Eesti Maaülikool
Estonian University of Life Sciences

DIETARY FIBER CONTENT OF CEREAL GRAINS INFLUENCED BY WEATHER, FERTILIZATION AND CROPPING SYSTEM

TERAVILJADE KIUDAINESISALDUSE SÕLTUVUS ILMASTIKUST, VÄETAMISEST NING VILJELUSVIISIST

BANAFSHEH KHALEGHDOUST

A Thesis
for applying for the degree of Doctor of Philosophy
in Agriculture

Väitekiri
filosoofiadoktori kraadi taotlemiseks
põllumajanduse erialal

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CONTENTS

ABBREVIATIONS 8
1. INTRODUCTION 9
2. REVIEW OF LITERATURE 12
2.1. Importance of cereal grains 12
2.2. Dietary fibers in cereal grains 13
2.2.1. Arabinoxylan 13
2.2.2. Beta-glucan 14
2.3. Factors affecting cereal grains dietary fiber content 15
2.3.1. Effect of weather condition 15
2.3.2. Effect of cropping system with different N fertilization 17
2.3.3. Effect of crop type and genotype 19
3. AIMS AND HYPOTHESIS OF THE THESIS 21
4. MATERIALS AND METHODS 23
4.1. Field experimental design 23
4.2. Dietary fiber analysis 25
4.3. Weather conditions during the experimental period (2014–2023) 25
4.3.1. Air temperature 25
4.3.2. Precipitation 26
4.4. Statistical analysis 28
5. RESULTS 29
5.1. Effect of weather on wheat, barley, and oat dietary fiber content 29
5.2. Effect of cropping system and nitrogen on wheat and barley dietary fibers 37
5.3. Effect of genotype on grains' dietary fibers in wheat, barley, and oat 39
6. DISCUSSION 42
6.1 The factors affecting wheat, barley, and oat dietary fiber contents 42

6.1.1. Effect of weather on wheat, barley, and oat dietary fibers .42
6.1.2. Effect of cropping system and nitrogen fertilization on wheat, barley, and oat dietary fibers 45
6.2. Effect of genotype on wheat, barley, and oat dietary fibers 48
7. CONCLUSIONS 50
REFERENCES 52
SUMMARY IN ESTONIAN.....67
ACKNOWLEDGEMENTS 73
ORIGINAL PUBLICATIONS.....75
ELULOOKIRJELDUS 110
CURRICULUM VITAE.....112
PUBLICATIONS 114

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Data collection	MK, **BK**	**BK**	**BK**
Data analysis	MK, **BK**	**BK,** MA	**BK**, MA
Preparation of manuscript	All	All	All

BK – Banafsheh Khaleghdoust, MA – Maarika Alaru, EL – Evelin Loit, All – all authors of the paper

ABBREVIATIONS

ANOVA	Analysis of Variance
AX	Arabinoxylan
BG	Beta-glucan
CV	Cultivar
EU	European Union
FAO	Food and Agriculture Organization
K	Potassium
LSD	Least Significant Difference
m	Meter
N	Nitrogen
NH_4NO_3	Ammonium Nitrate
Org	Organic
P	Phosphorus
r	Pearson's Correlation Coefficient
1000 KW	Thousand Kernel Weight
TDF	Total Dietary Fiber
TW	Test Weight

1. INTRODUCTION

The arable land on earth accounts for an estimated 1.5 billion hectares (Hens and Quynh, 2016) of which cereals occupy 40% (Biel et al., 2020). EU countries recorded an estimated 20% of the world's cereal production (FAO Food Outlook, 2016; Schils et al., 2018). A large volume of cereals in agricultural production is due to the popularity of cereal grains and their products in the diets of European nations (Lachman et al., 2012; Nogala-Kałucka et al., 2020).

The main structural components of cereal grain are the bran, germ, and endosperm (Balandrán-Quintana, 2018). In addition to protein, they contain calories, minerals, fibers, phytochemicals, and vitamins (Lafiandra et al., 2014; Zhu, 2017; Tieri et al., 2020; Biel et al., 2020) and are the dominant source of carbohydrates in the human diet, providing the primary source of energy and contributing significantly to protein intake (Lafiandra et al., 2014). In addition, cereal grains are a great source of dietary fiber. These carbohydrates are not digested and absorbed in the small intestine but pass perfectly to the colon and undergo complete or partial bacterial fermentation (Ibba et al., 2021).

The content and composition of dietary fibers in grains determine the quality of a cereal and its products (Biel et al., 2020). The intake of dietary fiber is important for human health improvement. For instance, adults are recommended to take approximately 30 g of dietary fiber daily (Reynolds et al., 2019). In cereal crops, arabinoxylans (AX) and beta-glucans (BG) are the main components of dietary fiber. AX and BG account for ~70% and 20% of dietary fiber content in whole grain wheat, respectively. On the other hand, AX and BG constitute approximately 20% and 70% of the total dietary fiber content in barley and oat grains, respectively (Prasadi and Joye, 2020). The dietary fiber content varies among different cereals: wheat contains 9-13%, barley 17-23%, and oats 7-11% of whole grains (Tullio et al., 2021). Nutraceutical benefits of cereals can be achieved by recovering the nutrients (Salazar-López et al., 2020) present in the whole grain. The hull, bran, and cereal germ are often removed from foods during cereal manufacturing as by-products (Galanakis 2022). Dietary fibers contribute to reducing the risk associated with colon cancer and heart disease. Fibers also reduce the post-prandial

glycemic response and intestinal transit time while contributing to the rise in fecal bulk (Prins and Kosik, 2023).

Cereal dietary fiber content in grains depends on many factors, including agricultural practices, environmental conditions, and genetic traits (Tiwari and Cummins, 2009; Takač et al., 2022). Understanding how organic farming with lower nitrogen fertilization affects the dietary fiber of cereals is crucial. The European Union's Farm to Fork strategy, part of the European Green Deal initiatives, aims to ensure sustainable food production and increase the agricultural land under organic farming to 25% by 2030 (European Commission, 2020).

As the EU shifts towards more diversified agricultural practices to meet these goals, promoting a diverse range of crops can enhance ecosystem resilience, improve soil health, and reduce reliance on chemical inputs. One effective strategy within this diversification approach is intercropping. Intercropping, the practice of growing two or more crops together, can improve nutrient use efficiency and pest management, which are particularly relevant to organic farming systems. The EU can support sustainable practices while addressing food security and climate change challenges by integrating intercropping into broader agricultural policies.

Global warming and unfavorable weather conditions have negatively affected the EU's cereal farming, particularly in 2020 and 2021, and more severely in 2022 (Popescu et al., 2022). Intercropping could mitigate the impact of climate change and global warming by enhancing crop resilience and stability (Maitra et al., 2023). Therefore, incorporating intercropping into organic farming practices aligns with the EU's political goals and strengthens the overall sustainability and productivity of cereal production.

In this thesis, the most important factors affecting dietary fibers (AX and BG) in the grain of three main Estonian cereals, wheat, barley, and oats, have been studied to evaluate and present the most performant agricultural practices for farmers to achieve both production and economic goals.

The novelty of this study is the time frame and assessment period of eight years of AX and BG content in winter wheat and spring barley

grown in cropping systems with different N fertilizations under different weather conditions. The chosen period was assumed to be ample time to show differences caused by environmental stresses. In addition, different genotypes of barley and wheat were analyzed to investigate potential epigenetic factors as well. The study also investigated the effect of intercropping on the content of Ax and BG in barley and oats.

Article I investigated the effect of organic and conventional cropping systems with different N fertilization rates during eight years on winter wheat and spring barley AX content, while **Article II** reported on their BG content. In addition, the content of AX and BG were characterized by different cereal genotypes. **Article III** studied the influence of legume intercropping during two years on AX and BG content in spring barley and oat grains.

2. REVIEW OF LITERATURE

2.1. Importance of cereal grains

Cereals are the most widely produced crops in the world and have multiple uses. They are considered the primary source of calories and protein in the diets of humans and livestock (Lafiandra et al., 2014). In 2024, global cereal production is 2.841 million tonnes, with wheat production at 789 thousand tonnes, barley production at 150 thousand tonnes, and oats production at 25 thousand tonnes (FAO, 2024). At the same time in Estonia, cereal production has been recorded at approximately 1,3 million tonnes, with wheat, barley, and oats production at 745, , 315, , and 135, thousand tonnes respectively (Statistics Estonia, 2025).

Wheat is among the earliest cereal crops cultivated in human history, and it produces high amounts of nutrients, calories, and dietary fibers in the diet of Western people (Gebruers et al., 2010). Wheat is the third most commonly produced cereal after rice and maize and the second-largest produced cereal for human consumption (World Economic Forum, 2022). The wheat genus has various species, such as common wheat (*Triticum aestivum* L.), spelt (*Triticum spelta* L.), and durum (*Triticum durum*). Common wheat is cultivated worldwide due to its high yield potential and high nutritional and baking qualities. Studies have shown that 19% of the total calorie intake in the human diet comes from wheat grain (Biel et al., 2020). Optimal growth and high wheat yields are achieved in environments with well-drained, fertile soils, moderate temperatures, and adequate moisture. A balanced provision of sunlight and nutrients is essential for this process (Poltoretskyi et al., 2020).

