020; Staton et al., 2019), and enhance overall crop productivity relative to conventional crop monocultures (Li et al., 2020).

Mixtures of wheat varieties are a viable strategy for sustainable products to help greater stability or reduced yield, promoting higher bread quality yields in organic farming compared to single systems (Kaut et al., 2009). Each wheat variety is susceptible to several stresses that can cause fluctuations in yield. Combining varieties with complementary traits can help yield stability where stresses occur unpredictably (Faraji, 2011). The superiority of cultivar blendings over pure-line cultivars has been observed in numerous crops by choosing cultivars that complement each other to perform important traits to meet specific production requirements (Faraji, 2011). There is abundant evidence that improving crop genetic diversity through cultivating a mixture of cultivars affects crop productivity and stability (Borg et al., 2018; Fletcher et al., 2019; Hoang et al., 2023, 2022; Huss et al., 2022; Kiær et al., 2009; Kong et al., 2023; Kristoffersen et al., 2020; Reiss and Drinkwater, 2018; Renard and Tilman, 2019; Vestergaard and Jørgensen, 2024).

In terms of productivity, wheat mixtures tend to outperform; however, it is often restricted to a moderate range between one and five per cent (Borg et al., 2018; Kristoffersen et al., 2020). A long trial by Kristoffersen et al. (2020) showed an increase of 1.4 % in yield under mixing different varieties compared to those grown in pure stands. Similarly, Borg et al. (2018) found a significant global average yield increase of 3.5 %, while Faraji (2011) showed an overall meta-estimate of wheat variety mixtures effect at 2.7 %. While most studies exhibit an overall positive impact on mixing varieties, Hoang et al. (2023, 2022) found that two-way variety mixtures yielded lower than the mean of the constituent varieties. This is similar to Dai et al. (2012), where mixtures of spring wheat varieties have no success in terms of grain yield compared with wheat varieties grown in monocropping systems.

Wheat-legume intercropping is a cropping practice with the advantage of the complementary use of nitrogen sources by the species used in the system (Bedoussac and Justes, 2010; Dahlin et al., 2020). On the other hand, wheat and legume mixtures enhance diversity in the ecosystem, positively affecting weed control, enhancing soil fertility, and increasing the N use efficiency. This can effectively increase grain yield and quality compared to the monoculture systems (Dvořák et al., 2022; Jensen et al., 2020; Monti et al., 2019; Voisin et al., 2014; Weih et al., 2021). A high amount of fixed N is provided into the system through the N-fixation of legume, which is available for cereals (Amossé et al., 2014; Bedoussac et al., 2015; Dvořák et al., 2022). However, the results of the intercropping system depend on factors such as environmental conditions, crop species, sowing and management practices

(Bedoussac et al., 2015; Brooker et al., 2015). According to previous studies, using a diversity of legume species in the wheat-legume intercropping system is considered a notable advantage (Alonso-Ayuso et al., 2014; Costanzo and Bàrberi, 2014). In the case of simultaneous intercropping, it may be necessary to consider additional management practices to address potential competition between legumes and cereal crops (Verret et al., 2017). These may include weed and disease control measures and mechanization of cultivation practices (Brooker et al., 2015). This appears mainly suitable for an increase in grain quality and yield through increased protein at harvest (Mohammed et al., 2013). Some authors have shown that simultaneous intercropping might limit or reduce the cereal yield in a case of vigorous growth or high density of the legume, resulting in competition for nutrients and light (Bergkvist, 2003; Dvořák et al., 2022; Lithourgidis et al., 2011). An alternate technique may be considered where the cereal and legume are sown in separate rows alternately (Tosti and Guiducci, 2010). An increase in the protein concentration of wheat grain in intercrops could be of economic benefit when selling wheat for breadmaking, as well as the aim of end-use for the main crop in the systems if the legume crops were also used effectively.

2 Aim and Hypothesis

2.1 Aim of dissertation

The debate on the suitability of mixtures of winter wheat varieties and mixtures of winter wheat varieties with different legume species in organic farming provided the impetus for this dissertation. Therefore, a three-year field study was conducted in the organic-certified field at Zvíkov, České Budějovice, Czech Republic to:

(1) To assess yield stability and wheat quality (protein content, wet gluten, gluten index, falling number, Zeleny sedimentation value and rheological parameters evaluation by Mixolab) of mixtures of winter wheat varieties in organic farming.

(2) To assess yield stability and wheat quality (protein content, wet gluten, gluten index, falling number, Zeleny sedimentation value and rheological parameters evaluation by Mixolab) of mixtures of winter wheat varieties and different legume species in organic farming.

The wheat grain samples were determined in the laboratory of the Department of Agroecosystems, Faculty of Agriculture and Technology, University of South Bohemia in České Budějovice, Czech Republic.

2.2 Working hypothesis

Growing winter wheat/legume mixtures will lead to stabilisation and increase grain yield, improving the technological quality of wheat grain in organic farming.

The statistical analysis of results will find the potential for sustainability of wheat organic cultivation in the Czech Republic.

3 Materials and Methods

3.1 Study site description

A three-year field study was conducted in the organic-certified farm during three growing seasons consecutive from 2019 to 2022 (2019/20, 2020/21, and 2021/22) at Zvíkov, České Budějovice, Czech Republic (48°58’26.4’’N, 14°37’43.5’’E, at an altitude of 460 m).

According to the USDA soil classification, the experiment’s soil field was Silt Loam. The physicochemical properties of the experiment’s soil with 18 % of sand (0.05–2 mm), 59 % of silt (0.05–0.002 mm), and 23 % of clay (<0.002 mm); pH(H_2O): 5.7, pH($CaCl_2$): 5.1, bulk density: 1.3 g cm^{-3}, total organic carbon (TOC): 1.97 %, NO_3^-: 48 mg kg^{-1}, NH_4+: 16.2 mg kg^{-1}, P: 59 mg kg^{-1}, K: 235 mg kg^{-1}, electrical conductivity: 0.0932 dS/m.

The mean annual air temperature throughout three growing seasons in Zvíkov is 9.1°C, higher than 1.9°C compared to a long-term period (1989 – 2019), and the total rainfall was 647 mm compared to 633 mm. The mean annual air temperature during the experiment was recorded at 9.5°C in 2019/20, 8.4°C in 2020/21, and 9.3°C in 2021/22 growing season. Each year's total rainfall was 699 mm, 633 mm, and 607 mm in the 2019/20 growing, 2020/21 growing, and 2021/22 growing seasons, respectively. In the 2020/21 growing season, heavy rain was recorded the whole time after sowing, and the temperature was lower than in January and February 2021 compared to the 2019/20 and 2021/22 growing seasons.

3.2 Field management

According to the principle of organic farming, the experiment did not apply pesticides or herbicides. The legume crop, red clover (cv. Bonus), was grown as a preceding crop for the experiment. Before ploughing, composted sheep manure was applied to the soil at 4 t ha^{-1}. The characteristics of organic fertilizer with 28 % content dry matter were 8.9 kg N t^{-1}, 5.4 kg P_2O_5 t^{-1}, and 17.7 kg K_2O t^{-1}.

3.3 Materials

The experimental material consisted of four winter wheat varieties (*Triticum aestivum* L., cv. Butterfly, Illusion, Lorien, and Vanessa) and five legume species (Faba bean - *Vicia faba* L., cv Merkur; Spring pea - *Pisum sativum* L., cv. Avatar; Winter pea - *Pisum sativum* L., cv. Balltrap; Incarnate clover - *Trifolium incarnatum* L., cv. Kardinál; Red clover - *Trifolium pretense* L., cv. Meadow).

3.4 Experiment design and treatment

The small plot experiment was arranged with a randomized complete block experiment design with three replicates, 15 m^2 for each plot. Two experiments were conducted, including (1) *wheat varieties mixtures* and (2) *wheat and legume mixtures*.

3.4.1 Wheat varieties mixtures experiment

All winter wheat varieties were used to assess grain yield stability, wheat quality, and environmental impacts for each variety and their mixtures. Four winter wheat varieties (Butterfly, Illusion, Lorien, and Vanessa) were sown in a classic row (12.5 cm) as control treatments, and eight mixtures were sown in mixed and row intercropped with 12.5 cm row spacing applied. In the mixed intercropping variant, winter wheat seeds were mixed before sowing. Winter wheat was sown for the experiment at a rate of 400 seeds m^{-2}.

3.4.2 Wheat and legume mixtures experiment

The experiment was established in which mixtures of wheat (Butterfly and Lorien varieties) and legume species were sown in different sowing methods and times. Butterfly and Lorien were sown alone as control treatments. The leguminous species included faba bean, incarnate clover, spring pea, winter pea, and red clover. Depending on the specific variant, the experiment was sown in a classic row (12.5 cm, wheat and leguminous seeds mixtures) and the row-row variant (wheat and leguminous in separate rows). Specifically, it was the autumn sowing of wheat and legumes (faba bean, incarnate clover, spring pea, winter pea) in the form (1) under-sowing in classic rows (mixed, the legumes remained in the plots for the entire growing season) and (2) sowing wheat and legumes in row-row variant (removing of legumes sown in springtime). In addition, two legume species (red clover and spring pea) were tested and intercropped in the plots without leguminous seed sown in autumn, both in a classic row and row-row in the springtime. Winter wheat varieties were sown at a rate of 400 seeds m^{-2}, faba bean at a rate of 30 seeds m^{-2}, spring pea and winter pea at a rate of 50 seeds m^{-2}, and incarnate clover and red clover at a rate of 300 seeds m^{-2}.

3.5 Evaluation of grain yield and quality

3.5.1 Agronomy characteristics

Agronomy traits as indexes grain yield and harvest parameters were assessed for each replication of experiments, including plant height (PH), spike number m^{-2} (No. m^{-2}), thousand kernel weight (TKW), hectoliter weight (HW), and wheat grain yield (t ha^{-1}). Whole plants were sampled from the centre of each replication, and grain samples were dried to a constant weight at 14 % moisture.

3.5.2 Baking quality

Within the wheat/legume mixtures experiment, all the wheat grain samples without legume grain were collected from each plot. PSY 20 (Mezos, Hradec Králové, Czech Republic) and Quadrumat Junior (Brabender, Duisburg, Germany) apparatus were used to mill the wheat flour samples. The properties of the resulting flour are determined by the parameters and methods below.

3.5.2.1 Hectoliter weight (HW)

Hectoliter weight measures the weight of a given volume of grain in kilograms per hectolitre (kg hL^{-1}). HW was measured using the Dickey-John GAC500XT apparatus.

3.5.2.2 Protein content (PC)

Kjeldahl technique (Kjeltec 1002 System, Tecator AB, Hoganas, Sweden) was used to calculate PC based on N * 5.7 (in dry matter).

