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Effect of cultural practices on growth and vigor of Kernza® in the field

Master´s thesis

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Master in Sustainable Agriculture and Food Security

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Declaration

I hereby declare that I have authored this master's thesis carrying the name „ Effect of cultural practices on growth and vigor of Kernza® in the field "independently under the guidance of my supervisor. Furthermore, I confirm that I have used only professional literature and other information sources that have been indicated in the thesis and listed in the bibliography at the end of the thesis. As the author of the master's thesis, I futher state that I have not infringed the copyrights of third parties in connection with its creation.

In Prague on 19th April, 2024 ____________________________

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Effect of cultural practices on growth and vigor of Kernza® in the field

Summary:

In agriculture, it's important to ensure that new technologies and practices don't harm the environment. These innovations should also be accessible and effective for farmers. Furthermore, they should contribute to improving food productivity. Wheat, among all primary crops, occupies a significant portion of the global arable land and makes a substantial contribution to overall global production. However, wheat cultivation also encounters challenges in soil health due to excessive or improper tillage, leading to issues such as soil erosion, disrupted structure, and degradation of organic matter. The transition to a perennial farming system, particularly in comparison to annual or biennial wheat cultivation, brings forth a range of social, environmental, and economic advantages. Intermediate wheatgrass (IWG) has substantial genetic similarity to wheat, but the grain characteristics and management of this species is not interchangeable with wheat and requires further investigation. This study examined five varieties of IWG growth and subsequent yield under distinct seasonal conditions and row spacing to generally understand the possibilities for this crop in Czech conditions. A field experiment was established with planting dates in Fall 2021 and Spring 2022 in Uhřineves, CZ as a randomized complete block design with three replications. The planting date and variety significantly influenced IWG yield however, row spacing did not show a significant main effect on yield. Additionally, we also observed a marginally significant interaction between planting date and row spacing suggesting a potential combined influence on yield. As the inaugural field-based study conducted in the Czech Republic, our research represents a pioneering effort to investigate the good agricultural practices and the economic viability of IWG. This study provides a unique opportunity to generate novel insights and scientific knowledge within the Czech agricultural context. By undertaking this pioneering study, we aim to lay the foundation for future research and practical applications in the field.

Keywords: Intermediate wheatgrass (IWG), Sustainable Agriculture, Perennial farming system, Yield, Row spacing, Variety, Planting date

Content

1 Introduction 6

2 Scientific hypothesis and aims of the thesis 8

3 Literature research 9
  3.1 Wheat 9
  3.2 Perennial vs Annual 10
  3.3 Challenges of Perennial system 12
  3.4 Intermediate Wheat Grass 12

4 Methodology 17
  4.1 Description of study area 17
  4.2 Management of study area 17
  4.3 Data collection 17
    4.3.1 Yield 17
    4.3.2 Weather 18
  4.4 Statistical analysis 19
    4.4.1 Data analysis techniques 19

5 Results 20
  5.1 Growing conditions 20
  5.2 Yield analysis 23
    5.2.1 Row spacing on yield 24
    5.2.2 Planting date on yield 25
    5.2.3 Variety 25

6 Discussion 27
  6.1 Environmental factors 27
  6.2 Agronomic Practices 27
    6.2.1 Row spacing 27
    6.2.2 Planting date 28
    6.2.3 Variety 29

7 Conclusion 30

8 Bibliography 31

9 List of abbreviations and symbols 37

10 Appendices I

1 Introduction

Every action we take to live on this planet needs to be sustainable for our future generations. Agriculture which provides food and serves the basic needs of humans can none the less be excluded from sustainability. The sustainability in farming systems first highlighted by Thomas Malthus in his 1798 publication "An Essay on the Principle of Population" as he contemplated whether the arithmetically increasing food production would meet the needs of the exponentially growing population in the future (Malthus 1798). Later in the year 1972, five parameters were studied to bring awareness about sustainability: population, food production, industrialization, pollution, and consumption of nonrenewable natural resources were studied to bring awareness about sustainability (Meadows et al. 1971). However, the idea of sustainability has gained prominence since the publication of the Brundtland Report 'Our Common Future' in 1987 (Velten et al. 2015). The Brundtland report emphasized the importance of the social, economic, and environmental aspects of Sustainable Agriculture which should not be based on methods that mine and deplete the soil, along with increasing not only the average productivity and incomes but also that of individuals lacking in resources (Report of the World Commission on Environment and Development 1987). Deriving from this report, a strong sustainability pertains to the conservation of resources that are deemed irreplaceable and must be safeguarded at any expense (Kuhlman & Farrington 2010).

In the agriculture system, sustainability also needs to ensure that the new technologies and practices should not have adverse effects on environment, are accessible to and effective for farmers and improves food productivity (Pretty 2008). To be sustainable, agriculture must meet the needs of present and future generations for its products and services, while ensuring profitability, environmental health, and social and economic equity. As a result, sustainable agriculture would contribute to all four pillars of food security – availability, access, utilization, and stability – in a manner that is environmentally, economically, and socially responsible over time (FAO 2014).

According to statistical data, four primary crops—rice, wheat, maize, and sugarcane—account for nearly half of global production in 2021, with wheat contributing approximately 8% (FAO 2023). Cereal crops occupy half of the total planted area, with approximately 219 million hectares (approximately 15%) of arable land dedicated to wheat cultivation in 2022 out of a total arable land area of 1.4 billion hectares (FAO Land Statistics, 2021). Production of the three main staple crop wheat, rice and maize contributes to nearly half of the global food calorie intake and two-fifths of protein consumption. Among these cereals, wheat holds a particularly vital position in safeguarding global food and nutrition security, providing one-fifth of global food calories and protein (Erenstein et al. 2022). As sustainability gains paramount importance in the new world, agriculture, including the extensive cultivation of wheat worldwide stands at the forefront, requiring careful consideration of social, environmental, and economic aspects to secure a sustainable future for generations to come (Robertson 2015).

When the forest land or natural ecosystems were converted into agriculture with multiple disturbances in the soil, it is said that the Soil Organic Matter (SOM) reduced from somewhere between 20 and 70%. Inorder to bring a transition from the large scale, energetically expensive input and high-volume output-based agriculture to an ecosystem friendly farming within the sustainable energy and biogeochemical boundaries of the planet, SOM would play the central role (Crews & Rumsey 2017). The increase in the SOM in the below surface can be possible with more plant productivity below the ground by the roots which is by its penetration into the soil (Six et al. 1998). In North America, it is widely believed that soil disturbance caused by tillage was a primary factor contributing to the historical loss of soil organic carbon (SOC). Another factor that contributes to the accumulation of Soil Organic Matter (SOM) underground is the stabilization and protection of organic compounds within soil aggregates (Baker et al. 2007). Both processes are promoted by perennial cropping systems, where roots penetrate the soil over time, causing fewer disturbances as weeds are controlled and less tillage is needed. This study primarily focuses on such a perennial crop known as Intermediate Wheatgrass, which is considered a relative of wheat due to its similar characteristics. Therefore, this study aims to explore the potential of Intermediate Wheatgrass as a sustainable alternative to annual wheat.