Barley has the highest production after wheat, rice, and maize (Boukid, 2024). It is the fourth-largest cereal crop produced globally (Geng et al., 2022). Much of barley production globally comes from the regions where cereals such as rice and maize do not grow adequately (Zhou, 2009). Approximately 70% of barley produced globally is used for animal feed, while 30% is utilized for human consumption, such as malt for alcoholic beverages and different food products (Newton et al., 2011). Barley is known for its high nutritional value content in groats and flakes (Biel et al., 2020). It exists in hulled and hullless varieties, hulled barley can be dehulled after harvest before processing (Newman and Newman, 2019).

However, the hull from barley is not easy to remove, as it is 'cemented' to the outer pericarp layer of the kernel (Djurle et al., 2016). Barley has a wider distribution and environmental adaptability globally due to its high tolerance to drought (Geng et al., 2022).

Oats (Avena sativa L.) are cereal crops cultivated globally, ranking sixth in production, following rice, maize, wheat, barley, and sorghum (Akasa, 2023). Oats are typically grown on marginal lands in regions where the environmental conditions are less appropriate for the optimal cultivation of wheat and barley, often requiring lower inputs regarding soil quality, water, and other agricultural resources (Ma, 2021). This makes oats viable in areas with more challenging growing conditions. Oats can be distinguished as hulled and hullless. Its hull, unlike barley, is much easier to remove. Oats is a nutritionally significant cereal crop that is rich in health-promoting dietary fibers (Zambrano et al., 2023).

2.2. Dietary fibers in cereal grains

Dietary fibre is defined as the edible part of plants, which is resistant to enzymatic digestion in the small intestine and includes cellulose, non-cellulosic polysaccharides (hemicellulose, pectic substances, gums, mucilages) and non-carbohydrate component (Marcotuli et al., 2020). Dietary fibers offer numerous health-related benefits, including improved digestive health, reduced cholesterol levels, and enhanced weight management. Dietary fiber consumption differs among countries, ages, and genders. It has been recommended that daily dietary fiber intake should be approximately 30 g to enhance adult health (Reynolds et al., 2019). Dietary fibers, beta-glucans, and arabinoxylans, are classified as non-starch polysaccharides; and are major structural components of the cell walls in cereal grains (Wang and Zhang, 2010).

2.2.1. Arabinoxylan

Arabinoxylan is a significant non-starch polysaccharide of cereal grains' cell walls, referred to as pentosan, consisting of pentose, arabinose, and xylose (Shah et al., 2021). Structurally, it consists of a linear backbone of β-(1,4)-linked D-xylopyranosyl residues, which can be substituted with α-L-arabinofuranosyl units at the C(O)-2 and/or C(O)-3 positions (Rosicka-Kaczmarek, 2016; Zannini et al., 2022). This complex carbohydrate is synthesized in the Golgi apparatus of plant cells and is

primarily accumulated in the bran, the outer layer of the grain (Comino et al., 2013; Weng et al., 2023). Wheat, rye, and triticale grains are regarded as the primary sources of AX, which is found in significant amounts in the cell walls of the endosperm (triticale) and aleurone layers (wheat, barley, and rye) (Becraft and Yi, 2011; Piro et al., 2023). The content of arabinoxylan varies among different cereals: wheat contains approximately 6-8% arabinoxylan, barley around 4-7%, and oats about 2-4% (Izydorczyk, Biliaderis, 2007; Weng et al., 2023). Despite the fact that AX is not a major component of caryopsis, it is the main structural polysaccharide of the cell walls, and it is the major portion of the dietary fiber in wheat flour (Skendi et al., 2011).

These fibers are crucial for the plant's structural integrity and offer numerous health benefits when included in the human diet (Zannini et al., 2022). AX influences several biotechnological processes. In bread baking, AX enhances water absorption, improves dough stability, and contributes to better loaf volume and texture. AX affects wort viscosity, filtration efficiency, and beer foam stability in brewing. AX acts as a prebiotic in animal feeds, promoting beneficial gut bacteria and improving livestock health and growth (Van Craeyveld, 2009; Kiszonas, et al., 2015). In the human body, AX minimizes blood cholesterol, enhances metabolic syndrome, and controls blood sugar levels and intestinal microorganisms when taken in a food diet (Sun et al., 2022). However, to obtain health benefits, the specific recommendation for AX consumption per day is 10 g (Stephen et al., 2017).

2.2.2. Beta-glucan

Beta-glucan (BG) is a second significant non-starch polysaccharide found in cereals such as barley, wheat, and oats. Structurally, it consists of linear polymers of D-glucose units linked by β-(1,3) and β-(1,4) glycosidic bonds (Lazaridou and Biliaderis, 2007; Cui and Wang, 2009). This polysaccharide is synthesized also in the Golgi apparatus of plant cells and is primarily accumulates in the endosperm (barley and oats) and aleurone (wheat) layers of the grain (Izydorczyk and MacGregor, 2000; Lazaridou and Biliaderis, 2007; Tiwari and Cummins, 2009; Sapirstein, 2016; Mejía et al., 2020; Singla et al., 2024). The content of BG varies among different cereals: barley contains approximately 3-11%, oats 3-7%, and wheat around 0.5-2% (Lazaridou and Biliaderis,

2007; Knudsen, 2014; Hu et al., 2015). A small amount of BG (2-3%) can also be found in rye (Maheshwari et al., 2017).

These polymers are crucial for maintaining the plant's structural integrity and provide a variety of health benefits (Mishra, 2020). BG consumption has many human health benefits, including reducing serum cholesterol and regulating blood glucose levels, obesity, and hypertension (Lazaridou and Biliaderis, 2007; El Khoury et al., 2012). β-glucan has a high water binding capacity, resulting in its physicochemical properties, such as solubility, viscosity, and gelation (Sun et al., 2021). In food industries, BG is used as a stabilizer, thickener, and texture enhancer (Mejía et al., 2020). Consequently, foods containing dietary fibers such as BG are regarded as naturally low in fat and convey lower calories, which are essential for human health (Elleuch et al., 2011). The European Commission reaffirms the health claim for the significance of BG in human health by stating that the daily intake of 3 g of barley BG helps lower blood cholesterol (European Commission, 2012).

2.3. Factors affecting cereal grains dietary fiber content

The dietary fiber content in cereal grains is affected by various factors, including the type of grain, genetic differences, and the environmental conditions during growth. The size and structure of the grain also play a crucial role. Processing methods like milling can significantly reduce dietary fiber content by removing the bran and germ rich in fibrous polysaccharides (Serna and Hernández, 2020). Whole grains, for example, retain more dietary fiber than refined grains. Agronomic practices, such as nitrogen fertilization, influence the fiber composition of cereals (Landberg et al., 2019). Additionally, organic farming practices, which avoid synthetic agrochemicals, can affect fiber content differently than conventional cropping systems due to different factors such as soil health and nutrient availability (Jefferson and Adolphus, 2019). These factors collectively determine cereals' total dietary fiber (TDF) levels, impacting their nutritional value and downstream health consequences and benefits.

2.3.1. Effect of weather condition

Weather conditions significantly impact the dietary fiber content in cereal grains. Factors such as temperature and rainfall during the

growing season can influence the levels of critical dietary fibers like BG and AX. In a recent study, weather conditions significantly affected the absorption and remobilization of nitrogen by cereal plants during the growing season (Sułek et al., 2024). The efficiency of N fertilizer in promoting grain and yield nutritional quality was influenced by the moisture condition during the tillering stage of a specific year (Bleidere et al., 2013). While high temperatures have negative effects on grain weight, they can increase some phenolic compounds (Martínez-Subirà et al., 2021) and, together with precipitation, can have a significant impact on dietary fibers (Andersson and Börjesdotter, 2011; Dickin et al., 2011). The grain tends to have larger levels of total dietary fiber components when grown in hot and dry climates (for instance, in Hungary) rather than in cool, wet conditions (in the UK) (Gebruers et al., 2010).

Early research suggested that heat stress and drought during flowering raise the concentration of AX (Hong et al., 1989), while high temperature and drought after flowering indicate an inverse effect (Laurentin and Douglas, 2003). Toole et al. (2007) suggested that the extent of substitution of the AX with arabinose (termed branching) in the cell walls of the starchy endosperm falls during the grain-filling stage. This happens more quickly when the plants are grown at a higher temperature with repressed water availability. Therefore, while some environmental factors can enhance AX concentration as a stress response, others can negatively influence its structure and amount, particularly during critical developmental stages. In another study, Zhang et al. (2010) found that high temperatures from flowering time also raised the AX concentration in the grain of wheat. Lempereur et al. (1997) studied drought as one of the environmental factors that increasingly impact AX content in wheat grains. However, the extent of stress also seems to be essential since the concentration of AX in wheat was raised by mild drought after flowering but declined under severe drought (Coles et al., 1997). As for oats, however, AX and other biopolymer compositions are highly affected by weather changes during the grain-filling stage of oats, with drier and warmer periods having lower values (Schmitz et al., 2020). Conversely, Dornez et al. (2008) revealed that weather conditions did not affect total AX levels at harvest.