3.5.2.3 Wet gluten (WG) and gluten index (GI)

The WG and GI were estimated by Glutomatic 2200 and Centrifuge 2015 (Perten Instruments, Hägersten, Sweden), according to ICC Standard No. 137/1, GI according to AACC/No. 38-12.02, ICC/No. 155 & 158, ISO 21415.

3.5.2.4 Falling number (FN)

Falling number was determined by Falling Number 1100 (Perten Inst., Sweden) according to AACC/No. 56-81.03, ICC/No. 107/1, ISO/No. 3093.

3.5.2.5 Zeleny sedimentation value (ZSV)

Zeleny sedimentation test is a technique based on the tendency of endosperm storage materials to expand and flocculate in a lactic acid solution that is extensively used to evaluate wheat gluten strength (Alemu et al., 2021). The SDZT4 apparatus was used to determine the Zeleny sedimentation value according to the ICC standard No. 116/1.

3.5.2.6 Rheological evaluation of dough by Mixolab

Mixolab (CHOPIN Technologies Mixolab II, Villeneuve-la-Garenne, France) was used to evaluate the rheological properties of wheat flour, such as the consistency of the dough during mixing and analyze the quality of the protein and starch, as well as assess the impact of enzymes, according to the ICC standard method No. 173-ICC 2006.

3.6 Statistical analysis

All statistical analyses were performed in JMP v. 14 (SAS, NC, USA) software. Tukey’s HSD (Honest Significant Difference) test was performed with a significance level of $P < 0.05$. In wheat variety mixtures experiment, statistical analysis was conducted to determine the effect of the growing season, winter wheat varieties mixtures, and sowing method. Principal component analysis (PCA) and correlation analysis were used to determine parameters' correlations. In wheat and legume mixtures experiment, statistical analysis was performed to assess the effect of growing season, winter wheat varieties, sowing method, and legume species management. Dunnett’s test was used to analyze the combined effect of the sowing method and wheat + legume species mixtures relative to wheat sown alone as the control for grain yield, PC, and WG each year. Dunnett’s test was used to analyze the effect of Butterfly/Lorien and legume species mixtures relative to Butterfly/Lorien sown alone as the control for grain yield and PC. Principal component analysis and correlation analysis were also used to determine parameters' correlations between grain yield, yield components and grain quality.

4 Results and Discussion

In this study, I focused on wheat grain yield and quality and separated these two parts based on the results of the wheat grain yield and grain quality effects of several winter wheat/legume mixes throughout three-year experiments. In the first part, wheat yield and grain quality results were reported under the effect of wheat varieties mixtures (Section 4.1). The next part evaluates the results of the influence of growing wheat with various legumes on the qualitative production parameters and wheat quality reported (Section 4.2).

4.1 Agronomic parameters, grain quality under effects of wheat variety mixtures

4.1.1 Plant height, yield components and grain yield

The growing season significantly affected grain yield, PH, TKW, spike number, and HW; however, the sowing method and the combination did not significantly affect grain yield, PH, TKW, spike number, and HW. In detail, the trend of season performance was 2020/21 < 2021/22 < 2019/20 for PH, TKW and the spike number. The grain yield and HW were higher in the 2019/20 and 2021/2022 seasons than in the 2020/21 season.

Plant height is an important agronomic characteristic that influences wheat morphogenesis and grain production. Genotype is the main factor influencing plant height. In this study, all winter wheat varieties in this experiment belong to medium plant height (KatalogSelgen, 2023; Konvalina et al., 2007). Plant height was lowest in the second cropping season at 67.6 cm, followed by the third at 84.7 cm and the first season at 92.1 cm. The difference in PH during the growing seasons, with the lowest in the second growing season, is affected mainly by weather conditions, which is our case. The sowing method and combination had no significant influence on PH. PH was affected under the sowing method (winter wheat was sown alone, wheat varieties mixtures - mixed intercropping, and wheat varieties mixtures - row-row intercropping), and PH ranged from 80.8 to 82.2 cm. PH in different wheat varieties and their mixtures fluctuate by 74.4 to 86.6 cm. PH of Vanessa and Illusion were lower, which is why their mixtures are lower than those of Butterfly, Lorien and their mixtures (Table 4.1).

Table 4.1 shows a significant difference between the growing season and sowing method, but there was no significant difference by combination in terms of spike number m^{-2}. The spike number m^{-2} in the second season was lowest at 290.1 spike m^{-2} compared to 354.3 spike m^{-2} and 414.1 spike m^{-2} in the third and the first seasons, respectively. Weather conditions are the main reason for lower grain germination and leaching nutrition by heaving rainfall in the second season, so lower plant density and nutrition result in lower tiller production and lower spikes m^{-2}. In the sowing method case, wheat variety sown in a separate row (row-row, 383.7 spike m^{-2}) indicated the spike number m^{-2} higher than in wheat sown alone (control, 330.8 spike m^{-2}) and mixed (344.1 spike m^{-2}). There was no significant influence under the effect of the combination. Vanessa had a lower plant height but a higher spike number m^{-2} than other varieties. This is mainly due to genetic factors.

Table 4.1: Winter wheat growth and grain yield under the effect of the growing season, sowing method, and wheat variety mixtures (combination).

Variant	Plant height (cm)	Spike number (No. m^{-2})	TKW (g)	Yield (t ha^{-1})	HW (kg hL^{-1})
Season					
2019/20	92.06±0.82^{a}	414.11±13.92^{a}	50.38±0.33^{a}	5.75±0.19^{a}	72.26±0.33^{a}
2020/21	67.57±1.16^{c}	290.28±12.32^{c}	41.34±0.57^{c}	2.52±0.10^{b}	67.90±0.50^{b}
2021/22	84.72±1.32^{b}	354.27±12.62^{b}	43.30±0.49^{b}	5.69±0.22^{a}	72.95±0.37^{a}
p-Value	***	***	***	***	***
Sowing method					
Control	80.84±2.32	330.83±15.03^{b}	45.62±0.88	4.84±0.32	71.30±0.63
Mixed	82.15±2.04	344.14±16.55^{b}	45.10±0.79	4.70±0.36	70.97±0.49
Row-Row	81.35±1.79	383.69±13.46^{a}	44.30±0.73	4.43±0.24	70.85±0.53
p-Value	ns	**	ns	ns	ns
Combination					
Butterfly	82.54±4.62	304.47±27.73	45.89±0.95	4.37±0.57	71.79±2.09
Illusion	79.87±4.05	320.63±34.29	45.97±1.45	4.94±0.71	73.92±0.67
Lorien	86.57±4.29	311.84±20.78	48.61±1.95	4.78±0.65	69.99±0.40
Vanessa	74.39±5.29	386.38±32.30	41.99±1.99	5.26±0.74	69.49±0.58
BuLoMi	86.00±3.69	282.02±30.21	47.12±1.29	4.45±0.74	71.29±1.25
BuVaMi	80.91±4.49	387.29±36.24	43.60±1.42	4.96±0.65	70.57±1.18
BuLoRo	85.78±3.69	345.82±21.97	46.03±1.22	3.85±0.47	70.73±1.27
BuVaRo	79.01±3.70	414.38±23.53	43.16±1.62	4.68±0.58	70.23±1.58
IlluLoMi	85.12±3.83	336.41±27.41	46.15±1.19	4.44±0.79	71.17±0.69
IlluVaMi	76.58±4.16	370.84±31.72	43.52±1.59	4.94±0.77	70.84±0.92
IlluLoRo	84.59±5.59	356.77±25.76	45.08±1.03	4.30±0.33	71.06±0.55
IlluVaRo	76.01±3.79	417.77±30.62	42.94±1.84	4.88±0.48	71.38±0.60
p-Value	ns	ns	ns	ns	ns

Mean ± standard error (SE). TKW, thousand kernel weight; HW, hectoliter weight; Different letters within the column show a statistical difference at *p*-Value < 0.05, Tukey HSD test was used. ns (non-significant); *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Table 4.1 indicated that TKW varied between 50.4 and 41.3 g among different wheat varieties and their mixtures. Similar to PH and spike number, the significantly highest TKW (50.4 g) was exhibited by the grain of wheat harvested in the first season, followed by the third harvested (43.30 g), while significantly lowest in the second harvested (41.3 g). In this study, there was no significant difference in winter wheat under the effect of the sowing method and combination in the case of TKW. In combination variants, the TKW ranged from 42.0 to 48.6 g, which was higher than the results of Tran (2021).

In this investigation, HW was significantly different under the effects of growing seasons and was not influenced by the sowing method and combination. The HW was lower in the 2020/21 growing season than in the 2019/20 and 2021/22 growing seasons. HW among sowing method and combination variants ranged between 69.5 and 71.8 kg hL^{-1}, lower than the suggested minimum test weight of 76 kg hL^{-1} for classed as the top wheat quality (Manley et al., 2009).

The effect of weather conditions results in reduced growth and development of wheat, so it influences our study's poor yield and yield component. This is similar to the survey by Konvalina et al. (2009), who reported that differences in variety and growing conditions influenced wheat yield. Identical to Tran's (2021) study, our results showed that grain yield was influenced by the growing season. The grain yield in the 2020/21 growing season was 2.5 t ha^{-1}, lower than 5.7 t ha^{-1} and 5.8 t ha^{-1} in the 2021/22 and 2019/20 growing seasons, respectively. While most studies exhibit an overall positive effect on mixing varieties, our study found that two-way variety mixtures yielded lower than the mean of the constituent varieties; however, there was no significant statistical difference. In detail, the grain yield in control was 4.84 t ha^{-1}, while mixing was 4.70 t ha^{-1} and 4.43 t ha^{-1} for mixed and row-row sowing. In the combination variant, the grain yield ranged between 3.85 t ha^{-1} and 5.26 t ha^{-1}.

4.1.2 Grain quality

One of the main objectives of modern agriculture is to obtain a satisfactory yield of wheat grain with high values for indicators of its quality, with protein content being the most important trait. In our experiment, under the effects of the growing season, sowing methods and combination, PC ranged from 8.31 to 9.60 % (Table 4.2). PC in the third growing season was 9.03 % lower than in the first and second growing seasons, at 9.22 % and 9.49 %, respectively. The influence of growing season on PC was also reported by Tran (2021); PC ranged from 10.8 to 11.8 %, higher than our study in the same location but with different cultivars.