2 Scientific hypothesis and aims of the thesis

The aim of the thesis was to compare parameters indicating health of IWG grown under different practices such as row spacing and planting timing. The main part of the thesis has been to take measurements and yield in the field.

Hypothesis:

1. Null Hypothesis (H0): IWG yield remains consistent across different spacing arrangements.
   Alternative Hypothesis (H1): IWG yield is influenced by spacing arrangements, and specific spacing configurations lead to variations in crop productivity.

2. Null Hypothesis (H0): There is no significant difference in IWG yield among different planting dates.
   Alternative Hypothesis (H1): IWG yield is significantly affected by planting time, with specific planting dates leading to higher or lower yields.

3 Literature research

3.1 Wheat

Cereals based foods including wheat are inexpensive, satisfying culinary preferences as well as caloric and essential nutrient needs for billions of people on all continents who consume them as a major food staple (Peña et al. 2014). Wheat is cultivated extensively in diverse climates, from temperate to subtropical zones. The international trade of wheat is pivotal for supporting the livelihoods of farmers, agricultural workers, and stakeholders throughout the wheat value chain along with ensuring global food security. The Green Revolution since the 1960s transformed world wheat production, benefitting both producers and consumers through low production costs and low food prices (Shiferaw et al. 2013).

Food and feed production worldwide heavily relies on wheat. However, current agricultural practices face numerous challenges including a shortage of land for cultivation, a desire to reduce the use of chemical pesticides and fertilizers, and the development of resistance towards employed pesticides and virulence towards host resistance in the most widely grown varieties. To keep up with the increasing human population, the yield trends of the major crops must align with the rapid growth rate. Major variation in wheat yields annually is mainly attributed to environmental factors, which are only expected to become less predictable with the ever-changing climatic conditions (Vestergaard & Jorgensen 2024). Current management practices rely heavily on high-energy inputs such as chemical fertilizers, herbicides, insecticides, and fungicides, mainly due to centuries of wheat breeding that focused on yield improvements resulting in crops cultivated in monoculture. This approach promotes the emergence of new virulent pathogen strains, especially if disease resistance in the considered crop is based on a simple genetic structure (Vestergaard & Jorgensen 2024).

Wheat production is estimated to rise by 11% by the year 2032 compared to 2022, reaching a total of 855 million metric tons (OECD-FAO 2023). Current management practices mainly rely on the application of external inputs such as pesticides for pest and disease control, mineral fertilizers to improve plant nutrition and biomass, and often irrigation to avoid water stress conditions. In combination with intensive soil tillage, these management practices may significantly reduce microbial diversity, whose key functions for crop production are widely recognized. Soil is a non-renewable natural resource. Its health is the result of biotic and abiotic processes and is linked to several interactions. These interactions have a significant impact on microbial activity, which supports essential soil processes. Microorganisms are the most abundant and diverse group among all organisms living in soils, playing crucial roles in maintaining ecological functions such as the decomposition of organic matter, energy flow, and nutrient cycling. However, microbial communities are highly susceptible to soil changes, such as disturbances due to tillage, irrigation, and fertilization, i.e., practices which are considered essential to achieve profitable crop yields (Romano et al. 2023).

Wheat cultivation also encounters challenges in soil health due to excessive or improper tillage, leading to issues such as soil erosion, disrupted structure, and degradation of organic matter (Power 2010). Fuel and energy-intensive conventional tillage methods contribute to environmental concerns and increased production costs. Reduced tillage or no-till systems, while promoting carbon sequestration, may pose weed management challenges. Issues such as water quality concerns, soil compaction, and disruptions to microbial and earthworm communities underscore the need for a balanced approach to tillage practices in wheat production, considering both productivity and environmental sustainability (Wuest et al. 2006).

China allocates 75% of its arable land to cereal grain cultivation. China, the world's largest wheat producer, accounts for approximately 17% of global wheat production (FAO 2022). Over time, the Nitrogen Use Efficiency (NUE) in crop production has declined from 32% in 1980 to 26% in 2005 (Ma et al. 2012). By reducing the nitrogen input and improvement of crop management, the NUE increased to 44% in 2018 (Shen et al. 2024). A study on the wheat production on irrigated soil in Iran, the largest shares of the total energy inputs were attributed to chemical fertilizer (37%) and diesel fuel (24.14%) (Ghorbani et al. 2011).

The reduced tillage in cold and dry climate conditions reduces the emission of greenhouse gases like N2O and CH4 as well helps with the Soil Organic Carbon sequestration (Krauss et al. 2017). When compared with the various tillage systems, the no-tillage system exhibited a 14% decrease in greenhouse gas emissions (CO2, N2O, and CH4) and global warming potential (Nasseri 2023). The reduced tillage also led to the yield reduction of wheat by 14% in Switzerland (Berner et al. 2008).

The persistent challenge of overproduction, driven by favorable weather conditions and technological advancements, results in market surpluses and subsequent price declines by discounting in the cotton sector (Wulff 2024). This could be even the case with the food industry as well. Another noteworthy issue arises from the intricate dynamics of international trade, where the influence of government policies and subsidies contributes significantly to the inherent volatility of wheat prices (Anwar & Iqbal 2020). The complexity of the situation is further compounded by fluctuations in currency values, shifts in global demand patterns, and the unpredictable impacts of geopolitical events (Vishwakarma et al. 2022).

In recent decades, the increase in inter-annual climate variability has led to instabilities in agricultural production, sometimes leading to food shortages and rises in global food prices. Projections predict an increase in frequency of extreme low yields due to adverse weather conditions, since current homogeneous varieties lack resilience to cope with climate instability as was demonstrated for European wheat varieties. Therefore, it is necessary to build sustainable systems that can ensure food security through the stabilization of agricultural production (van Frank et al. 2020).

3.2 Perennial vs Annual

Agriculture’s most fundamental problems can be traced back to its origins some 10,000 years ago, when humans began to replace diverse ecosystems dominated by perennial plants with simplified ecosystems that required frequent disturbance (Crews et al. 2016). However, after the industrial revolution, the farmers primarily cultivated and domesticated annual plants, overlooking the potential of thousands of perennial grasses, legumes, and other broadleaf plants. However, there is growing recognition of the benefits of perennial crops in assembling diverse agroecosystems. These systems have the potential to regenerate soils and fulfill crucial ecosystem functions. By minimizing soil disturbance and exposure periods, perennial crops are anticipated to enhance soil carbon balance, nutrient retention, soil water uptake efficiency, soil microbiome functions, and weed suppression (Crews et al. 2018).

Agriculture, depending on management practices, can yield various disadvantages, despite its contributions to socioeconomic development. These include the depletion of wildlife habitats, nutrient runoff, sedimentation of water bodies, emissions of greenhouse gases, and the risk of pesticide poisoning to humans and unintended species. Continuous soil tillage and prolonged absence of vegetation cover are linked to significant soil erosion, loss of soil carbon, and the leaching of nutrients into surface and groundwater (Power 2010).