Similar to AX, precipitation, and air temperature during the growing period has been indicated as the most critical factors influencing the content of BG in cereal grains. Several studies have shown that higher

rainfall correlated with lower BG content of barley and wheat grains (Güler 2003 a, b; Dickin et al., 2011; Howarth et al., 2021), while dry weather growing conditions positively affected the BG content in oats (Lazaridou and Biliaderis, 2007). However, Ehrenbergerová et al. (2008) and Chappell et al. (2017) demonstrated that rainy but cool weather during grain establishment and flowering had a negative effect on BG content in grain. Menkovska et al. (2017) reported that precipitation during the grain-filling period positively affected BG content in oat grains. Coles et al. (1991) indicate that drought stress decreases BG concentration in barley grain, while Güler (2003 a, b) found a negative influence of increased irrigation levels on barley BG content. In addition, research (Martínez-Subirà et al. 2021) conducted in Northeast Spain showed that high temperature does not affect the barely AX and BG profiles.

2.3.2. Effect of cropping system with different N fertilization

Among the agronomic factors, N fertilization significantly affects the yield and chemical composition of cereal grains (Sułek et al., 2024), as grain biochemical composition can be enhanced by manipulating N fertilization (Bleidere et al., 2013). Organic farming enhances natural processes that support plant growth while conserving soil and water. Unlike conventional farming, it avoids synthetic chemicals, reduces reliance on external resources, and improves farm profitability, creating a healthier environment and promote sustainable agriculture. While organically producd grains has the potential for higher profitability due to price premiums, it is subject to market volatility, higher production costs, and yield variability (Uematsu and Mishra, 2012; Smith et al, 2019; Esmaeilzadeh-Salestani et al, 2021; Parizad and Bera, 2023; Panday et al, 2024).

Previous studies have shown that dietary fiber content in grains can depend on the type of cropping system. For instance, it has been found that common wheat grown in conventional systems has higher AX values than in organic cropping, while the opposite is true for spelt wheat (Takač et al., 2022). At the same time, Menkovska et al. (2017) have shown comparable BG values for barley grains obtained from organic and conventional farming systems (Menkovska et al., 2017).

Intercropping represents an environmentally friendly and economically viable sustainable agricultural strategy, particularly in light of climate change (Tilman et al., 2011; Raza et al., 2021). This method increases crop productivity through enhanced crop diversity. The success of intercropping systems is affected by several critical factors, including functional diversity, species identity, species richness, and the dynamics of interspecific competition (Mahaut et al., 2020). Carefully selecting crops and species for intercropping can reduce interspecific competition and foster complementary interactions (Hauggaard-Nielsen and Jensen, 2005; Stomph et al., 2020). The integration of legumes with cereals in these systems is particularly advantageous, as legumes have the capacity to fix nitrogen from the atmosphere, thereby facilitating their growth and improving nitrogen utilization in cereal crops. Studies have shown that growing cereals alongside legumes (such as wheat/winter pea, wheat/beans, and maize/soybean) results in higher nitrogen content in cereal biomass and enhanced protein levels in grains compared to monoculture practices (Naudin et al., 2010; Raza et al., 2022).

Furthermore, including legumes in intercropping systems has been shown to improve quality indicators for cereal grains, such as the 1000 kernel weight (Benider et al., 2021). Intercropping, particularly with legumes, not only enhances crop productivity and nitrogen utilization but also has significant implications for dietary fiber content in cereals. This increase in dietary fiber is attributed to the complementary interactions between the crops, which optimize nutrient uptake and utilization (Ebbisa, 2022). The enhanced nutrient dynamics and reduced interspecific competition in intercropping systems create an environment conducive to higher dietary fiber accumulation in cereals (Saldívar and Hernández, 2020; Ebbisa, 2022).

Research indicates that nitrogen fertilization affects the content of BG and AX in cereals. While some studies suggest that N fertilization does not affect AX content in durum wheat (Lempereur et al., 1997), others have found that N application during grain filling increases AX content (Coles et al., 1997). Applications of nitrogen in the range of 70 to 140 kg N ha^{-1} have been shown to affect the content of AX significantly. Notably, higher application rates corresponded to further increases in AX levels. However, the extent of this effect was influenced by different environmental conditions (Sułek et al., 2024).

For BG, mineral N fertilizers have been reported to increase its content (Güler, 2003a; Noworolnik et al., 2014; Habiyaremye et al., 2021). A small amount of fertilization (50 kg N ha^{-1}) has an insignificant effect on BG content, while higher fertilization levels (100 kg N ha^{-1}) cause an increase (Fontana et al., 2009). For example, 100 kg N ha^{-1} has a larger effect on wheat, and 80 kg N ha^{-1} on barley (Güler, 2003a). In a study conducted on the husked oat genotype 'Lizette', the BG content was higher with increased nitrogen application rates (160 kg N ha–1) in 2011, 2012, and 2013. However, the opposite trend was observed in 2014 (Brunava et al., 2015). On the other hand, it was shown that different N applications did not significantly affect barley grain BG across all three years (2016-2018) and at two different locations (Almota and Genesee) in the USA (Habiyaremye, 2021).

2.3.3. Effect of crop type and genotype

The health benefits and functionalities of cereal grains are significantly influenced by the selection of grain varieties (Gebruers et al., 2010). Apart from environmental conditions and the cropping system, the content and properties of AX and BG are determined by genotype (Güler, 2003; Tiwari and Cummins, 2009; Dickin et al., 2011; Noworolnik et al., 2014; Rakszegi et al., 2014; Choi et al., 2020; Habiyaremye et al., 2021; Prins and Kosik, 2023). Genotypes have shown up to 70% differences in total AX content, with significant variations observed across different growing conditions (Shewry et al., 2020).

Studies have revealed that winter wheat generally has lower AX content than do spring wheat genotypes (Takač et al., 2022; Li et al., 2009). However, some research indicated that AX values can be similar in both winter and spring wheat (Selga et al., 2023). Different varieties tend to express different solubility of AX; therefore, the structure and content of AX vary widely among species, genera, varieties, and tissues, impacting the end-use properties and nutritional quality of the grain (Ebringerová et al., 2005).

The BG content is influenced by genotype as waxy barley genotypes and hull-less varieties have higher BG content than high amylose starch and hulled varieties (Ehrenbergerová et al., 2003; Izydorczyk and Dexter, 2008; Bleidere et al., 2013). Hull-less barley varieties with low amylose content can provide 1.5 to 4 times more BG compared to oats (Newman

and Newman, 2019). In oats, genotype does not significantly affect grain BG content (Fan et al., 2009). Naked oats lack glumes and have higher lipid and protein content but lower fiber and greater energy value than conventional oats (Biel et al., 2020). Primitive wheat and wild relatives, such as *Aegilops* species, have higher BG content, making them valuable for improving BG content in domesticated wheat (Marcotuli et al., 2019; Ivanizs et al., 2022).

3. AIMS AND HYPOTHESIS OF THE THESIS

Crop's dietary fibers, particularly AX and BG, are essential components of food grains that significantly contribute to human health and enhance the overall nutritional value of diets. Cereal grains such as wheat, barley, and oats play a pivotal role in ensuring food security and sustainable agricultural development while also serving as vital sources of AX and BG. Despite the well-documented nutraceutical benefits of these fibers, global dietary fiber intake remains insufficient (Jones, 2014; Mckeownet et al., 2022).

Addressing this deficiency requires optimizing the growing conditions for cereals to enhance their AX and BG content. At the same time, sustainable crop production practices could be adopted. European policymakers are increasingly advocating for organic farming systems, emphasizing crop diversification and reducing the reliance on synthetic fertilizers. Understanding how genetic, environmental, and agricultural management factors influence AX and BG levels in grains is crucial for aligning health and sustainability goals.

This thesis investigated the influence of weather conditions, cropping systems, and cereal-legume intercropping on cereal grains' AX and BG content. The study covered eight years of data on AX and BG content in winter wheat and spring barley grains under diverse cropping systems and environmental conditions. Additionally, it analyzed the AX and BG levels in barley and oat grains grown as pure stands and in legume-intercropped systems over a two-year period. Variations in fiber content across different winter wheat and spring barley genotypes were also characterized.

This thesis aimed to evaluate the two main dietry fibers, AX and BG, content changes in cereal grains based on the following factors:

A. Cropping systems with varying N fertilization leves (Article **I, II**)
B. Weather conditions (air temperature and precipitation) during crop growth (Article I, II, III)
C. Cereal crop genotype (Article I, II)
D. Cerel-legume intercroppoing system (Article III)

1. AX content is higher in cereal grains grown organically than conventionally; AX content in cereal grains depends on weather conditions, more specifically on drought (Article I).
2. BG content is greater in cereal grains from higher fertilized rates; BG content in cereal grains depends on weather conditions in specific growth stages (Article II).
3. BG and AX content is greater in barley and oats intercropped with vetch and field peas in contrast with pure stands (monoculture) of barley and oats (Article III).