Consistent with previous studies, in some cases, the mixtures improved grain PC and bread-making quality (Finckh et al., 2000), e.g., Vanessa has a low baking quality, but the mixtures enhanced in this study (Table 4.2, 9.26 % and 9.09 % PC was higher in mixtures BuVa and IlluVa than Vanessa grown alone). Chen et al. (2020) reported that PC was higher under two varieties of mixtures, but increases were relatively small (0.2 %) than individual components. Similarly, Lazzaro et al. (2018) indicated mixtures of cultivars also improved grain PC. PC was significantly different under wheat sown alone and their mixtures (Table 4.2), ranging from 8.3 to 9.6 %, and increases were relatively small in mixtures (0.15 % higher). Although Vanessa had a higher grain yield, it had the lowest PC at 8.3 %; however, its mixtures showed improved protein content in the wheat grain (PC > 9 %). The combination improved PC compared to individual varieties. Mixed and row-row did not significantly affect wheat grain PC and WG (Table 4.2).

Table 4.2: Grain quality of winter wheat under the effect of the season, sowing method, and wheat variety mixtures (combination).

Variant	Protein content (%)	Wet gluten (%)	Gluten index	Falling number (s)	ZSV (mL)
Season					
2019/20	9.22±0.10[ab]	15.93±0.64	70.30±2.28[b]	247.92±5.66[a]	17.78±1.07[b]
2020/21	9.49±0.07[a]	15.89±0.59	88.73±0.32[a]	240.31±2.64[ab]	22.04±0.77[a]
2021/22	9.03±0.08[b]	17.15±0.39	74.05±0.65[b]	227.53±4.52[b]	22.43±1.08[a]
p-Value	**	ns	***	**	***
Sowing method					
Control	9.15±0.10	16.22±0.77	79.77±2.16[a]	242.14±5.02	21.51±1.47
Mixed	9.28±0.08	16.47±0.42	73.11±2.37[b]	231.67±4.40	20.58±0.72
Row-Row	9.30±0.09	16.27±0.41	80.20±1.98[a]	241.94±4.36	20.52±0.77
p-Value	ns	ns	**	ns	ns
Combination					
Butterfly	9.60±0.06[a]	18.12±0.58[a]	81.15±4.07[ab]	258.33±13.20[a]	30.86±1.48[a]
Illusion	9.47±0.12[a]	17.86±1.57[a]	72.27±4.87[ab]	215.44±9.41[c]	25.22±2.04[ab]
Lorien	9.22±0.15[a]	16.65±0.54[ab]	75.70±3.29[ab]	239.67±4.89[abc]	20.31±0.79[bcd]
Vanessa	8.31±0.09[b]	12.26±2.13[b]	89.94±2.91[a]	255.11±3.19[ab]	9.67±0.82[e]
BuLoMi	9.48±0.11[a]	18.03±0.66[a]	68.02±4.93[ab]	239.44±6.29[abc]	23.38±1.83[bc]
BuVaMi	9.24±0.12[a]	14.97±0.62[ab]	75.88±4.01[ab]	254.89±7.15[ab]	20.39±0.93[bcd]
BuLoRo	9.52±0.25[a]	15.93±0.83[ab]	82.27±3.25[b]	245.78±5.62[abc]	24.72±0.50[ab]
BuVaRo	9.28±0.18[a]	15.06±1.19[ab]	85.09±3.04[ab]	267.67±8.61[a]	20.19±1.06[bcd]
IlluLoMi	9.40±0.15[a]	16.43±1.27[ab]	75.16±4.99[ab]	220.89±7.90[bc]	21.97±0.57[bcd]
IlluVaMi	9.00±0.20[ab]	16.46±0.38[ab]	73.37±5.29[ab]	211.44±6.69[c]	16.58±1.80[d]
IlluLoRo	9.22±0.15[a]	17.57±0.51[a]	75.47±2.99[ab]	219.22±8.04[c]	19.11±2.07b[cd]
IlluVaRo	9.18±0.12[a]	16.51±0.42[a]	77.97±5.74[ab]	235.11±2.70[abc]	18.06±1.29[cd]
p-Value	***	**	*	***	***

Mean ± standard error (SE). ZSV, Zeleny sedimentation value. Different letters within the column show a statistical difference at *p*-Value < 0.05, Tukey HSD test. ns (non-significant); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Wet gluten ranged from 15.9 % to 17.2 % under the effect of the growing season and from 16.2 % to 16.5 % under the impact of the sowing method (Table 4.2). Similarly, Tran (2021) indicated significant differences in the growing season and cultivars on WG and GI. Growing season, sowing method, and wheat variety mixtures significantly affected WG and GI (Table 4.2). Similar to PC, the lowest WG for Vanessa was sown alone (12.13 %), but that was enhanced in its mixtures (>15 %). In this study, WG was lower in Vanessa than in the Butterfly and Illusion varieties, and the combination of IlluVaRo increased WG compared to individual Vanessa sown. GI in each variety did not significantly differ with their combination, and only BuLoRo was lower than Vanessa (Table 4.2).

Weather conditions significantly influenced FN, a higher FN in the 2019/20 growing season and the 2020/21 growing season than the 2021/22 growing season. The FN ranged from 227.5 s to 247.9 s. Wheat grain for bread production should have FN of 250–350 s, the FN under the effect of harvested year and sowing method < 250 s in our results (Table 4.2). In wheat variety mixtures, FN was lower than 250 s (except for Butterfly and Vanessa, and their mixtures were in row-row and mixed-sown methods). FN in Illusion and its mixtures were lower than in Butterfly and Butterfly another variety mixtures. Illusion (215.44 s) and its combination (211.44 s, 220.89 s, and 219.22 s for IlluVaMi, IlluLoMi, and IlluLoRo, respectively) were lower than in Butterfly (258.33 s) and BuVa combination both mixed (254.89 s) and row-row (267.67 s) in the FN parameter (Table 4.2).

ZSV was lower in the first growing season (17.8 mL) than in the second growing season 22.04 mL) and the third growing season (22.4 mL). This contrasts with the findings of Tran (2021), who reported that the growing season did not influence the SDS test in the previous experiment location. A high correlation between PC and ZSV was found in this study (Table 4.5), similar to reports by Hoang et al. (2022), Kristensen et al. (2019), and Tran (2021). This indicates that Vanessa has a higher grain yield but lower grain quality; however, the improvement of those traits is enhanced in its mixtures. Vanessa had the lowest ZSV (9.67 mL), Butterfly had the highest ZSV (30.86 mL), and their mixtures had a higher ZSV of 20 mL (Table 4.2). We might confirm that grain quality could improve in the winter wheat variety mixtures, although grain yield depends on end-use.

4.1.3 Rheological of dough evaluated by Mixolab

Growing season significantly affected TC2, TC3, TC4, TC5, alfa, beta, and gamma but did not significantly difference WA, Time C1, amplitude, and stability (Tables 4.3a and 4.3b). WA, Time C1, and amplitude ranged from 61.36 to 61.42 %, 2.22 to 2.42 min, and 0.073 to 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 beta were higher in the 2019/20 growing season and 2020/21 growing seasons than in the 2021/22 growing seasons. In contrast, slope alfa was higher in the 2021/22 growing season compared to the first and second growing seasons. Slope gamma was higher in 2019/20 growing season than in the 2020/21 growing season and 2021/22 growing season. 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.

The results for rheological properties, water absorption (WA), dough development time (Time C1), amplitude, and stability investigated cultivars, and their mixtures are presented in the first stage (Mixolab application handbook, 2012). WA measures the amount of water a given flour can absorb. In this study, Butterfly showed a WA higher than other individual cultivars (Illusion, Lorien, and Vanessa). Butterfly and Lorien/Vanessa mixtures were higher than Illusion and Lorien/Vanessa mixtures (Table 4.3a).

Table 4.3a: Rheological properties of dough by Mixolab tested of wheat under the effect of the season, sowing method, and wheat variety mixtures (combination).

Variant	WA (%)	Time C1 (min)	Amplitude	Stability (min)	γ
Season					
2019/20	61.42±0.29	2.22±0.15	0.08±0.0029	5.38±0.16^{b}	-0.106±0.0045^{b}
2020/21	61.36±0.50	2.25±0.17	0.07±0.0029	6.64±0.20^{a}	-0.091±0.0035^{a}
2021/22	61.40±0.32	2.42±0.10	0.07±0.0026	4.96±0.16^{b}	-0.080±0.0041^{a}
p-Value	ns	ns	ns	***	***
Sowing method					
Control	61.55±0.55	2.16±0.13	0.08±0.003	5.19±0.16^{b}	-0.091±0.005
Mixed	61.25±0.27	2.31±0.12	0.07±0.003	5.74±0.22^{ab}	-0.095±0.004
Row-Row	61.38±0.23	2.43±0.17	0.07±0.002	6.06±0.22^{a}	-0.092±0.003
p-Value	ns	ns	ns	**	ns
Combination					
Butterfly	66.49±0.77^{a}	2.15±0.27^{bcd}	0.066±0.004^{cde}	5.77±0.34^{ab}	-0.099±0.01
Illusion	60.61±0.55^{c}	1.62±0.08^{d}	0.097±0.002^{a}	5.23±0.35^{b}	-0.106±0.01
Lorien	59.81±0.37^{c}	1.92±0.18^{cd}	0.090±0.006^{ab}	4.66±0.26^{b}	-0.091±0.01
Vanessa	59.29±0.28^{c}	2.95±0.24^{abc}	0.066±0.003^{cde}	5.09±0.24^{b}	-0.069±0.01
BuLoMi	62.64±0.42^{b}	2.14±0.19^{bcd}	0.071±0.005^{bcde}	6.06±0.50^{ab}	-0.094±0.01
BuVaMi	62.52±0.29^{b}	3.05±0.27^{ab}	0.054±0.003^{e}	5.20±0.50^{b}	-0.085±0.01
BuLoRo	62.58±0.22^{b}	2.26±0.27^{bcd}	0.069±0.005^{cde}	5.74±0.24^{ab}	-0.088±0.00
BuVaRo	62.67±0.28^{b}	3.51±0.40^{a}	0.060±0.003^{de}	6.27±0.40^{ab}	-0.085±0.01
IlluLoMi	59.91±0.18^{c}	1.93±0.10^{cd}	0.083±0.004 ^{abc}	5.33±0.29^{b}	-0.094±0.01
IlluVaMi	59.93±0.31^{c}	2.10±0.22^{bcd}	0.073±0.004^{bcde}	6.37±0.44^{ab}	-0.106±0.01
IlluLoRo	60.04±0.16^{c}	1.88±0.16^{cd}	0.081±0.005^{abc}	5.04±0.42^{b}	-0.095±0.01
IlluVaRo	60.22±0.13^{c}	2.09±0.16^{bcd}	0.077±0.004^{bcd}	7.19±0.40^{a}	-0.098±0.01
p-Value	***	***	***	***	ns

Mean ± standard error (SE). WA, water absorption; Time C1, time development of C1. Different letters within the column show a statistical difference at *p*-Value < 0.05, Tukey HSD test. ns, non-significant; **p* < 0.05; ***p* < 0.01; ****p* < 0.001.