The transition to a perennial farming system, particularly in comparison to annual or biennial wheat cultivation, brings forth a range of social, environmental, and economic advantages. A perennial system could reduce erosion, prevent carbon losses, decrease nutrient runoff into waterways, and capture nutrients deeper in the soil when they are limited. This results in lower farm costs and greater efficiency in agricultural grain crops (Soto-Gómez & Pérez-Rodríguez 2022).

From an economic standpoint, a perennial system offers multiple advantages over annual or biennial wheat cultivation. One notable benefit is the potential for reduced input costs, as established perennials often require fewer inputs such as seeds and labor. In a study conducted in Kansas, USA, the perennial system only consumed 8% of the infield energy cost compared to wheat resulting in significant cost savings for farmers (Glover et al. 2010). Furthermore, perennial crops may exhibit increased yields over time as they mature, providing a more consistent and potentially higher yield compared to annual or biennial crops. To achieve that, researchers are also pursuing crosses between existing annual grains with a wild perennial relative. Along with yield, it should also ensure sustainability of the yield maintaining the perennial nature of the crop (Baker 2017).

The perennial system also opens diversification opportunities, allowing farmers to explore and market various crops and its value-added products and byproducts, thereby enhancing economic resilience (DeHaan 2015).

On the environmental front, a perennial farming system excels in promoting sustainable practices. When compared to yearly rotation and traditional methods, perennial grain systems have the greatest potential for enhancing soil organic matter (SOM) and enhancing connections between plants and soil microbes in agricultural ecosystems (Audu et al. 2022). Perennial systems also contribute to increased biodiversity, providing habitats for beneficial insects, birds, and wildlife, thereby supporting ecosystem health and balance (Werling et al. 2014). Additionally, some perennial crops, with their deep root systems, contribute to water conservation by accessing moisture at deeper soil levels, reducing the reliance on irrigation and promoting sustainable water management practices (Angon et al. 2023). This leads to more resilient and sustainable agricultural practices. Even though perennials with their no till approach contribute to enhance the soil health by reducing erosion, promoting soil structure, and supporting microbial activity, the tillage is reduced only after they are established and allowed to grow for more years rather than replanting every year. This enhances the root and shoot growth and makes it even more difficult for the weeds to germinate and grow. The amount of soil disturbances by tillage in the first year for both annual and perennial crops would be the same or could be more depending on the type of weed control measures we undertake.

3.3 Challenges of Perennial system

Perennial agriculture, characterized by the cultivation of crops that live for multiple years, introduces a set of complexities distinct from conventional annual cropping practices. The longer growth cycles of perennial crops demand careful planning for pest and disease control (Cox et al. 2005). The research on perennial cropping systems encounters significant gaps across multiple domains. There is a critical need for focused efforts in agronomic practices optimization, economic viability evaluation, and addressing adoption challenges. Understanding the intricate relationships between perennial crops and its best cultivation requirements remains a persistent research gap, emphasizing the need for comprehensive studies. Bridging these research gaps is paramount for successful implementation and broader adoption of perennial cropping systems (Geels 2011).

The advantages of planting perennial forages could be utilized in soils with low water-holding capacity, limited fertility, or high salt content, often with infrequent irrigation. Additionally, they can be utilized in tropical and subtropical regions with adverse climatic factors, including subzero temperatures, chilling, or occasional frosts, as well as periodic defoliation resulting from machine harvest (Sanderson' et al. 1997). Perennial grain plants are well-suited for providing essential ecosystem services, yet their focus is not on maximizing seed production. This allocation pattern can be successful in stable environments where tolerating stresses is more advantageous than resorting to short life span and high reproductive efforts. To achieve seed yields comparable to annuals, perennial grains need to enhance their biomass production to levels observed in certain perennial grasses. If this objective can be accomplished, perennial crops could offer a more sustainable alternative to annuals (Vico et al. 2016).

3.4 Intermediate Wheatgrass

The wheat-relative intermediate wheatgrass *(Thinopyrum intermedium)* (Host) (IWG) is a winter-hardy cool-season perennial grass which can support the transition to agroecological systems because this perennial crop produces forage and grain for several years with great production potential, beneficial ecological properties, minimal soil disturbance and valuable grain for functional food (Pototskaya et al. 2022). Featuring large and deep roots, year-round soil cover, increased resource use efficiency and an extended growing season, the ‘perennialization’ of cropping systems would be one cornerstone of sustainable agriculture (Crews 2017). IWG has substantial genetic similarity to wheat, and Thinopyrum sp. have been sexually hybridized with wheat to integrate novel genes into modern wheat cultivars. Improved IWG populations have been released by The Land Institute (Salina, Kansas, USA) under the trade name Kernza® (DeHaan et al. 2018). Over a span of 4.5 years and five growing seasons in Kansas, USA, a research investigation showcased the consistent high water-use efficiency of perennial Intermediate Wheatgrass (IWG) across the entire growing period. IWG exhibited its peak evapotranspiration and net carbon uptake rates, notably surpassing those of annual crops. These outcomes offer valuable insights into the interconnection between hydrological and carbon cycles within these environments, shedding light on the comparative advantages and drawbacks in contrast to annual crops (de Oliveira et al. 2018).

Farmers can use IWG as a dual-purpose crop, utilizing it for both grain production and forage. While grain harvesting was occasionally forgone, the crop was consistently utilized for grazing or for harvesting hay and straw for bedding purposes (Lanker et al. 2020). Enhancing the profitability of IWG perennial grain involves integrating forage production, with favourable profits often resulting from the inclusion of hay harvests alongside grain and straw production. To enhance forage production, sowing with narrow row spacing often leads to the straw's value surpassing production expenses. Opting for row spacings of 15 or 30 centimetres tends to increase the production of straw and hay compared to wider rows. Consistently high net returns are observed when the hay is harvested during the fall season with this specific row spacing (Hunter et al. 2020). Another encouraging fact about the IWG to harvest for forage in fall is that the nutritive value of the forage from IWG is higher in the fall and spring especially suitable for the beef and lactating cows compared to when harvested in summer (Favre et al. 2019). These considerations collectively contribute to achieving enhanced yield of forage, which is a byproduct of growing IWG. On the forefront, the grain is the main product and needs to be the main focus to contribute directly towards food security.