4. MATERIALS AND METHODS

4.1. Field experimental design

The thesis was based on three field experiments. The first and third experiments were conducted at the Estonian University of Life Sciences located in Tartu County (called Eerika field) (58°21′53.2″N, 26°39′57.7″E), where the soil type was *Stagnic Luvisol* (**I, II, III**). The second experiment was conducted at the Estonian Crop Research Institute in Jõgeva County (58°45'56.1"N 26°24'26.5"E) , where the soil type was *Luvisol* (50 km north of the first field trial) (called Jõgeva field) (**I**, **II**).

The data were obtained for the first field experiment by analyzing winter wheat cv. 'Fredis' and spring barley cv. 'Anni' grain samples were collected from 2014 – 2021 from the long-term five-field crop rotation experiment (I, II). Crop rotation consisted of spring **barley** (*Hordeum vulgare L.*) with undersown red clover, red clover (*Trifolium pratense L.*), **winter wheat** (*Triticum aestivum L.*), pea (*Pisum sativum L.),* and potato (*Solanum tuberosum L.*) following one another in four replications. Conventional and organic cropping systems (60 m^{-2}) were fertilized respectively with mineral nitrogen (N) (N0, N1, N2, N3) and organic (Org0, Org1, Org2) fertilizers in parallel. The conventional treatments were control N0 ($N_0P_0K_0$), minerally (NH_4NO_3) fertilized N1 (N_{40}-$_{50}P_{25}K_{95}$), N2 (N_{80}-$_{100}P_{25}K_{95}$) and N3 ($N_{120\text{-}150}P_{25}K_{95}$). Spring barley was fertilized with lower N rates (N_{40}, N_{80}, N_{120}) and winter wheat with higher rates (N_{50}, N_{100}, N_{150}). Organic treatments consisted of organic control (Org0), off-season cover crops as green manure before spring barley, field pea and potato (Org1), and off-season cover crops with fully composted cattle manure (10 t ha^{-1}; Org2). In trial years, nitrogen amounts in manure for barley and wheat were between 44 and 54 kg $ha^{-1,}$ depending on manure dry matter content.

The data to evaluate the genotype effect on AX and BC content in grains was collected from a field experiment conducted in 2021. The effect was evaluated using the same cultivars, winter wheat cv. 'Fredis' and spring barley cv. 'Anni', in comparison with six winter wheat cultivars ('Ada', 'Creator', 'Perenaise', 'Julie', 'Širvinta', 'Kallas') and nine spring barley ('Maali', 'Grace', 'Planet', 'Feedway', 'Tuuli', 'Breustar', 'Irina', 'Quench'

and 'Anneli') cultivars. Spring barley received mineral fertilization at a rate of $N_{90}P_{24}K_{54}$ and winter wheat at a rate of $N_{152}P_{36}K_{75}$. All studied spring barley varieties were hulled two-row grains listed on the Estonian Plant Variety Register. All but 'Širvinta' (Estonian heritage wheat grain) wheat varieties were listed on the Estonian Plant Variety Register.

The third field experiment, conducted in 2022 and 2023, included two different cropping systems: the mono-cropping system and the intercropping system (Figure 1) (**III**). The experiment was carried out in a systematic block design consisting of four replicates (2 x 10 m) for every treatment. The intercropping system involved two treatments: barley 'Laureate' + vetch (*Vicia sativa*) 'Hanka' (the seed ratio 64:36) and oat 'Symphony' + field pea 'Casablanca' (83:17). No mineral fertilizers were added to the treatments (neither mineral nor organic), and no plant protection was used.

In 2022, crops were sown on May 25 and harvested on August 29, except for vetch and barley in the intercropping system, which was harvested on September 29. In 2023, crops were sown on May 12 and harvested on September 6. However, intercropped barley + vetch was not harvested due to the continuous growth of vetch. Seeds were harvested with a small experimental harvester Sampo, and seeds from intercropped systems were separated manually after harvest. Oat and barley yields were determined based on a 20 m^2 test plot per t ha^{-1}.

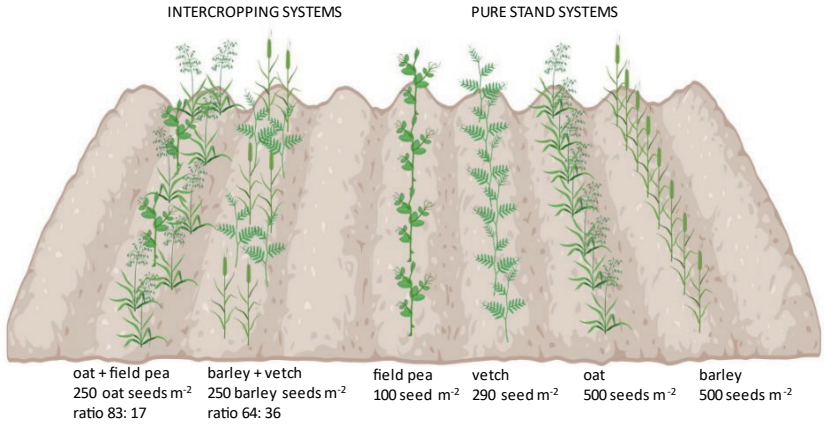


Figure 1. Experimental scheme and seeding ratios in 2022 and 2023. This figure was reproduced from Article **III** (Figure 1)

4.2. Dietary fiber analysis

After sample collection, the grains were milled using a Perten Laboratory Mill 3100 (Perten Instruments AB, Sweden). The content of AX and BG in milled samples of winter wheat (N =179) and spring barley (N = 221) grains obtained from the crop rotation experiment as well as spring barley (N = 10) and winter wheat (N = 7) grains obtained from Estonian Crop Research Institute and oat (N = 16) and barley (N = 16) grains obtained from the intercropping experiment were analyzed using appropriate procedures.

The content of AX was determined enzymatically with the microplate assay procedure for d-xylose (Megazyme International, Ireland Ltd). This procedure was conducted in triplicate using a Clariostar (BMG Labtech, Germany) absorbance microplate reader at a wavelength of 340 nm. The d-xylose percentage used to compute the AX content of the samples were literature-based values of 0.62, 0.75%, and 1% for winter wheat (**I**), barley (**I, III**), and oat (**III**), respectively.

The level of BG was determined using a Mixed-linkage BG assay kit (Megazyme, Ireland). The BG content was measured in wheat and barley (II) and barley and oat (III) at a wavelength of 510nm. The procedures were conducted using a Helios Omega UV-Vis Spectrophotometer (Thermo Scientific) (**II**) and a Clariostar (BMG Labtech, Germany) absorbance microplate reader (**III**).

4.3. Weather conditions during the experimental period (2014–2023)

4.3.1. Air temperature

Air temperatures during the growing periods of eight years (April to August from 2014 to 2021) were variable (Table 1). The post-winter period of winter wheat and vegetative period of spring barley from April to August was relatively colder between 2014 and 2017 (with an average temperature of 13.1°C) and similar to the long-term average of the growing period (1964–2021). Meanwhile, the period between 2018 and 2021 was warmer (with an average temperature of 14.5 °C). The most exceptional years were 2018 and 2021, with 2.6 and 1.7 °C higher

than long-term average mean temperatures during the growing period. 2015 and 2017 appeared coldest due to the unusually cold May, June, and July. Meanwhile, 2014 and 2020 were most similar to the long-term average of the growing periods. In 2022 and 2023, average temperatures were almost similar (14.1°C in 2022 and 14.2°C in 2023) and higher than the long-term average (13.1°C). The initial months of the growing periods had lower temperatures in 2022 (April 4.5°C and May 10.3°C) compared to 2023 (April 7.2°C and May 11.4°C). The late months from June to August had higher temperatures in 2022 (June 17.5°C, July 18.1°C and August 20.1°C) compared with 2023 (June 16.9°C, July 16.9°C and August 18.9°C) (Table 1).

4.3.2. Precipitation

The precipitation during the study's growing periods was fluctuating (Table 2). The growing period of 2021 showed the highest sum of precipitation, exceeding that of the long-term average by 85 mm due to the rainy months of May and August. Similarly 2014, precipitation was also high, caused by rain in May, June, and July. The driest years during the growing period were 2018, 2019, and 2023. They account for an estimated 100 mm less rain during plant growing period than the long-term average. Like the temperature, the 2016 and 2020 years were most similar to the long-term average. The sum of precipitation in 2022 and 2023 was 340 and 200 mm, respectively. Precipitation in May and June was much less in 2023 (10 mm and 27 mm) compared to 2022 (46 mm, 58 mm) and 60-year average (52 mm and 71 mm). Precipitation in July of 2022 was exceptionally high (145 mm) due to the heavy rain on July 16th (83 mm) and the same in 2023 with long-term averages (69mm and 69 mm, respectively). September of 2022 was cooler compared to the long-term average and an atypically dry month. In the second year (2023) we had atypically warm September (temperature was comparable to August) with close to average precipitation.

Table 1. Average air temperatures and long-term averages during the experimental period (2014 – 2023) in Eerika experimental field.