The first stage of Mixolab analysis supplies information about the mixing properties related mainly to gluten quality and the behaviour of the flour protein complex, which is impacted by thermomechanical changes (Lacko-Bartosova et al., 2021; Tran, 2021). The dough development time (Time C1) is an essential index known as the dough development or the gluten development time. Longer Time C1 was observed in mixtures (3.51 min, BuVaMi; 3.05, BuVaRo) than in individual cultivars and other mixtures. Time C1 ranged from 1.62 minutes to 3.51 minutes.

Similarly, the stability plays an important role in the first stage. The stability value normally ranges from 4.96 to 11.42 minutes (Mixolab application handbook, 2012). Table 4.3a shows the stability assessed by Mixolab for winter wheat varieties and their mixtures in between optimal values. This index reflects the resistance of dough against intensive mixing. The growing season, sowing method, and combination significantly affected stability. Stability showed higher significance in a row–row (6.06 min) than in mixed (5.74 min) and the control (5.19 min). This indicates that row–row was better than the control for the resistance of dough against intensive mixing. In terms of amplitude, Illusion/Lorien and their mixtures were higher than Butterfly/Vanessa and their mixtures.

TC2 and slope α were evaluated in the second stage. This stage occurs when the temperature is about 52 - 57 °C. This phase informs about the weakening of proteins due to protein denaturation through the changes in their quaternary, tertiary, and secondary structures during mechanical processing and temperature (Schmiele et al., 2017). Table 4.3b shows that the growing season significantly affected TC2, but the sowing method and individual wheat cultivars and their mixtures did not significantly influence TC2, TC2 range between 0.35-0.40 Nm. The slope alfa implying the speed of the protein network attenuating varied from -0.091 to -0.070 and was a significant difference. (Lacko-Bartosova et al., 2019; Schmiele et al., 2017; Tran, 2021), who reported that strong wheat flour has TC2 value higher than 0.4 Nm. If this value ranges from 0.5 to 0.6 Nm, it refers to good quality protein, the higher ability resistance of gluten to heating, and stronger gluten network. According to this issue, in this study, the value of TC2 (≤ 4) was lower than the optimal value for baking quality. This could be explained by PC and WG were related to TC2 (a positive correlation was demonstrated between PC, WG and TC2). TC2 was low because low PC and WG were found in our case (Table 4.5).

The third stage is characterized by TC3 and slope beta parameters, which means the starch gelatinization depicted in this stage when the temperature rises. Starch granules absorb water, and amylose molecules leak out, causing viscosity to increase (Schmiele et al., 2017). Table 4.3b shows that the growing season and combination significantly affected TC3 and slope beta. Under growing season influence, the third season was lower than the first and second growing seasons for TC3 and slope beta. Under combination factor, TC3 and slope beta were highest in Vanessa shows alone (1.80 Nm) and lowest in Butterfly (1.38 Nm). These results were slightly lower than the previous study (Tran et al., 2020) because different cultivars were used. The mixtures of winter wheat varieties were better than for this parameter in some mixtures compared to individual wheat was sown, e.g., BuLoMi, BuLoRo, BuVaRo, IlluLoMi, and IlluLoRo. Some mixtures also improved the slope beta (e.g., BuLoMi, BuLoRo, and IlluLoRo). Regarding the slope beta parameter, an indicator for the starch gelatinization speed, Vanessa and their mixtures had higher values, meaning they had a faster gelatinization process.

The parameters TC4 and slope gamma were assessed in the fourth stage. This phase relates to the heat stability of the starch gel as the temperatures increase over 80 °C and the resistance of starch against the enzymatic hydrolysis due to the amylase agent. The results of the assessment of different cultivars and their mixtures in Table indicate that the TC4 fluctuated from 0.70 to 1.20 Nm, and slope gamma was between -0.106 and -0.069. These results were similar to the findings of Tran (2021). The growing season significantly affected TC4 and slope gamma. The third season was lower than the first and second seasons for TC4. Slope gamma was lower in the first season than in the second and third seasons. The slope gamma displays the starch's enzymatic degradation speed and the starch gel's heating stability. Wheat and their mixtures significantly affected TC4 but did not influence slope gamma. Similar to TC3, TC4 was higher in Vanessa (1.2 Nm) and their mixtures, Butterfly was lower (0.70 Nm), followed by Lorien (0.96 Nm). The mixtures of wheat cultivars did not improve for the TC4 parameter (Table 4.3b).

Table 4.3b: Rheological properties of dough by Mixolab of winter wheat under the effect of the growing season, sowing method, and wheat variety mixtures (combination).

Variant	TC2 (Nm)	TC3 (Nm)	TC4 (Nm)	TC5 (Nm)	α	β
Season						
2019/20	0.37 ± 0.01^{ab}	1.67 ± 0.01^{a}	0.91 ± 0.02^{a}	1.57 ± 0.03^{ab}	-0.08 ± 0.001^{b}	0.51 ± 0.011^{a}
2020/21	0.38 ± 0.00^{a}	1.63 ± 0.03^{a}	0.94 ± 0.03^{a}	1.72 ± 0.05^{a}	-0.08 ± 0.002^{b}	0.53 ± 0.018^{a}
2021/22	0.36 ± 0.01^{b}	1.48 ± 0.02^{b}	0.81 ± 0.03^{b}	1.45 ± 0.06^{b}	-0.07 ± 0.002^{a}	0.43 ± 0.013^{b}
p-Value	**	***	**	**	***	***
Sowing method						
Control	0.37±0.01	1.59±0.03	0.92±0.04	1.59±0.08	-0.08±0.002	0.49±0.017
Mixed	0.36±0.01	1.58±0.02	0.85±0.03	1.53±0.05	-0.08±0.001	0.50±0.017
Row-Row	0.38±0.01	1.60±0.02	0.89±0.02	1.62±0.03	-0.08±0.002	0.49±0.014
p-Value	ns	ns	ns	ns	ns	ns
Combination						
Butterfly	0.38±0.01	1.38 ± 0.05^{d}	0.70 ± 0.09^{c}	1.13 ± 0.11^{d}	-0.08 ± 0.002^{ab}	0.41 ± 0.02^{c}
Illusion	0.38±0.01	1.57 ± 0.05^{bc}	0.81 ± 0.07^{ab}	1.45 ± 0.15^{bcd}	-0.09 ± 0.004^{c}	0.44 ± 0.03^{bc}
Lorien	0.35±0.00	1.63 ± 0.03^{abc}	0.96 ± 0.02^{b}	1.70 ± 0.09^{abc}	-0.08 ± 0.002^{abc}	0.53 ± 0.02^{abc}
Vanessa	0.38±0.01	$1.8\ \pm0.02^{a}$	1.20 ± 0.02^{a}	2.08 ± 0.05^{a}	-0.07 ± 0.001^{a}	0.60 ± 0.03^{a}
BuLoMi	0.36±0.01	1.50 ± 0.05^{cd}	0.77 ± 0.03^{ab}	1.35 ± 0.05^{cd}	-0.08 ± 0.003^{abc}	0.44 ± 0.02^{bc}
BuVaMi	0.37±0.01	1.63 ± 0.04^{abc}	0.92 ± 0.00^{b}	1.59 ± 0.07^{bc}	-0.07 ± 0.002^{a}	0.55 ± 0.03^{ab}
BuLoRo	0.36±0.01	1.50 ± 0.03^{cd}	0.81 ± 0.04^{ab}	1.47 ± 0.06^{bcd}	-0.08 ± 0.003^{ab}	0.45 ± 0.03^{bc}
BuVaRo	0.40±0.01	1.61 ± 0.03^{bc}	0.94 ± 0.02^{b}	1.67 ± 0.04^{bc}	-0.07 ± 0.001^{a}	0.48 ± 0.03^{abc}
IlluLoMi	0.35±0.01	1.57 ± 0.04^{bc}	0.85 ± 0.06^{ab}	1.59 ± 0.11^{bc}	-0.09 ± 0.002^{bc}	0.47 ± 0.04^{abc}
IlluVaMi	0.36±0.01	1.64 ± 0.04^{abc}	0.86 ± 0.06^{ab}	1.57 ± 0.11^{bc}	-0.08 ± 0.003^{abc}	0.54 ± 0.03^{abc}
IlluLoRo	0.37±0.01	1.59 ± 0.02^{bc}	0.86 ± 0.03^{ab}	1.59 ± 0.07^{bc}	-0.09 ± 0.003^{c}	0.46 ± 0.02^{bc}
IlluVaRo	0.39±0.01	1.69 ± 0.02^{ab}	0.95 ± 0.02^{b}	1.76 ± 0.06^{ab}	-0.08 ± 0.004^{abc}	0.56 ± 0.03^{ab}
p-Value	ns	***	***	***	***	***

Mean ± standard error (SE). TC2, torque C2; TC3, torque C3; TC4, torque C4; TC5, torque C5. Different letters within the column show a statistical difference at *p*-Value < 0.05, Tukey HSD test. ns, non-significant; *$p < 0.05$; **$p < 0.01$; *** $p < 0.001$.

In the last stage, the fifth, dough was cooled to 58-60°C, and the parameters C5 were assessed (Schmiele et al., 2017). Similar to TC3 and TC4, TC5 was affected by growing season and combination. TC5 was lower in the third season (1.45 Nm), followed by the first (1.57 Nm) and second seasons (1.72 Nm). Vanessa presented the highest C5 values, probably because low grain quality (PC) resulted exhibited high retrogradation in the cooling phase and a higher degree of starch gelatinization in the heating phase. A higher TC5 value means a shorter life of products. To improve the anti-retrogradation effect on starch, additives could be added to longer-lasting products (e.g., SPH added to prevent staling in bakery products (Schmiele et al., 2017)). In this study, mixtures of wheat varieties improved TC5.

4.1.4 Correlation analysis and principal component analysis (PCA)

Table 4.4: Correlation between grain yield, yield components and grain quality of winter wheat varieties and their mixture.

Variant	Yield	PC	WG	GI	FN	ZSV
Yield	-	-0.46^{**}	ns	-0.53^{**}	ns	ns
Plant height	0.78^{**}	ns	ns	-0.65^{**}	ns	ns
Spike number	0.74^{**}	-0.35^{*}	ns	ns	ns	-0.49^{**}
TKW	0.55^{**}	ns	ns	-0.58^{**}	ns	ns
Hectoliter weight	0.71^{**}	ns	ns	-0.62^{**}	ns	ns

TKW, thousand kernel weight; PC, protein content; WG, wet gluten; GI, gluten index; FN, falling number; ZSV, Zeleny sedimentation value. ns (non-significant); $^{*}p < 0.05$; $^{**}p < 0.01$.