Although Intermediate Wheatgrass (IWG) serves as both a grain and forage crop, the researchers and policy makers primarily focus on its grain aspect. The rising concern about IWG could be that the forage yield of IWG increases year to year however the grain yield is comparatively less with wheat. Another point noted in a study conducted in Minnesota, US was a decrease in the yield of IWG after the third year of harvest (Puka-Beals et al. 2022). The yield of IWG have also been reported to decline in a study conducted in New York over three years which also states that the strip tillage helps to increase the yield compared to the no-till fields (Law et al. 2020). While strip tillage may boost yields, it still disturbs some soil and may not offer the same level of ecological benefits as no-till practices. In the study conducted in Ohio, US, it was found that the cutting of forage led to increased forage and grain yield, which could be attributed to enhanced nutrient cycling and availability. This is supported by the observation that forage harvest did not affect root C:N ratios but resulted in an increase in mineralizable C (Pugliese et al. 2019). The soil microbial and fungal activities increased when the roots of the IWG are left in the ground without disturbing for 3 or more years (Bergquist et al. 2019). The yield of the IWG in subsequent years is mainly accounted to the resource allocation to the individual plants in the subsequent years (Hunter et al. 2020). In another yield-based study in

Wisconsin, US, the late fall planting did impact the yield in the initial year; however, the following year compensated for this delay (Olugbenle et al. 2021). Even with the intercropping systems with legumes, both the grain and the forage of IWG achieved similar yield compared to the monoculture of IWG (Priscila et al. 2022). Selecting a cropping system is detrimental in planning the weed control measures. A study in West Germany on the IWG found that the weeds covered the 90% of the plots in the first year of planting however, IWG outcompeted them in the subsequent year (Liste & Muskolus 2023).

The Land Institute, KS recommends a set of good agricultural practices to grow IWG. In the preparation phase, a well-drained soil would help its deeper rooting system to penetrate. Since IWG is relatively drought tolerant, a good moisture after planting would help for stand establishment. Introducing IWG after a winter annual grain could lead to contamination of the crop, however after a legume or spring grain would counter the risk of contamination and promote healthier growth due to reduced competition for resources and potentially different pest and disease pressures. A general rule of thumb is to sow seeds at a depth approximately two to three times the diameter of the seed. Planting the IWG 0.25-0.5 inch deep would give good germination than planting too deep. If the fall planting is carried out two weeks prior to the normal winter wheat planting time, this would help reduce competition with weeds. Spring Planting could depend on the moisture in the soil however, there would not be any grain production in the first summer. 10 lbs per acre (18 plants per square foot) (78260 plants/acre) would give a seeding rate with 6 inches spacing between the seeds which could help with good aeration, weed suppression and competition. Harvesting when the head is 70% ripe and drying the grain soon after would help for uniformity in the moisture content as the seeds may not ripen evenly within the head (Peters 2021). Contrary to these recommendations, a 5-year study way back in 1969 on three row spacings (76 cm, 102 cm and 152 cm) and fertilization on dry land in the US said that the Nitrogen based fertilizer application increased the yield of both the grain and the forage of the IWG irrespective of the row spacing and independent of the Phosphorous applied to the plants. This study also pointed out that without fertilization, row spacing had no significant influence and the need for Nitrogen based fertilizer increases every year with the increase in the requirements of proteins (Black & Reitz 1969). Recommendation of a set of good agricultural practices would be premature for Intermediate Wheatgrass (IWG) at this stage of acceptance globally. Given its relatively recent introduction into agricultural systems and ongoing research, it is essential to await further scientific evidence and field trials taking even the agroclimatic factors into consideration.

Along with the good agricultural practices, the ideal agroclimatic conditions as well contributes to the growth and development of IWG. The grain yield of IWG exhibits a positive correlation with the accumulation of vernalization units from seeding throughout fall, winter, and spring. Late summer seeding as well increases the yield of IWG. (Jungers et al. 2022). Vernalization (4-5°C), combined with the interplay of growing degree days and day length (13-14h) during spring is optimal for the IWG flowering and grain production (Duchene et al. 2021). Most IWG varieties are facultative dual induction grass with moderate vernalization requirements and require cool temperatures and (or) short days for primary induction followed by warm temperatures and (or) long day secondary induction. (Ivancic et al. 2021).

Vernalization is the process by which plants are induced to flower after exposure to prolonged periods of cold temperatures. Adequate moisture availability plays a crucial role in supporting this process by facilitating the development of healthy plants. IWG adapts in regions receiving 330 mm or more of annual precipitation and demonstrates an optimal performance at elevations ranging from 1100 to 2700 m.a.s.l (Hybner 2012). Studies on region specific agroclimatic advantages does play a crucial role in developing a crop program for IWG, however, knowing the origin of the crop gives a deeper understanding of the same.

The primary geographic origin for food-grade IWG is being identified between the Black Sea and Caspian Sea, particularly in the Stavropol region of Russia. There are also likely smaller contributions from collections as distant as Kazakhstan in the east to Turkey in the west. However, currently, IWG grain cultivars are being developed in multiple breeding programs around the world. This finding suggests potential areas for future germplasm collections as well as in-situ conservation initiatives (Wagoner et al. 2023). Crop-specific studies, with a particular emphasis on popular species such as IWG, and perennial grains, are crucial. IWG, in addition to its perennial growth cycle and deep-rooted characteristics, contributes to no-tillage practices and promotes environmentally sustainable farming. This perennial nature also enhances microbial biomass and water sorption (Rakkar et al. 2023). In the near future, IWG may be considered as a practical alternative to annual wheat and even could be planted on all land used for production. This cropping system can help to resolve many of the problems that limit the sustainability of agriculture today and the producers can realize the improved soil, water, and air quality (Scheinost et al. 2001). However, in-depth investigations are necessary to comprehend their adaptation to diverse environments, predictability of yields, and region specific good agricultural practices. Successfully cultivating IWG relies on pivotal factors like agroclimatic conditions, soil moisture, and environmental considerations. The timing of planting, row spacing, and choice of varieties are some of the key elements requiring careful planning in IWG cultivation.

An online survey conducted in France and the US reported that 57 % were interested to grow perennial crops for environmental benefits however 41% mostly conventional farmers needed more information pertaining to profitability (Wayman et al. 2019). The first agronomic study conducted on IWG in a Scandinavian country took place in Sweden, marking a significant milestone for research in the region. In Sweden, researchers studied IWG and found that when grain crops are grown alongside legumes, the plants can better obtain nitrogen and water. This is important because climate change is causing droughts to become more severe. By improving nitrogen and water availability, crops are better equipped to withstand the increasingly extreme drought conditions (Martensson et al. 2022). Interest in perennial grains is increasing in many areas, but there hasn't been much research or practical use of them in Western Europe yet. This makes it hard to connect the progress of perennial grains with local farming opportunities (Duchene et al. 2019). Despite increasing research interest in Intermediate Wheatgrass (IWG) in the United States and parts of Europe, there remains a notable lack of research focused on the Czech environment. The unique climatic and soil conditions in the Czech Republic present specific challenges and opportunities for IWG cultivation that may not be adequately addressed by research conducted elsewhere. Therefore, there's a clear need for targeted research efforts within the Czech Republic to assess IWG's performance under local conditions and tailor agronomic practices accordingly. Such localized research can provide valuable insights into IWG's potential contributions to sustainable agriculture in the Czech Republic, addressing issues like soil erosion, water conservation, and biodiversity enhancement. Moreover, such research can inform policymakers and farmers about the feasibility and potential benefits of incorporating IWG into crop rotations, supporting the development of environmentally friendly and economically viable agricultural practices in the region.