	The average air temperature per month,°C					
	April	**May**	**June**	**July**	**August**	**year average**
2014	6.5	12.0	13.4	19.4	16.7	13.6
2015	5.4	10.3	14.2	15.7	17.0	12.5
2016	6.1	14.0	15.9	17.9	16.1	14.0
2017	3.4	10.4	14.0	16.0	16.7	12.1
2018	7.2	16.0	15.9	20.8	18.8	15.7
2019	7.7	11.4	18.6	16.4	16.7	14.1
2020	4.8	9.5	18.4	16.3	16.8	13.2
2021	5.3	10.9	19.8	22.2	15.8	14.8
2022	4.5	10.3	17.5	18.1	20.1	14.1
2023	7.2	11.4	16.9	16.9	18.9	14.3
Long term average temperature	5.1	11.4	15.5	17.4	16.1	13.1

Table 2. Monthly precipitation and long-term averages during the experimental period (2014 – 2023) in Eerika experimental field.

	The sum of precipitation per month, mm					
	April	**May**	**June**	**July**	**August**	**year sum**
2014	13	84	103	71	113	385
2015	69	62	39	61	41	273
2016	52	2	125	82	42	301
2017	52	16	94	61	106	328
2018	28	8	61	14	59	170
2019	3	60	51	41	58	213
2020	50	32	117	69	64	332
2021	29	108	19	17	217	390
2022	31	46	58	145	60	340
2023	23	10	27	69	71	200
Long term average precipitation	33	52	71	69	79	304

4.4. Statistical analysis

Results were expressed as means ± standard error and analyzed using one-way and two-way analysis of variance (ANOVA) with the statistical software package Statistica version 13 (TIBCO Software Inc, USA) and Microsoft Excel. In case of significant differences ($P < 0.05$), Fisher's least significant difference test (LSD post-hoc test) was used to determine the differences among means. Pearson's correlation coefficient r was calculated to measure the strength of a linear association between the variables studied.

5. RESULTS

The fiber content in grains was mainly influenced by weather conditions, which calculate proportion of variance from two-way anova accounted for 79% of the total variance of AX and 90% of BG. In comparison, the second factor fertilization contributed only 2% to the variance in both dietary fibers. Latter means that neither the crop production systems nor the nitrogen treatments significantly influenced the composition of the grains.

The average values recorded for AX and BG in wheat and spring barley across different treatments were as follows: AX ranged from 1.90 to 5.90 g 100 g^{-1}, while BG values ranged between 0.30 and 5.80 g 100 g^{-1}. The variation between years was 3.85 and 2.41 g 100 g^{-1} for AX and 4.55 and 4.80 g 100 g^{-1} for BG, respectively. Moreover, it was found that winter wheat grains had slightly higher AX levels than spring barley. On average, the AX content in winter wheat (4.74 g 100 g^{-1}) was significantly higher ($P = 0.025$) than that in spring barley (4.23 g 100 g^{-1}). Oat had a considerably higher content of AX (5.00 to 8.08 g 100g^{-1}). The difference between oat and barley in their BG content was less (12% on average) than between their AX values (33% on average).

When considering all trial years and N treatments, the AX content for winter wheat varied between 4.54 and 5.12 g 100 g^{-1}, while spring barley ranged from 4.00 to 4.51 g 100 g^{-1}. The average BG content in spring barley, across different N treatments over the trial years, was recorded between 3.74 and 5.60 g 100 g^{-1}, whereas for wheat, it ranged from 0.40 to 0.57 g 100 g^{-1} and oat ranged from 2.26 to 3.66 g 100g^{-1}.

5.1. Effect of weather on wheat, barley, and oat dietary fiber content

The findings show that the highest source of variation for AX and BG was the weather conditions, (plant growing period air temperatures and precipitation)($p<0.001$) (Figures 2 and 3) compared with N treatments. The years 2017 and 2020 indicate the lowest AX content as a mean of N treatments in winter wheat grain (3.24 and 2.84 g 100 g^{-1}, respectively), while the highest (6.68 g 100 g^{-1}) was in 2016. AX content remained between 4.31 and 5.75 g 100 g^{-1} in the other years. For the AX content

in spring barley grain, similar to wheat, the highest content was in 2016 (5.59 g 100 g^{-1}), while the lowest was in 2020 (2.79 g 100 g^{-1}).

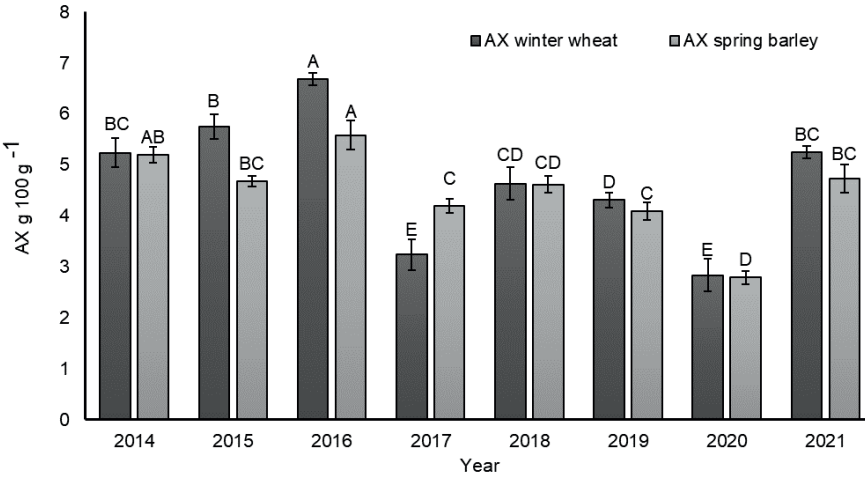
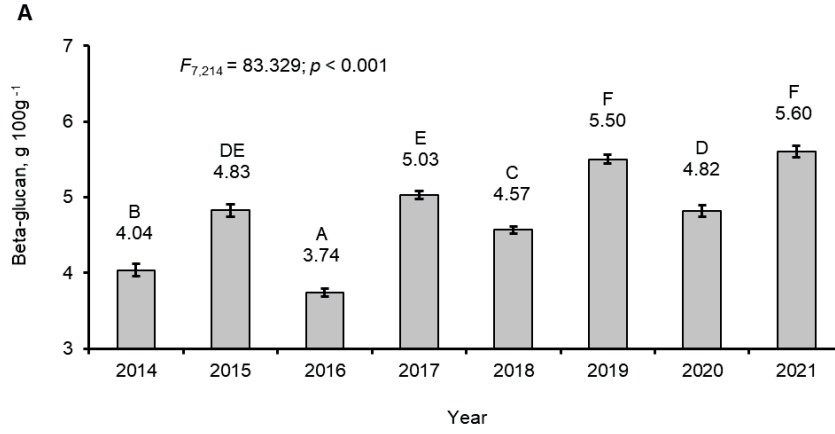


Figure 2. The AX content as an average of N treatments in winter wheat and spring barley flours in different years, g 100 g^{-1}. Letters on the bars refer to significant differences between years of the same species ($P < 0.05$). This figure was reproduced from Article I (Figure 1).



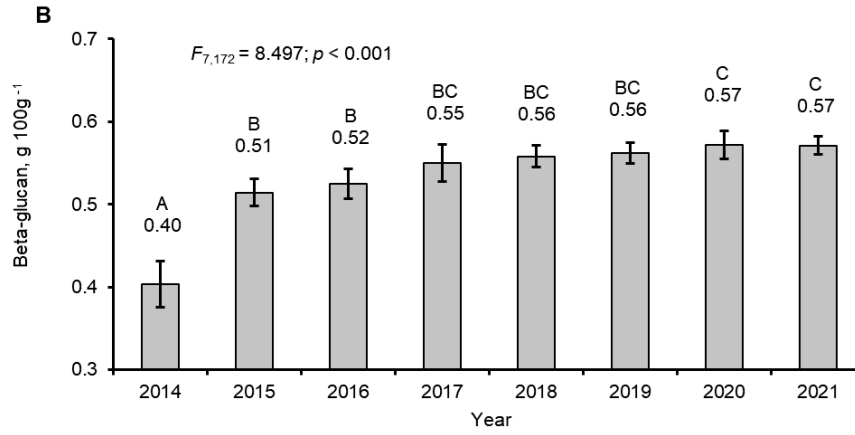


Figure 3. The BG content as an average of N treatments in spring barley flours in different years, g 100 g^{-1} (A), and winter wheat flours in different years, g 100 g^{-1} (B). Letters on the bar refer to significant differences (P < 0.05). These figures were reproduced from Article II (Figure 1).

The AX content in grains was affected by the temperatures of May (tillering/elongation stage) and July (grain filling period) (Figure 4). The years with lower average air temperature in the grain filling period of winter wheat (15.7–16.4 °C) have lower AX contents that varied between 2.84 and 5.75 g 100 g^{-1}. At higher temperatures in July, i.e. between 18 and 22.4 °C, the AX fluctuated between 5.25 and 6.68 g 100 g^{-1}. The same scenario was evident with the spring barley grain filling period (starting 2–3 weeks later), where at lower temperatures (16.1 - 17.2 °C) and at higher temperatures (19.9–23 °C), AX content varied between 2.74 and 3.87 g 100 g^{-1} and between 4.21 and 5.14 g 100 g^{-1}, respectively. The amount of precipitation had no significant effect on the AX content of the grains.