Results of correlation analysis and PCA provided an overview of multidimensional experimental data, which was then investigated in more detail using mixed model analysis. The relationships between wheat grain yield and yield components have been researched in many works (Jaenisch et al., 2022). Studies on correlation analysis and principal component analysis between wheat grain yield and yield components generally show highly varying results, which can be attributed to varying factors, e.g., experimental and environmental conditions (Koppensteiner et al., 2022). However, almost all previous studies indicated a positive correlation between grain yield and yield components (Hoang et al., 2022; Jaenisch et al., 2022; Kong et al., 2023; Koppensteiner et al., 2022; Mohammadi et al., 2012; Protić et al., 2009). Jaenisch et al. (2022) reported that kernel m^{-2} had greater importance in increasing grain yield than kernel weight. A positive relationship of kernels m^{-2} explained the variation in grain yield, while no relationship between kernel weight and yield occurred. Averaged across cultivars, increased grain yield with increases in kernels m^{-2}. A positive correlation ($r = 0.47^{**}$) between grain yield and the number of plants m^{-2} and a strong correlation between grain yield and the number of spikes m^{-2} were established ($r = 0.55^{**}$) reported by Protić et al. (2009). Similarly, correlation analyses investigated the relationships between grain yield and other important yield components in bread wheat genotypes under supplemental irrigation and dryland conditions

(Mohammadi et al., 2012). Similar to previous studies, our study found a positive correlation between yield and yield components (Table 4.4). Table 4.4 showed a high positive correlation between PH (r = 0.78**), Spike (r = 0.74**), and a mean positive correlation between TKW (r = 0.55**) and yield, which indicated that the yield components significantly affected the grain yield, greater plant height, and spike number, and TKW increased the grain yield. Kong et al. (2023) confirmed that grain number per spike had no significant correlation with grain yield, but grain yield showed a significant positive correlation with TKW and spike number.

Wheat grain proteins have particular chemical and physical properties, making it a dominant cereal. Raising wheat grain yield and protein is an essential objective in wheat production. These two traits have been challenging to improve simultaneously due to the negative relationship observed in the current study (Twizerimana et al., 2020). Mitura et al. (2023) reported a low yield but a high content of PC and WG, regardless of the different wheat cultivars grown in different farming systems. In this study, grain yield was negatively correlated with baking quality parameters. The results are presented in Table 4.4. A high positive correlation exists between grain yield and HW (r = 0.71**). A mean negative correlation was found between yield, PC (r = -0.46**), and GI (r = -0.53**). This study had no significant correlation between grain yield and WG, FN, and ZSV.

Table 4.5: Correlation coefficients between grain yield and quality indicators of winter wheat under winter wheat varieties and their mixtures.

Variant	Yield (t ha^{-1})	PC (%)	WG (%)	GI	FN (s)	ZSV (mL)
Water absorption	ns	0.38*	ns	ns	ns	0.57**
Time of C1	ns	ns	ns	ns	0.46**	ns
Torque C2	ns	ns	ns	0.44**	0.55**	ns
Torque C3	ns	ns	-0.49**	ns	0.33*	-0.67**
Torque C4	ns	ns	-0.58**	0.43**	0.55**	-0.53**
Torque C5	ns	ns	-0.56**	0.48**	0.41*	-0.52**
Alfa	0.33*	-0.36*	ns	ns	0.38*	-0.19
Beta	ns	ns	-0.43**	0.35*	0.33*	-0.42*
Gamma	ns	ns	ns	ns	ns	ns
Amplitude	ns	ns	ns	ns	-0.44**	ns
Stability	-0.54**	0.40*	ns	0.49**	ns	ns

PC, protein content; WG, wet gluten; GI, gluten index; FN, falling number; ZSV, Zeleny sedimentation value. ns (non-significant); *$p < 0.05$; **$p < 0.01$.

Table 4.5 shows the correlation between grain yield and Mixolab parameters of wheat. TC2 was related to protein quality, while TC5, TC4 and TC3 were related to starch characteristics. Flours with high gluten or glutenin showed higher TC2. This was expected since ZSV and GI have been related to gluten and glutenin content (Singh et al., 2019). In this study, PC was positively correlated with stability and WA and negatively correlated with slope alfa. At the same time, WG was negatively correlated with TC3, TC4, TC5, and slope beta. On the contrary, GI had a mean positive correlation with TC2, TC4, TC5, beta, and stability (Table 4.5 and Fig. 4.4). FN had a positive correlation with all Mixolab parameters (except WA, slope gamma and stability), but it had a negative correlation with amplitude (r = -0.44*). This is similar to the findings of Leszek et. al (2016), FN was highly correlated with TC3 (r = 0.73**), amylolytic activity (TC4, r = 0.94**), retrogradation (TC5, r = 0.94**) and pasting speed (slope beta, r = 0.55**). ZSV had a positive correlation with WA and a negative correlation with TC3, TC4, TC5, slope alfa, and slope beta because ZSV had been related to the gluten protein. Table 4.5 shows that grain yield was negatively correlated with stability (r = -0.54*). This was expected because of the high grain yield and low grain quality, as discussed before in this study.

4.2 Agronomic parameters and grain quality of wheat under effects of winter wheat and legume mixtures

4.2.1 Plant height, yield components and grain yield

Growing season and wheat cultivars significantly affected grain yield and yield components (Table 4.6). Similar to the wheat variety mixtures experiment, the trend of season performance was as follows: 2019/20 > 2021/22 > 2020/21. This is the same because two experiments were conducted at the same time and location. The grain yield, TKW, PH, and spike number were lower in the 2020/21 growth season than other growth seasons caused by the effect of weather conditions. The weather conditions significantly affected grain yield and yield components. In this study, the rainfalls were relatively higher than in the first cropping season; however, heavy rainfalls in September were recorded in the second season higher than in the first and third cropping seasons (Section 3.1). This high rainfall was the major factor related to the erosion of the topsoil, leaching nutrients, and affecting the germination of seeds, and a lower seed germination rate was recorded in the second season. PH, Spike, TKW, and grain yield were highest in 2019/20 at 96.44 cm, 370.76 spike number m^{-2}, 52.49 g, and 6.01 t ha^{-1}, followed by 2021/22 growing season at 88.02 cm, 327.35 spike per m^2, 44.39 g, and 4.89 t ha^{-1}, respectively. PH, Spike, and TKW were lowest in 2020/21 at 66.69 cm, 209.35 spike number m^{-2}, 43.95 g, and the grain yield was 2.03 t ha^{-1}, lower than two times with the 2021/22 growing season and three times compared to the 2019/20 growing season (Table 4.6).

The sowing method did not significantly affect grain yield and yield components, the grain yield ranged from 4.15 to 4.58 t ha^{-1} (Table 4.6). Wheat and different legume species mixtures significantly affected wheat grain yield. Wheat and legume (sown in the springtime) mixtures had lower grain yield than wheat control (sown alone) and wheat and legume (sown in the fall) mixtures (Table 4.6). In detail, wheat + spring pea/red clover (sown in the springtime, 3.9 t ha^{-1}) mixtures were lower wheat control (Butterfly and Lorien sown alone) and wheat + Faba bean/spring pea mixtures (sown in the fall, 4.5 t ha^{-1}). The grain yield in wheat + spring pea/red clover sown in the springtime (Wheat+Sps/Wheat+Re) were lower by 15 % and 13 % than wheat control (control), and wheat + legume crops were sown in the fall (Wheat+Fa, In, Sp, and Wi), respectively (Table 4.6).

Table 4.6: Effect of the season, sowing method, wheat cultivar, and leguminous species on production and quality parameters of winter wheat.

Variant	Plant height	Spike number	TKW (g)	Yield (t ha^{-1})	HW (kg hL^{-1})
Season (A)					
2019/20	96.44±0.48^{a}	370.76±7.87^{a}	52.49±0.35^{a}	6.01±0.10^{a}	73.24±0.31^{a}
2020/21	69.69±0.89^{c}	209.35±7.24^{c}	43.95±0.35^{b}	2.03±0.08^{c}	66.41±0.54^{b}
2021/22	88.02±0.53^{b}	327.35±5.98^{b}	44.39±0.19^{b}	4.89±0.10^{b}	72.48±0.73^{a}
Sowing method (B)					
Control	84.56±3.10	308.16±16.83	47.25±1.10	4.58±0.42	70.89±1.06
Mixed	84.37±1.40	302.25±11.09	46.78±0.47	4.37±0.20	70.76±0.51
Row-row	85.12±1.02	300.83±7.35	47.01±0.17	4.15±0.17	70.61±0.66
Wheat cultivar (C)					
Butterfly	82.09±1.14^{b}	294.56±9.05^{b}	46.27±0.28^{b}	4.17±0.16	72.92±0.45^{a}
Lorien	87.34±1.08^{a}	310.41±7.62^{a}	47.62±0.54^{a}	4.44±0.17	68.50±0.53^{b}
Legume mixtures (D)					
Control	84.56±2.16	308.16±11.73	47.25±0.77	4.58±0.30^{a}	70.89±0.74
Wheat+Fa	86.00±1.96	301.74±14.59	47.22±0.78	4.52±0.32^{a}	71.09±0.80
Wheat+In	85.20±2.09	307.53±17.21	46.69±0.89	4.40±0.28^{ab}	70.88±0.84
Wheat+Sp	85.03±2.09	315.27±15.88	47.01±0.90	4.48±0.36^{a}	71.72±0.84
Wheat+Wi	84.87±2.16	302.51±17.40	46.62±0.77	4.34±0.28^{ab}	69.48±1.67
Wheat+Sps	83.59±2.19	290.54±15.57	47.75±0.83	3.96±0.34^{ab}	69.99±0.88
Wheat+Re	83.78±2.32	291.65±17.42	46.09±0.71	3.87±0.34^{b}	70.94±0.81
ANOVA					
Season	***	***	***	***	***
Sowing method	ns	ns	ns	ns	ns
Wheat cultivar	**	ns	*	ns	***
Legume mixtures	ns	ns	ns	*	ns

Mean ± standard error (SE). Wheat, winter wheat; Fa, faba bean; In, incarnate clover; Sp, spring pea; Wi, winter pea; Sps, spring pea was sown in springtime; Re, red clover; 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 < 0.05; **p < 0.01; and ***p < 0.001.

4.2.2 Grain quality

Growing season and wheat cultivars significantly affected HW. Similar to wheat variety mixtures, in this experiment, HW was lower in the 2020/21 growing season than in the 2019/20 and 2021/22 growing seasons (Table 4.6). In this study, HW was affected by growing season and cultivar but was not affected by the sowing method and wheat and legume mixtures. Generally, HW was low (about 70 kg hL^{-1}) and well below the threshold requirement of 76 kg hL^{-1} for top-quality milling wheat (Bilsborrow et al., 2013; Manley et al., 2009).