4 Methodology

4.1 Description of Study Area

The agricultural experimentation took place at CZU Research Station in Uhříněves, Prague 22, Czech Republic. A small plot field trial was established at the experimental field to look at planting date, row spacing, and variety. Experimental units 1.5 m by 3 m was distinctly marked and assigned a unique three-digit identity number. All treatments were replicated 3 times. The treatment arrangement was a 3-way factorial of row spacing, planting date, and variety with 2 levels of row spacing, 2 planting dates, and 5 varieties for a total of 20 unique combinations. The establishment of all treatments within the first planting date was September 30, 2021, and the second planting date on March 29, 2022. While the first planting date vernalized and produced grain in 2022, the first date that both had a grain yield was in 2023, the subject of this evaluation.

4.2 Management of Study Area

The land preparation procedure involved tilling of the field. All weeds were removed to create a clean environment for the crops. It was sown using the machine seed drill attached to a tractor (Figure 2). The temperature and precipitation was recorded from the nearest weather station in Uhříněves on a monthly basis from the month of planting (Table 1). The Land Institute (TLI) supplied with the varieties. The five different varieties tested here were TLI-C5, TLI-701, TLI-702, TLI-703, and TLI-34715. The two levels of row spacing were: narrow spacing of 20 cm and a wide spacing of 40 cm between plants. Additionally, we maintained a seed density of 10 kg per hectare across the field.

4.3 Data Collection

4.3.1 Yield

Unlike wheat, IWG never fully dries and the maturity is not as even because it still has some wildness and it is also a perennial crop. Maturity for harvest can be determined overall brown color and the bend of the ears. Harvesting was carried out at a height of 35 cm above ground level to minimize biomass and ensure accurate yield assessment. Harvesting occurred when plant was at least 70 % dry. Plots were maturing evenly and so a straight-cutting method was deemed appropriate. A Wintersteiger Classic (Wintersteiger, Austria) small-plot combine was used to harvest all plots on August 21, 2023. Each experimental unit was individually harvested, and the grain of each plot individually bagged and labelled. After immediately calculating the fresh weight, it underwent an 11 day drying period in the farm's designated area. It followed a cleaning phase utilizing a tabletop grain cleaner to measure the final, clean weight of the IWG crop.

Figure 1: The Wintersteiger Combined Harvester in operation in the IWG field. Pictures taken on 21-Aug-2023 by Sreejith Thodamkannath.

4.3.2 Weather

The weather data used was collected from the IPRAGU66 weather station located in Praha - Uhříněves, Prague. Positioned at 50.032° N latitude and 14.599° E longitude, with an elevation of 288 meters above sea level (m.a.s.l), this station provided meteorological information useful for our study in this area.

Table 1. Weather data during the growth of IWG from planting of first date to the harvest of whole experiment.

	2021		**2022**		**2023**	
Month	Rainfall (in mm)	Avg. Temp. (°C)	Rainfall (in mm)	Avg. Temp.(°C)	Rainfall (in mm)	Avg. Temp.(°C)
Jan	NA	NA	207.26	1.00	29.47	2.30
Feb	NA	NA	737.38	3.30	72.89	1.80
Mar	NA	NA	10.67	4.00	80.02	5.00
Apr	NA	NA	61.47	6.70	52.32	6.80
May	NA	NA	27.94	15.10	20.57	13.00
Jun	NA	NA	135.12	19.30	47.76	17.50
Jul	NA	NA	38.09	19.20	39.36	20.20
Aug	NA	NA	79.25	19.90	75.69	19.40
Sep	15.74	15.50	57.91	12.70	15.23	17.70
Oct	404.61	8.30	21.56	11.00	NA	NA
Nov	72.13	3.40	43.43	3.80	NA	NA
Dec	13.20	0.40	33.79	0.40	NA	NA
	505.68	**6.90**	**1453.87**	**9.70**	**433.31**	**11.52**

4.4 Statistical Analysis

4.4.1 Data Analysis Techniques

The yield data of the IWG were subject to the analysis of variance (ANOVA), to compare the data collected from different planting dates, varieties, and spacing arrangements from the 60 study plots. The data analysis involved partitioning the data for main effects and testing for interactions. Each of the 60 plots, characterized by unique combinations of planting date (Spring or Fall), variety (one of five), and spacing (wide or narrow), underwent factorial analysis to examine the individual effects of each factor. We utilized factorial ANOVA in R Studio to test for interactions between factors and determine whether their effects were dependent on each other. Statistical analyses were performed using R Studio and Microsoft Excel. (*R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Microsoft Corporation (2022). Microsoft Excel. Redmond, Washington, USA.)*

5 Results

5.1 Growing Conditions.

The growing conditions for the planted IWG from the September 2021 to August 2023 were typical to the agro climatic conditions in Czech Republic. In September 2021, prior to fall planting, the area received a precipitation of 15.74 mm which allowed the sowing (Figure 2) and germination. From October through December 2021, after fall planting, received the precipitation of 489.94 mm which ensured adequate moisture during the germination, establishment, and growth stages of the IWG. The rainfall of 404.61 mm received in October 2021 helping for the growth and development of the crop before entering winter from November 2021 to February 2022. In March 2022, before spring planting, the precipitation was recorded at 10.67 mm, making the soil moisture at par for planting (Table 1). After the Spring planting, from March to May 2022, the temperature picked up along with an interim rainfall for the sustenance of the crop.

Figure 2: Sowing of IWG using a machine seed drill attached to a tractor. Pictures taken on 11-May-2023 by Sreejith Thodamkannath.

The average temperature from September through December 2021 was 6.9°C, while for the entire year 2022, it was 9.7°C enabling photosynthesis during the summer and spring. Prior to harvest in August 2023, the area experienced a precipitation of 418.08 mm from January through August 2023, with an average temperature of 10.75°C during the same period sufficient for the growth, development, and flowering of the IWG. Approximately 182 days of vernalization units were accumulated from September 2021 to March 2022, and approximately 211 days from April 2022 to August 2023 satisfactory prior to the harvest (Table 1). The fall planted crop had accumulated a GDD (Growing Degree Days) of 4505.1 which is 417.3 more than the spring-planted crop which is 4087.8 GDD (Table 2). Following sowing, the plants initially faced competition from weeds, however, the overall growth and vigor of the planted IWG were satisfactory by the beginning of the summer of 2023. This observation highlights the resilience and vigor of the crops grown in the study, despite the presence of weeds from the previous year. The fact that all plots exhibited competitive growth in 2023 suggests that the crops were able to effectively outcompete the weeds and establish themselves successfully. (Figure 3). All plots matured evenly and were ready for harvest in August (Figure 4).

The total GDD at the time of harvest for the fall-planted crop was 4505.1, while for the spring-planted crop, it was 4087.8 GDD (Table 2).