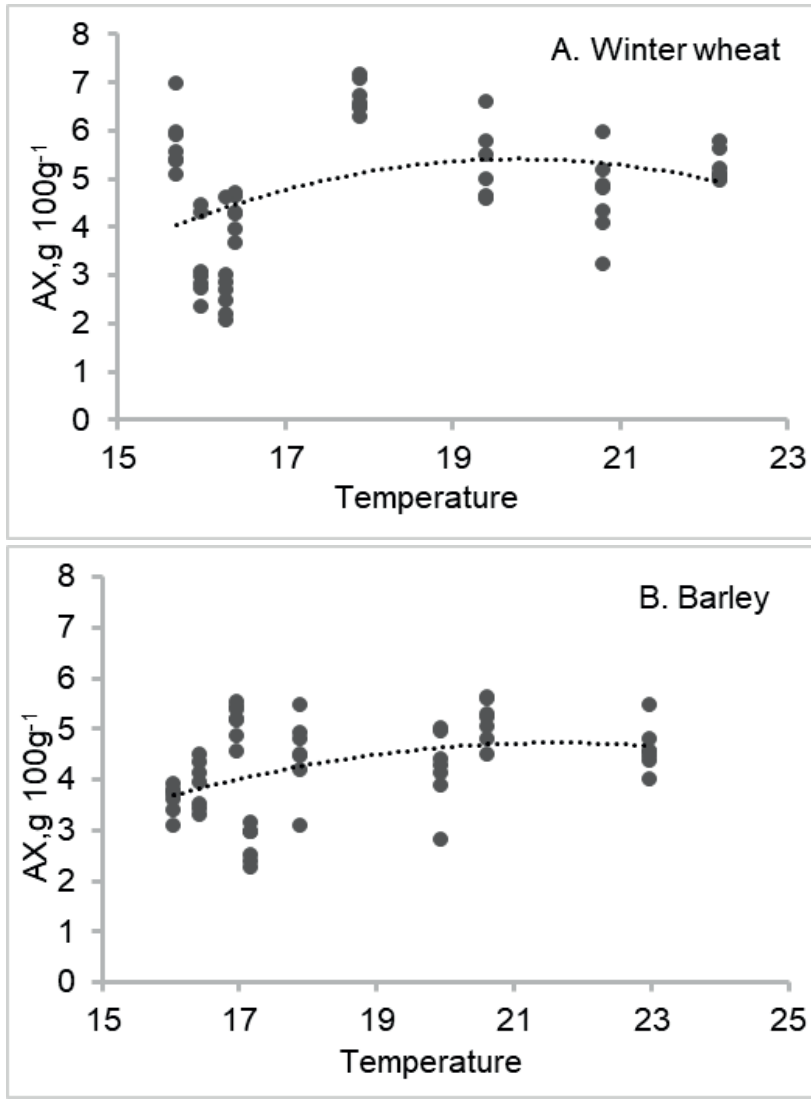


Figure 4. AX content in winter wheat (A) and spring barley (B) depending on air temperatures during the grain filling period; A. winter wheat: R^2=0.13 P<0.05; B. spring barley: R^2= 0.17; P<0.01. This figure was reproduced from Article I (Figure 2).

Winter wheat BG content had a positive relationship with air temperature in the grain filling period (r = 0.28, p < 0.05, respectively) and a negative correlation with the sum of precipitation in June (r = –0.23, p < 0.05) and July (r = –0.35, p < 0.01) (i.e., in the flowering and grain filling period of wheat (Figure 5A). The BG content of barley indicated a negative correlation with air temperature in May (tillering and stem

elongation stage of barley; r = 0.48, p < 0.01). Spring barley BG content had a negative correlation with the sum of precipitation in June and July (flowering and grain filling stages of barley; r = 0.67, p < 0.001; r = 0.85, p < 0.001, respectively; (Figure 5B).

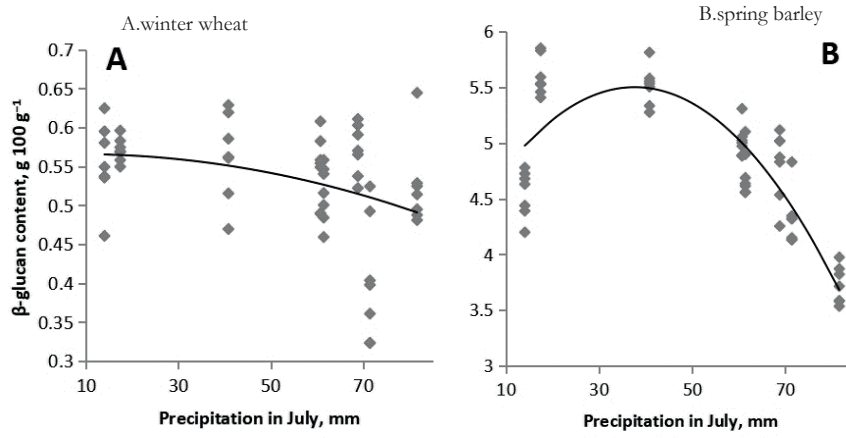


Figure 5. The influence of precipitation in grain filling period on BG content of winter wheat (A) and spring barley (B). Winter wheat: R^2=0.13*P*<0.01; spring barley: R^2=0.72; *P*<0.001. This figures was reproduced from Article II (Figures 3A and 3B).

Year did not affect the AX content of barley and oat grains in the intercrop system (Table 3). BG content was also the same in 2022 and 2023 in all pure stand crops, but there is a significant difference between intercropped treatments. Barley + vetch treatment revealed a significantly lower BG amount (2.68 g $100g^{-1}$) in 2022 than in 2023 (3.10 g $100g^{-1}$), as was also noted in the oat + field pea treatment, 2.34 g $100g^{-1}$ and 2.66 g $100g^{-1}$ in 2022 and 2023, respectively.

In 2022, the grain yield for barley was similar to 2023 (Table 3). In contrast, oat showed an opposite trend, with a yield of 1.71 t ha^{-1} in 2022 increasing significantly (p<0.05) to 2.50 t ha^{-1} in 2023. Oat yield in the mixture was also higher in 2023, being statistically significant. The average yield of barley was 1.94, less than the Estonian country-wide average (3.0 t ha^{-1}), while the pure-sown oat average yield was 2.10 t ha^{-1}, equal to the Estonian average. However, the intercropped oat average yield was 1.39 t ha^{-1}, which was 43% less compared to the pure-sown system.

Table 3. Grain yield and their BG and AX g 100 g^{-1} content in treatments in 2022 and 2023 experimental years. This table was reproduced from Article III (Table 1)

Cropping type	Year	Grain yield, t ha^{-1}	BG	AX
Barley	2022	1.99 ± 0.18B**	3.16 ± 0.09[a*]	4.65 ± 0.27[b]
Barley	2023	1.89 ± 0.09B	3.35 ± 0.14[a]	3.84 ± 0.38[b]
Barley + vetch	2022	1.21 ± 0.06b	2.68 ± 0.04[b]	4.18 ± 0.12[b]
Barley + vetch	2023	-	3.10 ± 0.09[a]	4.58 ± 0.46[b]
Oat	2022	1.71 ± 0.05B	2.62 ±.0.10[b]	6.71 ± 0.49[a]
Oat	2023	2.50 ± 0.13A	2.80 ± 0.05[b]	6.65 ± 0.55[a]
Oat + field pea	2022	0.98 ± 0.04b	2.34 ±0.33[c]	6.44 ± 0.16[a]
Oat + field pea	2023	1.80 ± 0.15a	2.66 ±0.10[b]	5.96 ± 0.35[a]

*Different letters in columns denote significant differences between treatments. **Significant differences in grain yield of pure stand and intercropping systems are indicated by uppercase and lowercase letters, respectively.

The study showed a positive strong correlation between barley BG and 1000 kernel weight (KW) ($R=0.82$; $p<0.001$), test weight (TW) ($R=0.83$; $p<0.001$), and yield ($R=0.63$; $p<0.001$) while a moderate correlation with the year ($R=0.52$; $p>0.05$). There was no correlation between oat BG and 1000 KW nor between oat BG and yield. Only barely 1000 KW correlated with its yield ($R=0.50$, $p<0.001$) (Figure 6). AX content of barley and oat fibers did not show any correlations with the quality parameters mentioned.