Different wheat cultivars significantly affected baking quality. Butterfly cultivar performed better in PC, WG, GI, FN, and ZSV than Lorien. This is similar to studies of Konvalina et. al (2009), Tran et. al (2020), and Tran (2021), whose reported cultivars affected grain yield and quality. In this study, PC, WG, GI, FN, and ZSV in Butterfly were higher than by 0.6 %, 1.51 %, 6.09, 15,3 s, and 10.4 mL than Lorien, respectively (Table 4.7).

PC in wheat grain was significantly influenced by the growing season and cultivar (Table 4.7). The grain with the highest PC significantly was obtained in 2020/21 (10.18 %). The significantly highest PC was characteristic of the grain of the Butterfly (10.05 %), in which the content of this component was higher by 0.6 % than in the grain of Lorien. In this site, grain yield did not change (Table 4.7), and PC was unaffected by removing (row-row) and keeping legume (mixed) crops compared to wheat sown alone (sowing method, Table 4.7).

PC was significantly affected in winter wheat and legume mixtures; WG, GI, FN, and ZSV were unaffected in winter wheat and legume mixtures (Table 4.7). Our results in Table 4.7 are consistent with these findings and showed that the lowest PC in wheat grain was found in control pure sown wheat and was significantly different from the intercropped wheat.

Wheat and legume mixtures significantly increased PC by 4 % compared to the sole wheat (Table 4.7). The highest PC in Wheat + Spring pea sown in springtime (Wheat+Sps, 10.06 %), followed by Wheat + red clover (Wheat+Re, 9.86 %). Among the legume species and wheat mixtures, PC was not statistically significantly affected, but there was a slightly lower PC value for Wheat + legume species sown in the fall mixtures compared to wheat + legume sown in the springtime.

WG and GI were significantly influenced by the growing season and cultivar (Table 4.7). Wheat grain from the 2020/21 harvests had significantly higher GI values (86) than grain harvested in 2019/20 (73.59) and 2021/22 growing seasons (69.5) (Table 4.7). A significant effect of weather conditions on GI values has also been indicated (Ceseviciene et al., 2009; Mazzoncini et al., 2015; Sulek and Cacak-Pietrzak, 2018).

Significant cultivar variation was observed in WG and GI (Table 4.7). The significantly higher WG was characteristic of the wheat grain of the Butterfly cultivar (18.76 %) than Lorien (17.25 %). The higher significant GI value was characteristic of the wheat grain of the Butterfly cultivar (79.41) than Lorien cultivar (73.32). WG ranged from 17.39 to 18.96 % and was not significantly differentiated under the effect of wheat and legume species mixtures (Table 4.7).

Significantly, the lowest amylolytic enzyme activity (FN 241 s) was characteristic of the grain harvested in 2020/21 due to the lower amount of rainfall during the maturation and harvesting of wheat from the field compared to the multi-year average (Section 3.1). In the other two years of the study, amylolytic enzyme activity was higher than in 2020/21 (FN ranging from 251-269 s). Significantly higher FN values were obtained for the grain of Butterfly (261 s) than Lorien (246 s) (Table 4.7). The influence of the cultivar factor on FN values has also been indicated (Mitura et al., 2023; Sulek and Cacak-Pietrzak, 2018; Tran, 2021). FN was not affected by wheat and legume species mixtures (Table 4.7).

Table 4.7: Effect of the season, sowing method, wheat cultivar, and leguminous species on quality parameters of winter wheat.

Variant	PC (%)	WG (%)	GI	FN (s)	ZSV (mL)
Season (A)					
2019/20	9.78±0.05^{b}	18.07±0.16	73.59±1.23^{b}	269.39±3.92^{a}	24.44±0.53^{c}
2020/21	10.18±0.08^{a}	18.25±0.34	86.01±1.07^{a}	241.18±2.30^{c}	26.45±0.44^{b}
2021/22	9.29±0.05^{c}	17.7±0.19	69.5±1.07^{c}	251.12±2.85^{b}	28.47±0.90^{a}
Sowing method (B)					
Control	9.41±0.09	17.39±0.42	78.43±2.62	249.00±7.20	25.58±1.52
Mixing	9.83±0.06	18.34±0.21	74.70±1.42	259.14±2.94	26.48±0.59
Row-row	9.79±0.07	17.87±0.23	77.34±1.19	250.29±2.92	26.72±0.60
Wheat cultivar (C)					
Butterfly	10.05±0.05^{a}	18.76±0.19^{a}	79.41±1.00^{a}	261.55±3.00^{a}	31.65±0.38^{a}
Lorien	9.45±0.06^{b}	17.25±0.18^{b}	73.32±1.15^{b}	246.25±2.24^{b}	21.25±0.19^{b}
Legume mixtures (D)					
Control	9.41±0.06^{c}	17.39±0.30	78.43±1.83	249.00±5.01	25.58±1.06
Wheat+Fa	9.77±0.11^{abc}	18.25±0.34	73.58±2.25	256.31±4.70	26.55±1.10
Wheat+In	9.62±0.10^{bc}	17.45±0.36	79.53±1.82	250.03±5.22	26.44±1.08
Wheat+Sp	9.71±0.13^{abc}	17.99±0.33	77.95±2.05	255.44±5.34	26.50±1.10
Wheat+Wi	9.82±0.11^{abc}	18.96±0.44	72.51±2.37	261.14±5.35	26.63±1.03
Wheat+Sps	10.06±0.13^{a}	17.83±0.39	74.91±2.12	250.42±5.05	26.43±0.97
Wheat+Re	9.86±0.09^{ab}	18.16±0.38	77.65±1.92	254.94±5.13	27.04±0.97
ANOVA					
Season	***	ns	***	***	***
Sowing method	ns	ns	ns	ns	ns
Wheat cultivar	***	***	***	***	***
Legume mixtures	**	ns	ns	ns	ns

Mean ± standard error (SE). PC, Protein content; WG, wet gluten; GI, gluten index; FN, Falling number; ZSV, Zeleny sedimentation value; Wheat, winter wheat; Fa, faba bean; In, incarnate clover; Sp, spring pea; Wi, winter pea; Sps, spring pea was sown in springtime; Re, red clover. Different letters within the column show a statistical difference at *p-Value* < 0.05, Tukey HSD test. ns (non-significant); *p < 0.05; **p < 0.01; and ***p < 0.001.

Highly significant differences were noticed with respect to ZSV in harvested years and wheat cultivars. ZSV varied from 21.25 to 31.65 mL. Higher ZSV was observed in Butterfly (31.47 mL) than in Lorien (21.25 mL). Higher ZSV was the third growing season (28.47 mL), followed by the second (26.45 mL) and first growing season (24.44 mL) (Table 4.7). ZSV was not significantly affected by the sowing method and the wheat and legume species mixtures in this study (Table 4.7). In terms of wheat and legume mixtures, in accordance with the conclusions of Gooding et al. (2007) and Dvořák et al. (2022), our results showed that wheat mixed with legumes reached higher ZSV; however, it did not significantly statistically differ from wheat sown alone from our site.

4.2.3 Rheological of dough evaluated by Mixolab

Table 4.8a: Effect of the season, sowing method, wheat cultivar, and leguminous species on rheological parameters evaluated by the Mixolab.

Variant	WA (%)	Time C1 (min)	Gamma	Amplitude	Stability
Season (A)					
2019/20	62.35±0.28^{c}	2.55±0.09^{a}	-0.094±0.00^{b}	0.073±0.00	5.41±0.13^{b}
2020/21	64.75±0.47^{a}	1.86±0.07^{c}	-0.086±0.00^{ab}	0.075±0.00	6.15±0.12^{a}
2021/22	63.15±0.40^{b}	2.25±0.06^{b}	-0.084±0.00^{a}	0.076±0.00	4.93±0.11^{c}
Sowing method (B)					
Control	63.15±0.91	2.036±0.16	-0.095±0.01	0.078±0.01	5.211±0.25
Mixing	63.40±0.36	2.239±0.07	-0.085±0.00	0.074±0.00	5.672±0.13
Row-row	63.53±0.35	2.258±0.08	-0.088±0.00	0.074±0.00	5.414±0.11
Wheat cultivar (C)					
Butterfly	66.69±0.19^{a}	2.37±0.08^{a}	-0.084±0.00^{a}	0.063±0.00^{b}	5.77±0.10^{a}
Lorien	60.15±0.10^{b}	2.07±0.04^{b}	-0.091±0.00^{b}	0.087±0.00^{a}	5.22±0.11^{b}
Legume mixtures (D)					
Control	63.15±0.63	2.04±0.11	-0.095±0.01^{b}	0.078±0.0	5.21±0.17
Wheat+Fa	63.64±0.66	2.31±0.14	-0.084±0.00^{ab}	0.071±0.00	5.26±0.19
Wheat+In	63.55±0.66	2.04±0.12	-0.088±0.00^{ab}	0.075±0.00	5.60±0.24
Wheat+Sp	63.51±0.64	2.30±0.13	-0.087±0.01^{ab}	0.075±0.00	5.51±0.20
Wheat+Wi	63.44±0.63	2.28±0.13	-0.078±0.00^{a}	0.077±0.00	5.60±0.20
Wheat+Sps	63.26±0.58	2.14±0.12	-0.093±0.00^{b}	0.079±0.00	5.52±0.20
Wheat+Re	63.39±0.54	2.41±0.12	-0.090±0.00^{ab}	0.068±0.00	5.77±0.21
ANOVA					
Season	***	***	*	ns	***
Sowing method	ns	ns	ns	ns	ns
Wheat cultivar	***	**	**	***	**
Legume mixtures	ns	ns	*	ns	ns

Mean ± standard error (SE). Wheat, winter wheat; Fa, faba bean; In, incarnate clover; Sp, spring pea; Wi, winter pea; Sps, spring pea was sown in springtime; Re, red clover; WA, water absorption. Different letters within the column show a statistical difference at *p-Value* < 0.05, Tukey HSD test. ns (non-significant); *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$.

Season and cultivar were significantly affected rheological parameters (Table 4.8a and 4.8b). WA ranged from 62.35 to 64.75 %, and the highest value was found in the 2020/21 growing season, followed by the 2021/22 and 2019/20 growing seasons. On the contrary, Time C1 was highest in the 2019/20 growing season (2.55 min) and the lowest (1.86 min) in the 2020/21 growing season. The stability indicated the highest in the 2020/21 growing season (6.15 min), then the 2019/20 growing season (5.41 min), and lowest in the 2021/22 growing season (4.93 min).