Table 2. Growing Degree Days (Base temperature: 5°C)

Month	GDD -2021	GDD - 2022	GDD- 2023
Jan		0	0
Feb		0	0
Mar		0	0
Apr		51	54
May		313.1	248
Jun		429	375
Jul		440.2	471.2
Aug		461.9	446.4
Sep	315	231	381
Oct	102.3	186	
Nov	0	0	
Dec	0	0	
TOTAL	**417.3**	**2112.2**	**1975.6**

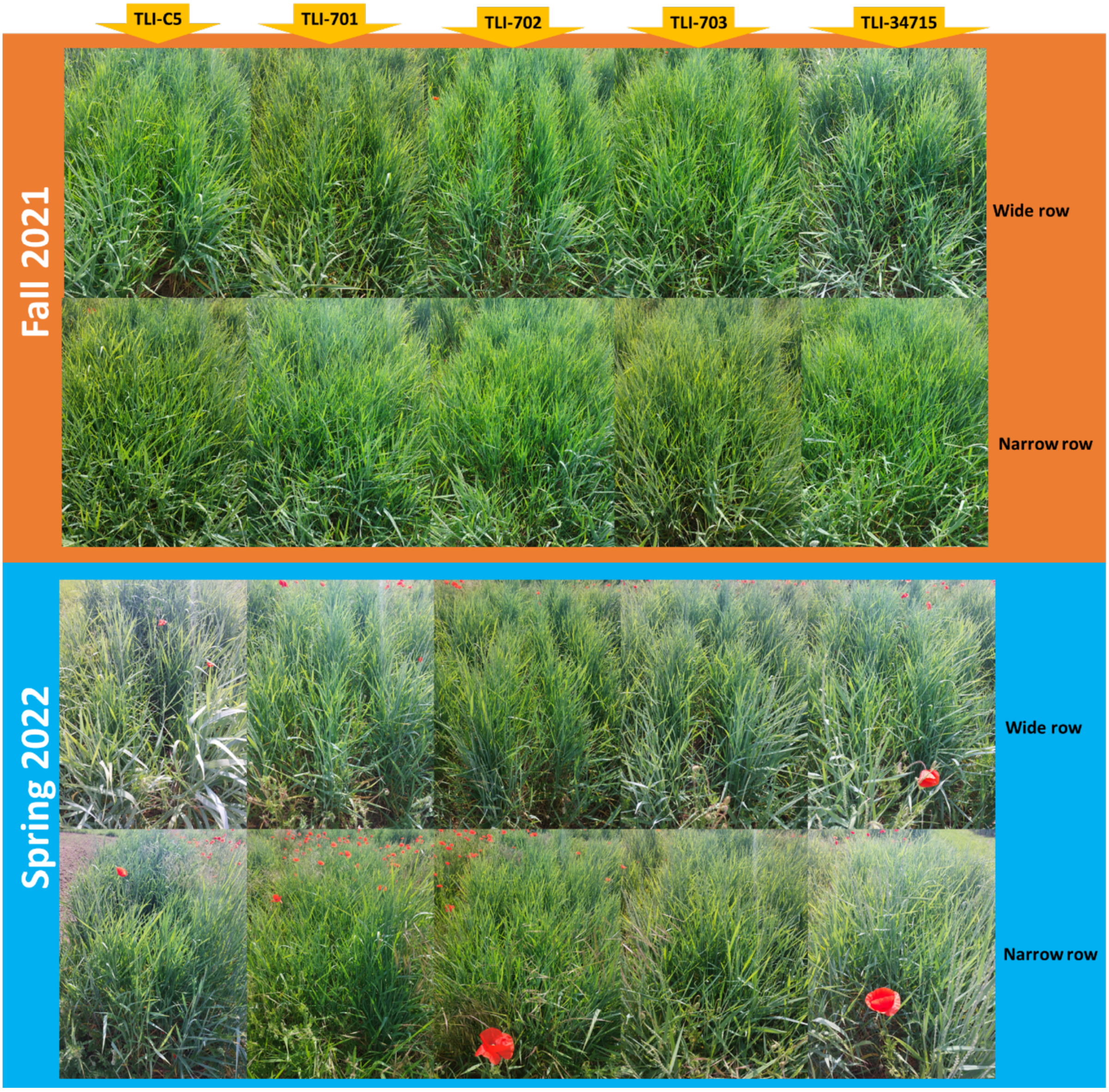

Figure 3. Growth of IWG varieties of Kernza (TLI-C5, TLI-701, TLI-702, TLI-703, TLI-34715) which were planted in different row spacings (wide or narrow) and planting timings (spring or fall). Pictures taken June 5, 2023 by Theresa Piskáčková, used with permission.

Figure 4: Spring planted IWG growing for 15 months and acquired a GDD of 4087.8. Pictures taken on 21-August 2023 by Sreejith Thodamkannath

5.2 Yield Analysis

Grain yield was harvested per plot and after initial drying and seed cleaning, the grain weight was between 350 and 1000 g per plot. The hypothesis were tested if the variation in yield could be accounted for by the treatments tested, variety, spacing, or date. In the 3-way factorial analysis, both planting date and variety exhibited statistical significance. However, there was no significant effect observed for row spacing (Table 3). Moreover, no interactions were identified between the main effects in the analysis (Table 3).

Table 3: Only main effects of planting date and variety were observed significant.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Planting date	1	249615	249615	21.115	3.63e-05	***
Variety	4	185273	46318	3.918	0.00832	**
Row spacing	1	9882	9882	0.836	0.36556	
Planting date: Row spacing	1	39015	39015	3.300	0.07608	.
Planting date: Variety	4	57427	14357	1.214	0.31832	
Variety: Row spacing	4	23927	5982	0.506	0.73151	
Residuals	44	520160	11822			
-	-	-	-	-	-	-
Significant codes	0 ‘***’	0.001 ‘**’	0.01 ‘*’	0.05 ‘.’	0.1 ‘ ’	1

5.2.1 Rowing spacing on yield

The mean yield within the narrow row spacing was 635.33 grams, with a standard deviation of 85 grams. For wide row spacing, the mean yield was 661 grams, with a standard deviation of 95 grams. The range of yields in narrow spacing was from 380 grams to 880 grams, while in wide spacing, it was from 350 grams to 990 grams.