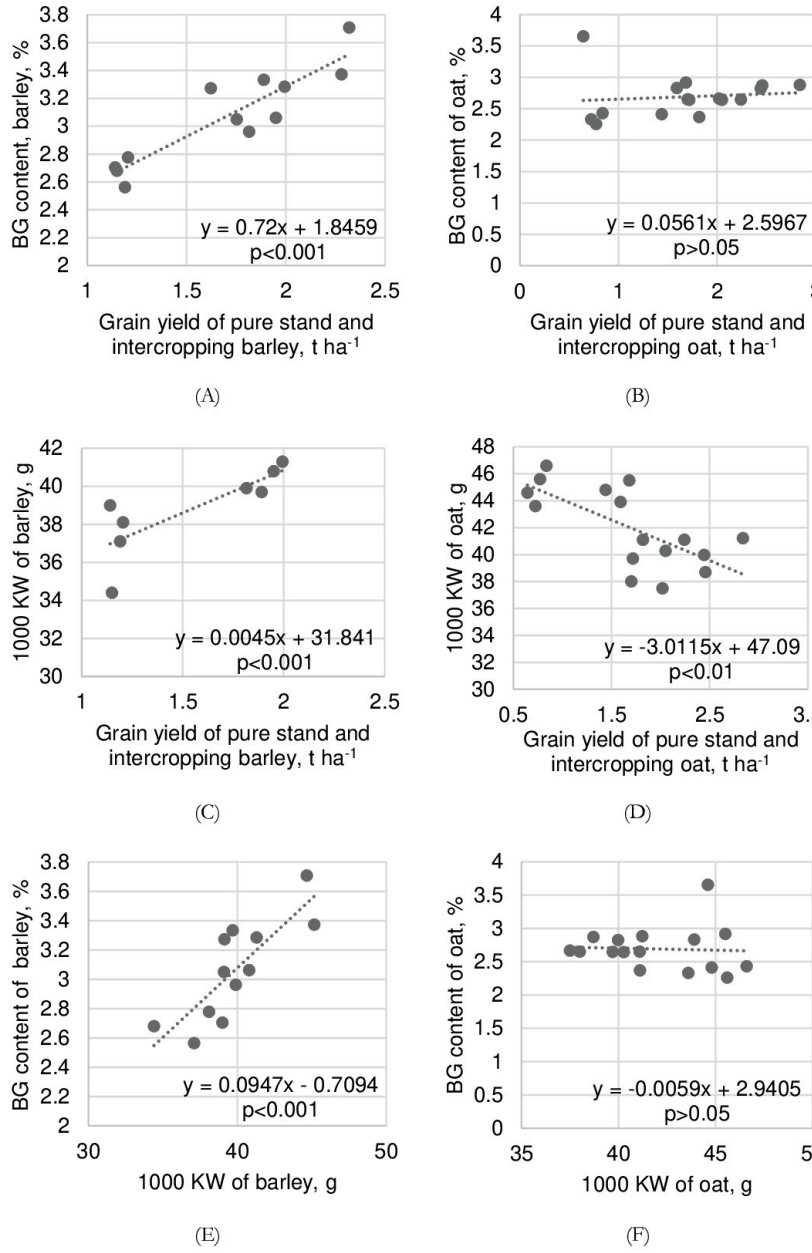


Figure 6. BG content in barley (A) r=0.63, P<0.0.1; and oat (B) r=0.11, P>0.05; depending on grain yields; 1000 kernel weight of barley (C) r=0.64, P<0.01; and oat (D) r=0.69, P<0.01; depending on grain yields; BG content of barley (E) r=0.82, P<0.001; and oat (F) r=0.05, P>0.05; seeds depending on 1000 kernel weight. This figure was reproduced from Article III (Figure 4).

In 2022- 2023, an average of 1000 KW (41.2 and 37.2 g, respectively) and TW (58.2 and 51.0 kg hl-l, respectively) of barley in pure stand and intercropping system were significantly different, as was also 1000 KW between oat and oat + field pea treatments (40.5 and 43.6 g respectively) (Figure 7). When comparing these values between years, then, 1000 KW was similar in all treatments (barley + vetch 1000 KW was not measured in 2023). TW was significantly higher in oat pure stand and oat + field pea in 2023 compared to 2022, while for barley pure stand, TW was similar.

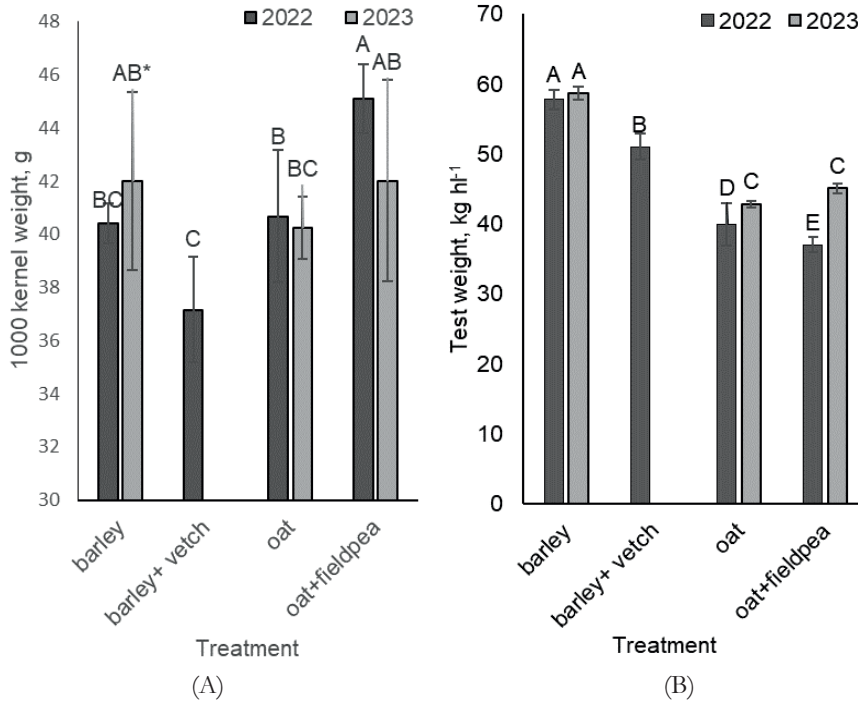


Figure 7. Comparison between (A) 1,000 kernel weight (g) and (B) test weight (kg hl^{-1}) in each treatment in experimental years. *Different letters on the vertical bars denote significant differences between treatments of the same parameter measured. This figure was reproduced from Article III, Figures 5a and 5b.

5.2. Effect of cropping system and nitrogen on wheat and barley dietary fibers

Results revealed that the levels of crop yield depends on the cropping system and the fertilizer treatments used (for winter wheat $r = 0.43$, $P < 0.001$ and $r = 0.51$, $P < 0.001$, respectively; for spring barley $r = 0.56$, $P < 0.001$ and $r = 0.65$, $p < 0.001$, respectively). Over the trial years, winter wheat produced an average grain yield ranging from 4.66 to 4.98 t ha^{-1} in the conventional systems (N1-N3). This yield was as much as 45% greater than what was observed in the all organic treatments (Org0-Org2). For barley, the grain yield ranged from 3.32 to 3.80 t ha^{-1} in conventional systems, which was up to 57% higher than that found in organic treatments.

The results indicated that the winter wheat grain yield had a negative correlation with the AX content of the grains ($r = 0.27$, $P < 0.05$), unlike that of spring barley, with an insignificant correlation with AX values (Table 4). The AX content in spring barley negatively correlates with 1000 kernel weight but positively correlates with test weight values ($r = 0.50$, $P < 0.001$ and $r = 0.29$, $P < 0.05$, respectively). On the other hand, spring barley's average 1000 kernel weight negatively correlated with air temperature in July ($r = 0.38$, $P < 0.01$).

The BG content of wheat and barley grains was not significantly influenced by the types of crop production systems or N treatments. However, a combined effect of the temperature during the grain filling period, N fertilization, and cropping systems appeared to affect the AX content in both crops. When the temperature during the grain filling period was lower, the AX content averaged lower for both crops compared to higher temperatures. The cropping systems had a significant effect on AX values exclusively for winter wheat grains at the lower temperature, where the average AX content in conventional treatments was significantly lower than in organic treatments (Table 3). For barley, the AX content was not significantly affected by the cropping system.

Table 4. AX correlations with cereal yields, its quality indicators, and environmental Factors. Table 4 was reproduced from Article I (Table 2)

Variable	Mean ± Std. Err.	Correlation coefficient r forAX
Winter wheat		
Yield t ha^{-1}	3.66 ± 0.22	-0.27
1000 KW, g	45.4 ± 0.68	ns
Test weight, kg hl^{-1}	779 ± 4.31	0.22
Temperature, °C	13.8 ± 1.15	0.24
Precipitation, mm	299 ± 10	ns
Spring barley		
Yield t ha^{-1}	2.55 ± 0.17	ns
1000 KW, g	44.1 ± 0.72	-0.5
Test weight, kg hl^{-1}	629 ± 5.39	0.29
Temperature, °C	13.8 ± 1.15	ns
Precipitation, mm	299 ± 10	ns

Cropping type had no effect on the AX content of oats and barley. Like AX, the BG content of pure stand and intercropping oat treatments did not differ. Nevertheless, BG concentration in barley pure stand has a significantly higher value (3.25 g $100g^{-1}$) compared to barley in intercropping (2.90 g $100g^{-1}$); the percentage of variance for barley cropping type was 38% (Figure 8). However, BG in barley+ vetch was similar to that found in the oat treatment. BG concentration was the same for both barley (2.56–3.74 g $100g^{-1}$) and oat (2.26–3.66 g $100g^{-1}$) in the intercropping system.