Table 4.8b: Effect of the season, sowing method, wheat cultivar, and leguminous species on rheological parameters evaluated by the Mixolab.

Variant	TC2 (Nm)	TC3 (Nm)	TC4 (Nm)	TC5 (Nm)	Alfa	Beta
Season (A)						
2019/20	0.377±0.01[a]	1.58±0.01[a]	0.92±0.01[a]	1.59±0.02[a]	-0.079±0.0[b]	0.49±0.01[a]
2020/21	0.352±0.00[b]	1.43±0.02[b]	0.71±0.02[c]	1.31±0.05[c]	-0.084±0.0[c]	0.44±0.01[b]
2021/22	0.358±0.00[b]	1.44±0.02[b]	0.82±0.03[b]	1.50±0.06[b]	-0.077±0.0[a]	0.43±0.01[b]
Sowing method (B)						
Control	0.364±0.01	1.50±0.05	0.83±0.05	1.42±0.10	-0.079±0.00	0.47±0.02
Mixing	0.364±0.00	1.49±0.02	0.83±0.02	1.50±0.04	-0.080±0.00	0.45±0.01
Row-Row	0.360±0.00	1.48±0.01	0.80±0.02	1.45±0.04	-0.080±0.00	0.45±0.01
Wheat cultivar (C)						
Butterfly	0.372±0.00[a]	1.37±0.01[b]	0.66±0.02[b]	1.15±0.03[b]	-0.079±0.0[a]	0.40±0.01[b]
Lorien	0.353±0.00[b]	1.60±0.01[a]	0.97±0.01[a]	1.78±0.03[a]	-0.082±0.0[b]	0.50±0.01[a]
Legume mixtures (D)						
Control	0.364±0.01	1.50±0.03	0.83±0.04	1.42±0.07	-0.079±0.0	0.47±0.01
Wheat+Fa	0.364±0.01	1.49±0.03	0.83±0.04	1.49±0.08	-0.08±0.0	0.46±0.02
Wheat +In	0.361±0.01	1.50±0.03	0.82±0.04	1.47±0.08	-0.08±0.0	0.45±0.01
Wheat+Sp	0.366±0.01	1.47±0.03	0.82±0.04	1.47±0.08	-0.08±0.0	0.44±0.02
Wheat+Wi	0.366±0.01	1.49±0.03	0.83±0.04	1.50±0.07	-0.08±0.0	0.44±0.01
Wheat+Sps	0.354±0.01	1.47±0.02	0.80±0.04	1.45±0.07	-0.08±0.0	0.44±0.01
Wheat+Re	0.361±0.01	1.49±0.02	0.80±0.04	1.47±0.07	-0.079±0.0	0.47±0.01
ANOVA						
Season	***	***	***	***	***	***
Sowing method	ns	ns	ns	ns	ns	ns
Wheat cultivar	***	***	***	***	**	***
Legume mixtures	ns	ns	ns	ns	ns	ns

Mean ± standard error (SE). Wheat, winter wheat; Fa, faba bean; In, incarnate clover; Sp, spring pea; Wi, winter pea; Sps, spring pea was sown in springtime; Re, red clover; TC2, torque 2; TC3, torque 3; TC4, torque 4; TC5, torque 5. Different letters within the column show a statistical difference at *p-Value* < 0.05, Tukey HSD test. ns (non-significant); *p < 0.05; **p < 0.01; and ***p < 0.001.

The slope alfa values showed that in the first season (-0.08) and the third growing season (-0.077) were similar and higher than in the second growing season (-0.084). Similar results were obtained for TC2, TC3, TC4, TC5, and beta, which were higher in the 2019/20 growing season than those in the 2020/21 and 2021/22 growing seasons. Also, the lowest slope gamma is indicated in the 2019/20 growing season (-0.095) compared to the 2020/21 (-0.082) and 2021/22 growing seasons (-0.084) (Table 4.8a).

The difference in wheat cultivar significantly affected the rheological properties of dough. The higher number was indicated in Butterfly on WA, Time C1, alfa, stability, and TC2 at 7.02 %, 0.25 min, 0.004, 0.65 min, and 0.018 Nm than in Lorien, respectively. TC3, TC4, TC5, and slope beta in Butterfly were 0.255 Nm, 0.33 Nm, 0.687 Nm, and 0.11 lower than Lorien (Table 4.8b).

There were no significant differences in the sowing method and wheat and legume species mixtures on the rheological properties evaluated by Mixolab (Tables 4.8a and 4.8b). In the combination of individual cultivars, there were no significant differences in rheological parameters evaluated by Mixolab for Butterfly and Lorien cultivars intercropped with legume in different sowing methods and sowing times (except Stab, TC5, and beta for Lorien cultivar).

4.2.4 Correlation analysis and principal component analysis

Table 4.9 shows the correlation between grain yield, grain quality and Mixolab parameters of wheat and legume species mixtures.

Table 4.9: Correlations are significant at p < 0.05 between wheat yield, baking quality, and rheological parameters of dough evaluated by Mixolab under the effect of winter wheat and legume mixtures.

Variant	Yield	PH	Spike	TKW	HW	PC	WG	GI	FN	ZSV
Yield	1.00	0.61**	0.78**	0.39*	ns	-0.61**	ns	ns	ns	ns
WA	ns	-0.82**	-0.40*	-0.50**	0.85**	0.81**	0.68**	0.51**	0.65**	0.99**
Time C1	ns	-0.40*	ns	ns	0.47*	0.51**	0.47*	ns	0.46*	0.61**
TC2	ns	-0.56**	ns	-0.68**	0.61**	0.45*	0.46*	0.40*	0.78**	0.64**
TC3	ns	0.79**	ns	0.43*	-0.82**	-0.84**	-0.67**	-0.50**	-0.60**	-0.98**
TC4	0.41*	0.81**	0.40*	0.42*	-0.83**	-0.85**	-0.66**	-0.51**	-0.55**	-0.98**
TC5	ns	0.81**	ns	0.44*	-0.84**	-0.83**	-0.66**	-0.51**	-0.57**	-0.98**
Alfa	-0.57**	-0.71**	-0.55**	-0.47*	0.43*	0.57**	0.45*	ns	ns	0.58**
Beta	ns	0.69**	ns	ns	-0.71**	-0.80**	-0.64**	-0.45*	-0.56*	-0.92*
Gamma	ns	ns	ns	-0.42*	ns	ns	0.41*	ns	0.71**	0.45*
Amplitude	ns	0.70**	ns	0.49*	-0.79**	-0.70**	-0.62**	-0.51**	-0.62**	-0.92**
Stability	ns	-0.49*	ns	-0.40*	0.57**	0.54**	0.48*	ns	0.59**	0.54**

PH, plant height; Spike, spikes number m^{-2}; TKW, thousand kernel weight; PC, protein content; WG, wet gluten; GI, gluten index; FN, falling number, ZSV, Zeleny sedimentation value; WA, water absorption; TC2, torque 2; TC3, torque 3; TC4, torque 4; TC5, torque 5; α, slope alfa: attenuating rate of protein in warming; β, slope beta: starch gelatinization rate; γ, slope gamma: enzymatic degradation rate. ns (non-significant); *p < 0.05; **p < 0.01.

The results were similar to the experiment of wheat variety mixtures; grain yield had a positive correlation with yield components but a negative correlation with grain quality. This is also reported in the previous studies by Dhaka et al. (2012), Hoang et al. (2023), and Tran (2021). This was expected because of the high grain yield and low grain quality, as discussed before in this study. Table 4.9 shows that WA, Time C1, and TC2 positively correlated with PC, WG, GI, FN, and ZSV while negatively correlated with TC3, TC4, TC5, and slope beta. Slope gamma and alfa had a positive correlation with grain quality. TC2 was also considered as the start of pasting of dough. Flours with high gluten or glutenin showed higher TC2. TC2 was positively correlated to ZSV and GI. This was expected since

ZSV and GI have been related to gluten and glutenin content (Singh et al., 2019). The study correlation between grain quality and rheological properties was also indicated in the previous investigation (Hoang et al., 2023; Lacko-Bartosova et al., 2019; Peña et al., 2005; Singh et al., 2019; Tran, 2021).

5. Conclusion

Finding strategies to deal with limited nutrient (nitrogen) sources in sustainable farming practices has been difficult since they are limiting factors for grain yield and quality, especially in organic farming. The key to fixing this issue is to draw attention to suitable farming systems, such as intercropping systems for wheat/legume mixtures. This research study was carried out over three years to evaluate wheat/legume mixtures' effect on wheat yield and grain quality. In general, the results from two experiments showed that:

In wheat varieties mixtures experiment, the results show that the grain yield significantly differed among the growing seasons. In this study, winter wheat varieties mixtures did guarantee stable grain production. The positive effect of growing a higher grain yield-lower quality variety and a less good-higher quality variety was demonstrated when a certain averaging occurred. The yield stabilized but was lower, while the bakery quality improved. 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 alone. 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 alone. The Illusion variety and its component mixtures did not increase the rheological properties of the mixtures. To achieve comprehensive 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. For example, using more than two winter wheat varieties mixtures instead of two-way was used in this study.

In wheat and legume species mixtures, the results show that the intercropping of winter wheat and legume caused no wheat grain yield variation or rheological properties of dough tested by Mixolab but an increased wheat protein content. This study is an opening for further research on the effect of changing leguminous species on baking quality and nutrient content in many wheat varieties. Butterfly shows greater grain quality but lower grain yield than Lorien, so a study blending Butterfly with Lorien and legume mixtures could be an option in future research to improve simultaneous grain yield and grain quality. Legumes (red clover and spring pea) sown in the springtime had slightly higher PC in wheat grain but lower wheat grain yield than wheat mixed with other legume species sown in the fall.

Growing season and genotype (cultivars/varieties) significantly affected grain yield, yield components, baking quality, and rheological properties tested by Mixolab. The grain yield improves if the growth and development of wheat are ensured. Plant height (PH), the spike number m^{-2} (spike number), and thousand kernel weight (TKW) positively affect grain yield. Higher grain yield due to higher PH, spike number, and TKW. Grain yield had a positive correlation with yield components but a negative correlation with baking quality and rheological properties, as tested by Mixolab. Protein content (PC) and wet gluten (WG) had a positive correlation with water absorption (WA) and TC2 and a negative correlation with TC3, TC4, and TC5. This confirms that grain yield and grain quality are the main characteristics of wheat, but it is challenging to contribute them together in one wheat cultivar.

The data on plant and soil nutrition information were limited in this study, so it needs to be further investigated in future studies to more comprehensively evaluate the effects of mixtures, especially between wheat and legumes on wheat yield and quality. From there, it is useful for farmers and policymakers to make optimal decisions that promote sustainable agriculture. Based on the results, this can be implemented into the cropping system related to organic farming on arable land in South Bohemia, Czech Republic.