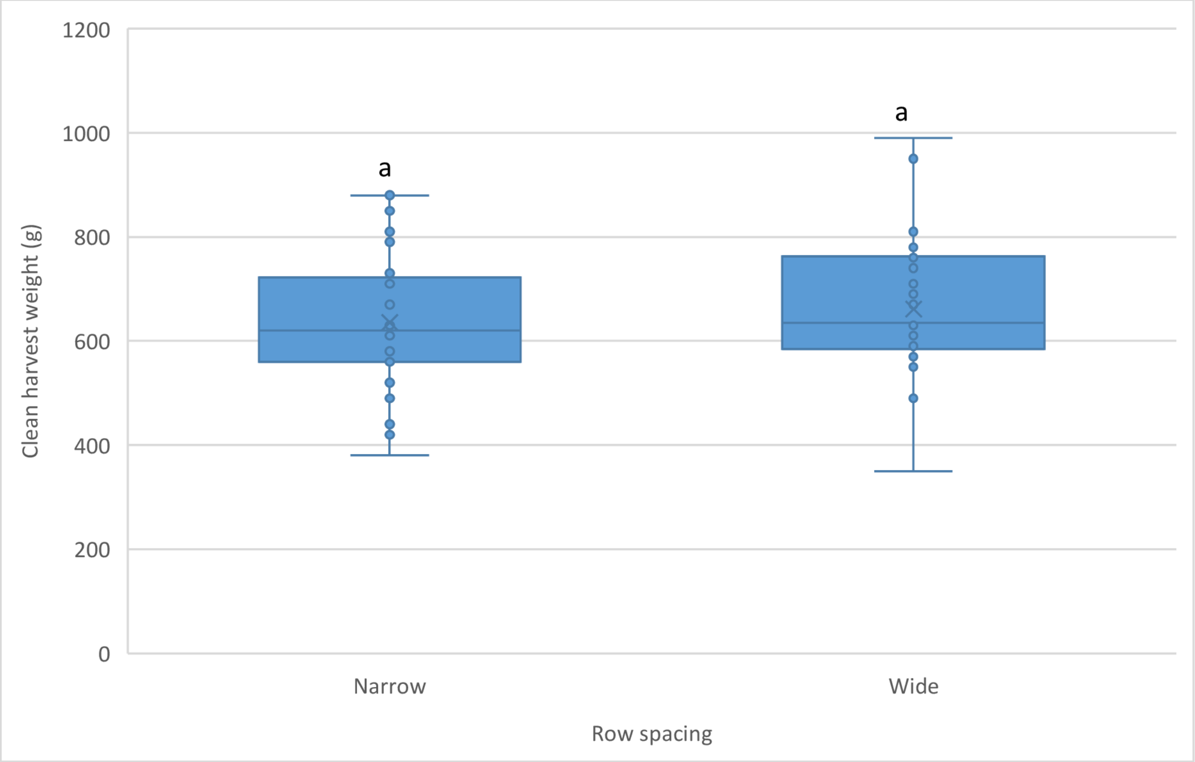

Figure 4: Clean seed weight in grams after 2 weeks drying affected by row spacing (box plot)

5.2.2 Planting date on yield

In Figure 2, the average yield from all fields is higher during spring (712.67 grams) than during fall (583.67 grams). The mean yield for fall is 610 grams, with a standard deviation of 60 grams. The mean yield for spring is 790 grams, with a standard deviation of 85 grams. The range of yields in fall is from 350 grams to 950 grams, while in spring, it is from 490 grams to 990 grams. Two outliers are identified in fall at 950 grams and 990 grams. Similarly, for spring, the outliers are at 490 grams and 880 grams. Overall, there is variability in the samples planted in spring compared to those planted in fall.

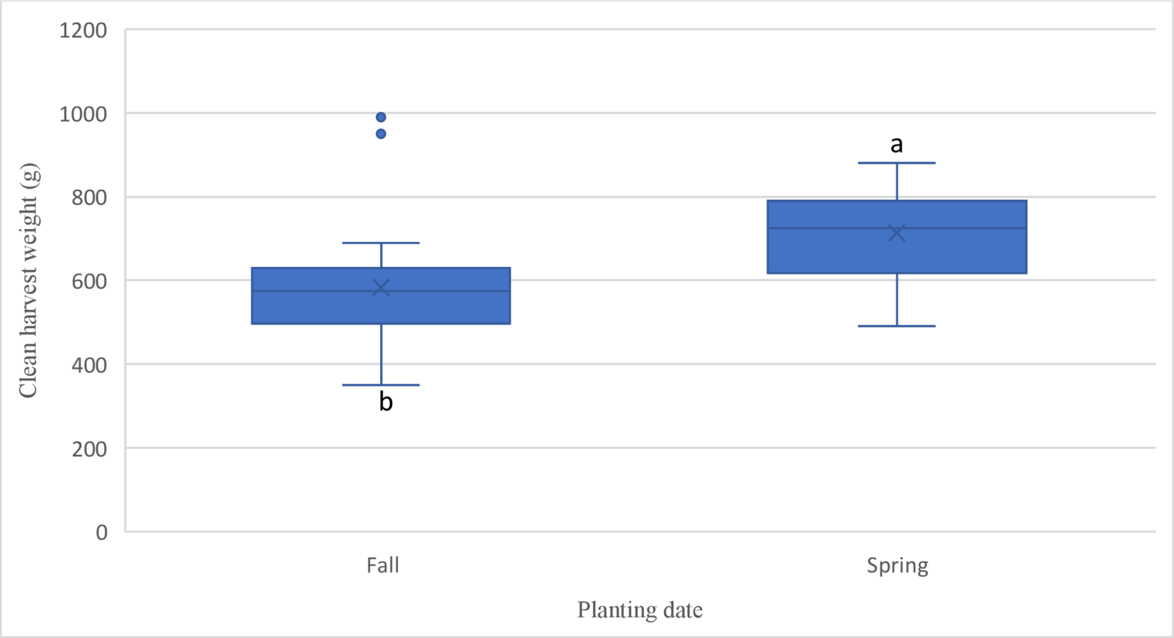

Figure 5: Harvest weight of clean seed after 2 weeks drying affected by planting date (box plot)

5.2.3 Variety

The highest estimated yield per hectare is for the 703 variety which is over 1600 kgs/ha.(Table 5). The significance of variety can be inferred from the average yield per square meter and the Tukey's HSD values associated with each variety. Varieties with different letters in the Tukey's HSD column exhibit statistically significant differences in their average yield per square meter. For example, Variety 703, labeled "a" in the Tukey's HSD column, demonstrates the highest average yield per square meter (164.44) compared to other varieties. In contrast, Varieties C5 and 702, labeled "bc," and Variety 34715, labeled "ab," exhibit lower average yields. These differences in yield indicate that the choice of variety significantly influences the productivity of the crop.

Table 4: Estimated yield on variety

Variety	Average yield per metre square	Tukey’s HSD	Estimated yield per Hectare (Ha) in grams	Estimated yield per Hectare in Kgs	Standard deviation	Standard error
C5	130.74	bc	*1307407.41	1307.41	157.01	**45.32**
701	150.74	ab	1507407.41	1507.41	155.90	**45.01**
702	133.33	bc	1333333.33	1333.33	101.00	**29.15**
703	164.44	a	1644444.44	1644.44	136.91	**39.52**
34715	140.93	ab	1409259.26	1409.26	62.59	**18.07**

*means that differences reported with some letters are not statistically significant based on Tukey's Honestly Significant Difference (HSD) test at a significance level of α = 0.05.

6 Discussion

As a new crop of interest, basic agronomic research is needed on IWG in each new region it expands. The agronomic research on IWG has a short history overall (DeHann et al. 2018) and has only a few research teams in Europe so far. This study is on the yield from the first IWG planted in the Czech Republic and so several effects needed to be generally evaluated. Although a complete factorial of three parameters were tested, no interactions were seen in the yield between all three factors variety, planting date, and row spacing, so each main effect will be discussed separately. Our study aimed to elucidate how these factors individually contribute to IWG yield, providing valuable insights for optimizing cultivation practices. Even though, many farmers would prefer to grow IWG for hay production than grain considering the economic returns, (Lanker et al. 2020), our study focussed only on the grain yield and not the biomass of IWG.

The ANOVA results indicate that planting date and variety significantly influence IWG yield, with both factors exhibiting statistically significant effects ($F = 21.115$, $p < 0.001$ for planting date; $F = 3.918$, $p = 0.00832$ for variety). However, row spacing did not show a significant main effect on yield ($F = 0.836$, $p = 0.36556$). Additionally, we observed a marginally significant interaction between planting date and row spacing ($F = 3.300$, $p = 0.07608$), suggesting a potential combined influence on yield.

6.1 Environmental factors

The vernalization (4-5°C), combined with the interplay of growing degree days and day length (13-14h) during spring is considered optimal for the IWG flowering and grain production in the study in Switzerland (Duchene et al. 2021). In our study, the average temperature below 5°C during winter and the growing degree days would have helped to fall planted IWG to yield better than Spring.

Our study assumes that IWG can establish and yield successfully at an elevation of 288 meters above sea level with an annual precipitation of 1400 mm. This assumption deviates from guidelines provided by studies in the Northwestern United States, which recommend a rainfall threshold of 330 mm and an elevation range between 1100-1700 meters above sea level for IWG planting (Hybner 2012). While our findings suggest potential for IWG cultivation under these conditions, further research is needed to validate our assumptions and assess broader applicability.

6.2 Agronomic Practices

6.2.1 Row Spacing

Even though, the narrow row spacing of 15-30 cm generally has a better yield over 3 years of growth in terms of the hay and straw production, the grain was not harvested. (Hunter et al. 2020), in our study, the initial harvest indicates that row spacing didn't significantly impact the yield. The study conducted in 1969 on the row spacing and fertilization found that row spacing does not have any influence on the yield and nitrogen based fertilizer does have. Our study did not use any synthetic fertilizer to boost the yield and the row spacings were narrower (20 cm, 40 cm) compared to that study (76 cm, 102 cm and 152 cm). However, for a perennial crop like IWG, a more extended study over a few years is essential to truly comprehend the influence of row spacing on the overall growth, sustenance, competition, and yield of the crop before considering widespread commercial farming.

These results contribute to a deeper understanding of the factors influencing IWG production and provide valuable guidance for growers seeking to enhance crop yield and overall agricultural productivity. However, additional research is needed to investigate other factors under various agroclimatic conditions that could impact IWG yield and help to formulate more comprehensive cultivation approaches.

6.2.2 Planting Date

The key emphasis is on the planting date, showing that it plays a more significant role than row spacing and variety when analysing yield comparisons.

The planting recommendation for growing IWG in the upper Midwest of the United States is to plant in the spring (Peters et al. 2021) and is based on the results of several experiments in that region (Hybner 2012). While extensive research has been done in the United States across many regions, (Hybner 2012) and some research has been done in Europe in the north (Germany) (Liste & Muskolus 2023) and west (France) (Soto-Gómez & Pérez-Rodríguez 2022), no evaluation of planting date has been done in central Europe. Conditions in this region are most close to the region of Germany with similar precipitation of 503 mm, respectively. On the other hand, that region is much warmer to here with an average yearly temperature of 10°C, compared to our average temperature of 9.7°C (Table 1). In other locations IWG has been planted to be a little earlier than winter wheat or a little later than spring wheat. Here we also planted to correspond with that timing.

As a perennial crop, IWG will have several yields over its lifetime and the expectation is that the yield will diminish over time (Law et al. 2020). And the plant must overcome winter before it will yield grain. The yield taken for analysis of this thesis was the second grain yield for fall planted IWG and the first grain yield for spring planted IWG. Our results indicate that grain yield from the plants planted in spring 2022 were higher than the yield from plots planted in fall 2021 (Figure 5).

Planting IWG two weeks before winter facilitates vernalization in crops like wheat and barley. This process ensures a transition from the vegetative to the reproductive phase, ultimately leading to higher yields. However, it's noteworthy that planting in spring, with optimal moisture for establishment, resulted in surpassing yields compared to fall planting. The research conducted in Minnesota, Montana, and Kansas states in the US found that the grain yield of IWG shows a positive correlation with the accumulation of vernalization units and late summer seeding (Jungers et al., 2022). Similarly, our study indicated that planting in the fall leads to increased accumulation of vernalization units, resulting in higher yields compared to spring planting. However, like the aforementioned study, additional investigation is required into other climatic factors that impact yield, such as photoperiod and snow cover.

6.2.3 Variety

In terms of yield, the chosen five varieties did exhibit a significant and distinguishable outcome compared to the overall IWG yield. This observation also emphasizes the necessity of considering other factors suitable to the region when selecting a specific variety for the crop. Breeding and hybridization programs are carried out in various parts of the world with focus to improved on the yields and adaptability of the crop to various agroclimatic conditions, in order to make the crop globally cultivatable for sustainable agriculture.

7 Conclusion

- This study revealed significant effects of planting date and variety on crop yield, as evidenced by the highly significant F-values and associated p-values in the analysis of variance. Specifically, planting date emerged as a crucial determinant of yield, with considerable variance attributed to variations in the timing of planting. Additionally, the choice of crop variety demonstrated a significant impact on yield
- In contrast, row spacing did not exhibit a statistically significant effect on yield, as indicated by the non-significant F-value and p-value. However, it is essential to consider practical implications of row spacing that may not be captured by statistical analysis alone.
- The interaction effects between planting date and row spacing, as well as planting date and variety, were not statistically significant, suggesting that the influence of these factors on yield is largely independent of each other. Nonetheless, further investigation into potential interactions between these variables may yield valuable insights into optimal crop management practices.
- Moreover, the examination of Growing Degree Days (GDD) and rainfall patterns across the two years revealed substantial variability, with notable differences observed between years. This variability shows the dynamic nature of weather conditions and their potential impact on crop performance.
- Overall, the findings highlight the significance of planting date and variety selection in optimizing crop yield, while also emphasizing the need for careful consideration of weather patterns and their potential implications for agricultural productivity.

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9 List of abbreviations and symbols

ABBREVIATIONS	
ANOVA	Analysis of Variance
GDD	Growing Degree Days
IWG	Intermediate Wheatgrass
NUE	Nutrient Use Efficiency
SOM	Soil Organic Matter
TLI	The Land Institute