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7. Appendixes

Appendix 1. List of publications (2021- September 2024)

1. Bernas, J., **Hoang, T.N.,** Ghorbani, M., Amirahmadi, E., Ali, S., Baloch, S.S., Mukosha, E.M., Murindangabo, Y.T., Konvalina, P., Bernasová, T., and Nedbal, V. (2024). Hotspot detection in the cultivation of organic winter wheat variety mixtures. The International Journal of Life Cycle Assessment. https://doi.org/10.1007/s11367-024-02360-4 | **IF: 4.9, Q1**.
2. **Hoang, T.N.**, Konvalina P., Kopecký, M., Ghorbani, M., Nguyen T.G., Bernas J., Murindangabo, Y.T., Capouchová, I., Sangin S., and Hlásná Čepková P. (2024). Assessing the Quality and Grain Yield of Winter Wheat in the Organic Farming Management under Wheat-legume Intercropping Practice. Helyon. https://doi.org/10.1016/j.heliyon.2024.e31234 | **IF: 3.4, Q1**.
3. Amirahmadi, E., Ghorbani, M., Moudrý, J., Bernas, J., Mukosha, C., and **Hoang, T.N.** (2024). Environmental Assessment of Dryland and Irrigated Winter Wheat Cultivation under Compost Fertilization Strategies. Plants, 13 (4):509. https://doi.org/10.3390/plants13040509 **| IF: 4, Q1**.
4. Murindangabo, Y.T., Kopecký, M., Perna, K., Konvalina, P., Bohata, A., Kavkova, M., Nguyen, T.G., and **Hoang, T.N.** (2023). Correction to: Relevance of Entomopathogenic Fungi in Soil–Plant Systems. Plant and Soil 495(1-2). https://doi.org/10.1007/s11104-023-06325-8 **| IF: 3.9, Q1.**
5. Murindangabo, Y.T., Kopecký, M., **Hoang, T.N.**, Bernas, J., Parajuli, T., Dhakal, S., Konvalina, P., Ufitikirezi, J.D.D.M., Kaneza, G., Khanal, B.R., Dhakal, S.C., and Shrestha, A.K. (2023). Comparative Analysis of Soil Organic Matter Fractions, Lability, Stability Ratios, and Carbon Management Index in Various Land Use Types within Bharatpur Catchment, Chitwan District, Nepal. Carbon Balance and Management, 18 (1). https://doi.org/10.1186/s13021-023-00241-1 | **IF: 3.9, Q2.**
6. Murindangabo, Y.T., Kopecký, M., Konvalina, P., Ghorbani, M., Perna, K., Nguyen, T.G., Bernas, J., Babar Baloch, S., **Hoang, T.N.**, Eze, F., and Ali, S. (2023). Quantitative Approaches in Assessing Soil Organic Matter Dynamics for Sustainable Management. Agronomy, 13 (7), 1776. https://doi.org/10.3390/agronomy13071776 **| IF: 3.3, Q1.**
7. Murindangabo, Y.T., Kopecký, M., Perna, K., Nguyen, T.G., Ghorbani, M., Konvalina, P., Bohata, A., Kavkova, M., **Hoang, T.N.**, Kabelka, D., and Klenotová, E. (2023). Enhancing Soil Organic Matter Transformation through Sustainable Farming Practices: Evaluating Labile Soil Organic Matter Fraction Dynamics and Identifying Potential Early Indicators. Agriculture, 13(7), 1314. https://doi.org/10.3390/agriculture13071314 **| IF: 3.3, Q1.**
8. **Hoang, T.N.**, Minamikawa, K., Tokida, T., Wagai, R., Phuong, T.X.P., Dong, T.T.H., and Tran, D.H. (2023). Higher Rice Grain Yield and Lower Methane Emission Achieved by Alternate Wetting and Drying in Central Vietnam. European Journal of Agronomy, 151. https://doi.org/10.1016/j.eja.2023.126992 **| IF: 4.5, Q1.**
9. **Hoang, T.N.**, Konvalina, P., Kopecký, M., Ghorbani, M., Amirahmadi, E., Bernas, J., Ali, S., Nguyen, T.G., Murindangabo, Y.T., Tran, D.K., and Sangin, S. (2023). Assessing Grain Yield and Achieving Enhanced Quality in Organic Farming: Efficiency of Winter Wheat Mixtures System. Agriculture, 13 (5). https://doi.org/10.3390/agriculture13050937 **| IF: 3.3, Q1.**
10. Ghorbani, M., Amirahmadi, E., Konvalina, P., Moudrý, J., Kopecký, M., and **Hoang, T.N.** (2023). Carbon Pool Dynamic and Soil Microbial Respiration Affected by Land Use Alteration: A Case Study in Humid Subtropical Area. Land, 12 (2), 459. https://doi.org/10.3390/land12020459 **| IF: 3.2, Q2**.
11. Bedrníček, J., Lorenc, F., Jarošová, M., Bártová, V., Smetana, P., Kadlec, J., Jirotková, D., Kyselka, J., Petrášková, E., Bjelková, M., **Hoang, T.N**., and Bárta, J. (2022). Milk Thistle Oilseed Cake Flour

Fractions: A Source of Silymarin and Macronutrients for Gluten-Free Bread. Antioxidants, 11(10). https://doi.org/10.3390/antiox11102022 | **IF: 6.0, Q1.**

12. **Hoang, T.N.**, Kopecký, M., and Konvalina, P. (2022). Winter Wheat Mixtures Influence Grain Rheological and Mixolab Quality. Journal of Applied Life Sciences and Environment. 54, 417–428. https://doi.org/10.46909/journalalse-2021-036.
13. Tran, D.H., Nguyen, T.G., and **Hoang, T.N.** (2022). Performance and population growth rate of cassava pink mealybug, Phenacoccus manihoti Matile-Ferrero (*Hemiptera: Pseudococcidae*) under different potassium fertilization regimes. Agriculture and Natural Resources. 56, 791-796. https://doi.org/10.34044/j.anres.2022.56.4.13
14. Tran, T.X.P., Nguyen, T.G., **Hoang, T.N.,** Tran., D.H., Nguyen., T.N., Do., M.C., and Tran, L.S., (2022). Efficiency of biochar in rice cultivation Thua Thien Hue. Hue Journal of Agricultural Science and Technology. 6 (3), 3196-3204. http://10.46826/huaf-jasat.v6n3y2022.959 (Vietnamese).
15. Tran, T.X.P., **Hoang, T.N.,** Ho, D.K., Ton, T.C., Phung, V., Truong., P.H., and Nguyen, H., (2021). Situation of rice production and treatment straw after harvest in Thua Thien Hue province. Hue Journal of Agricultural Science and Technology. 5 (3), 2720-2726. http://10.46826/huaf-jasat.v5n3y2021.857 (Vietnamese).
16. Kopecký, M., Kolář, L., Váchalová, R., Konvalina, P., Batt, J., Mraz, P., Menšík, L., **Hoang, T.N.**, and Dumbrovský, M. (2021). Black Carbon and Its Effect on Carbon Sequestration in Soil. Agronomy, 11(11), 2261. https://doi.org/10.3390/agronomy11112261 | **IF: 3.3, Q1.**

<u>Conference presentations (proceedings abstract, paper):</u>

Konvalina, P., **Hoang, T.N.**, Yalcin, A. (2023). Impact of Wheat/legume Mixtures on Crop Yield and Grain Quality under Organic Management. Conference: 11th International Scientific Conference Rural Development 2023. September 26-28, Lithuania.

Hoang, T.N., Kopecký, M., Kabelka, D., Dang, K., Suchy, K., Konvalina, P. (2022). Effects of Winter Wheat and Legumes Mixtures on Grain Yield and Baking Quality in Organic Farming, Conference: XVII Congress of the European Society for Agronomy 2022. 29 August – 02 September 2022, Potsdam, Germany.

Hoang, T.N., Kopecký, M., Ghorbani, M., Murindangabo, Y.T., Dang, K., Suchy, K., Konvalina, P. (2022). Wheat and Legume Mixtures Influence Grain Quality, Conference: TAE 2022 – 8th International Conference on Trends in Agricultural Engineering, September 20-23. Praha, Czech Republic.

Hoang, T.N., Minamikawa, K., Hosen, Y., Shirato, Y., Tokida, T., Dang Hoa, T. (2022). Alternate Wetting and Drying Reduced Greenhouse Gas Emissions While Increased Rice Yield in a Paddy Field in Central Vietnam, 2022, 8th International Conference on Sustainable Energy and Environment (SEE 2022): The Road to Net-Zero: Energy Transition Challenges and Solutions 7-9 November, Bangkok, Thailand.

Appendix 2. Training and supervision plan during the study

Name	Trong Nghia Hoang	
Department	Agroecosystems	
Supervisor	doc. Ing. Petr Konvalina, Ph.D.	
Period	19th October 2020 until 30th December 2024	
PhD courses		(Year/Semester)
Agroecology *(Agroekologie)*		2021/SS
Bioclimatology *(Bioklimatologie)*		2021/SS
Plant and Environment *(Rostlina a prostředí)*		2021/WS
Methodology of Scientific Work *(Metodologie vědecké práce)*		2021/WS
English Language *(Anglicky jazyk)*		2022/SS
Scientific seminars		
Over the Horizon and for Mutual Acquaintance **USB Conference** of Doctoral Students, České Budějovice, Czech Republic (3 – 4th November 2021).		2021/WS
European Society for Agronomy **(ESA) 2022, XVII Congress**, Potsdam, Germany (29 August – 2 September 2022).		2022/SS
TAE 2022 – 8th International Conference on Tren in Agricultural Engineering, Prague, Czech Republic (20 – 23rd September 2022).		2022/SS
Over the Horizon and for Mutual Acquaintance 2nd **USB Conference** of Doctoral Students, České Budějovice, Czech Republic (7 – 8th December 2022).		2022/WS
Department seminar, České Budějovice, Czech Republic		2023/WS
Traning		
University of Agricultural Sciences and Veterinary Medicine, Iasi, Faculty of Agriculture, Department of Pedotechnics, Romania		16th May 2021 - 30th June 2021
Leibniz Centre for Agricultural Landscape Research (ZALF), Germany		1st Aug 2022 - 30th Sept 2022
Agriculture and Forestry University, Faculty of Agriculture, Nepal.		1st March 2023 - 30th April 2023
Gyeongsang National University, College of Agriculture & Life Sciences, Korea.		1st Sept 2023 - 30th Sept 2023
Lectures		
Organic Plant Production		2021-2024
Quality, Processing and Marketing of Bioproduction		2021-2024