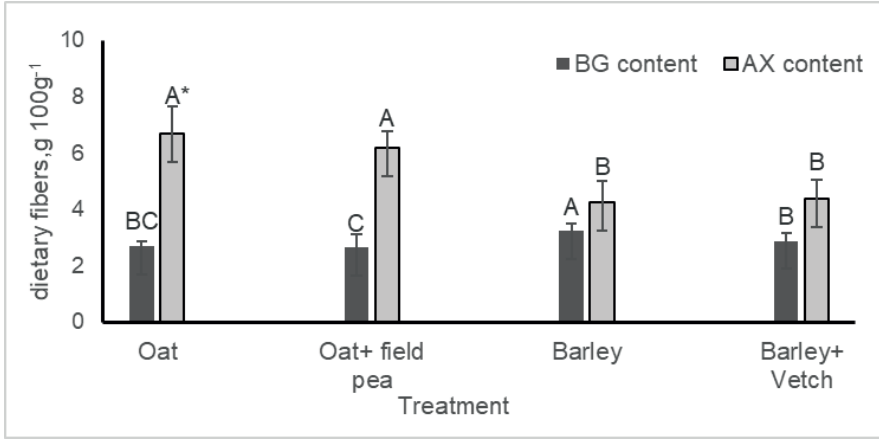


Figure 8. Average 2022-2023 BG and AX contents (g 100 g^{-1}). *Different letters on the vertical bars denote significant differences between treatments of the same fiber. Figure 8 was reproduced from Article III (Figure 3).

5.3. Effect of genotype on grains' dietary fibers in wheat, barley, and oat

Mean varietal AX content was 3.76 and 5.18 g 100 g^{-1} for winter wheat and spring barley, respectively, with barley having lower varietal mean AX content ($P < 0.001$) (more than 25%) compared to wheat cultivars. In the intercrop system, oats had an appreciably higher value of AX (5.00−8.08 g 100g^{-1}) compared to that found in barley (3.14−5.20 g 100g^{-1}) (Figure 8), irrespective of pure stand or mixed stand.

Wheat BG content measured was below that of barley kernels (~10 times lower), and BG content in barley was significantly higher than in both oat treatments. The difference between oat and barley in their BG concentration was less (12% on average) than between their AX values (33% on average).

The result of AX content of winter wheat cultivar 'Fredis' and spring barley 'Anni' grown in the same year but in different locations with similar N fertilizer rates (N_{150} and N_{80} respectively) were comparable, being 5.04 and 5.91 g 100 g^{-1} for winter wheat ($P = 0.28$) and 2.82 and 4.20 g 100 g^{-1} for spring barley ($P = 0.07$) in Eerika and Jõgeva fields respectively. All the seven studied winter wheat cultivars have almost similar AX contents ($P = 0.663$), ranging from 4.37 g 100 g^{-1} in 'Perenaise' to 5.91 g 100 g^{-1} in 'Fredis' (Figure 9).

Similarly, the AX content of the ten spring barley cultivars was not affected significantly by genotype (P = 0.089); the values were between 2.83 and 4.77 g 100 g^{-1}, with 'Anneli', 'Breustar' and 'Anni' having the highest values of 4.77, 4.56 and 4.20 g 100 g^{-1} respectively.

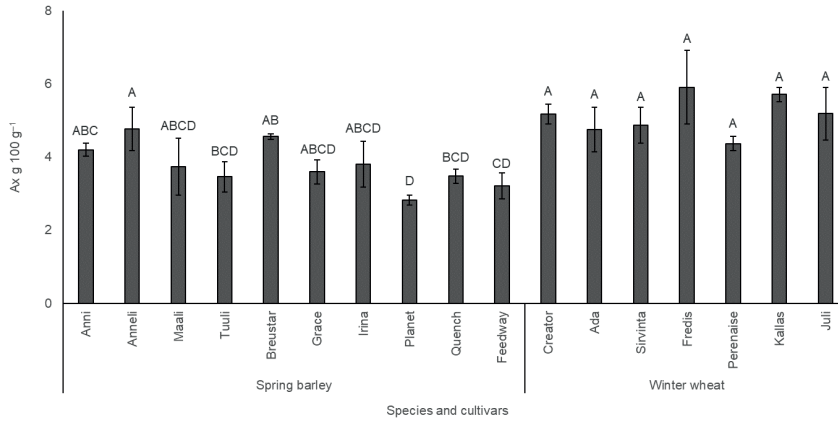


Figure 9. Average content of AX in different barley and wheat cultivars, g 100 g^{-1}. This Figure was reproduced from Article I (Figure 3). The letters on bars refer to a comparison between cultivars of the same species; bars with the same letter are not significantly different (p <0.05).

In the Jõgeva field, barley BG content was influenced significantly by genotype, with the lowest and highest values recorded from 'Maali' and 'Anni' respectively (Table 5). There was up to 1.51 g 100 g^{-1} differences between the cultivars. The wheat 'Fredis' BG content in the Eerika field fluctuated between 0.32 and 0.63 g 100 g^{-1} during eight trial years. Like barley, genotype also affected wheat BG values in the Jõgeva field experiment, with small but significant varietal differences (0.14 g 100 g^{-1}).

Barley cultivar 'Anni' BG content was affected significantly by location in this experiment (p < 0.001; Table 5). We compared the BG content of the 'Anni' cultivar obtained from the Jõgeva field (BG values in 2021 at the same level of N quantities – 100 kg ha^{-1}) with that of the Eerika field. Mean BG results over repetitions were 4.51 ± 0.192 and 5.85 ± 0.366 g 100 g^{-1} in Jõgeva and Eerika fields, respectively.

Table 5. Content of BG (g 100 g^{-1}) in spring barley and winter wheat genotypes in 2021 from Jõgeva and Eerika fields. This table was reproduced from Article II (Table 3)

Barley genotype	BG (g 100 g^{-1})	Wheat genotype	BG (g 100 g^{-1})
Maali	3.14 ±0.03 d*	Ada	0.47 ± 0.03 b
Grace	3.26 ±0.21 d	Creator	0.53 ± 0.05 a
Planet	3.36 ±0.17 cd	Perenaise	0.55 ± 0.01 a
Feedway	3.37 ±0.13 cd	Širvinta	0.55 ± 0.01 a
Tuuli	3.46 ±0.07 cd	Julie	0.55 ± 0.00 a
Breustar	3.49 ±0.13 cd	Fredis	0.55 ± 0.05 a
Irina	3.78 ±0.18 bcd	Kallas	0.55 ± 0.03 a
Quench	3.52 ±0.15 bc	Fredis (Eerika field)	0.55 ± 0.02 a
Anneli	3.92 ±0.18 bc		
Anni	4.51 ±0.19 b		
Anni (Eerika field)	5.85±0.29 a		

*Mean values (averaged from replicates in the same field and same year) are presented with ±SE.
n: different lowercase letters in the columns indicate significant differences; values with the same letter are not significantly different ($P < 0.05$).

The BG content of barley grains was positively correlated with grain 1000 KW (Figure 10) and TW values ($r = 0.64$, $r = 0.54$, respectively, $p < 0.001$, for both values). Neither 1000 KW nor TW affected the BG content of winter wheat 'Fredis'.

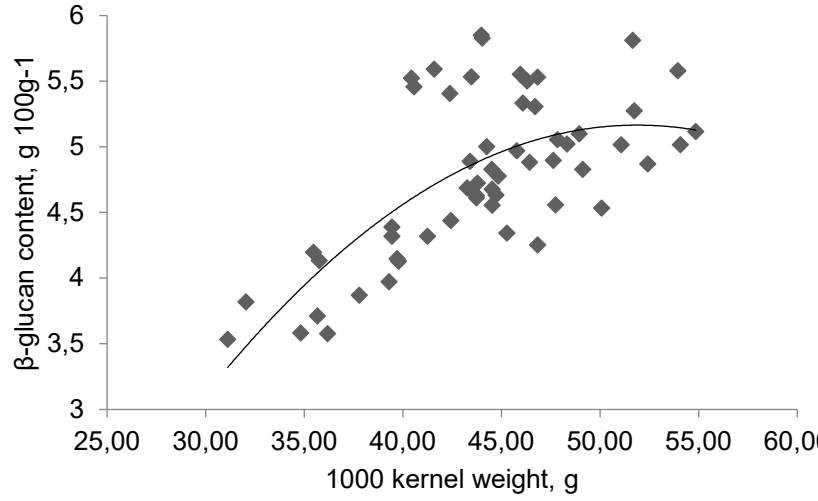


Figure 10. Relationship between 1000 KW and BG content in spring barley 'Anni' from Eerika field; $R^2 = 0.41$, $p < 0.001$; $n = 55$. This Figure was reproduced from Article II (Figure 4)

6. DISCUSSION

6.1 The factors affecting wheat, barley, and oat dietary fiber contents

Dietary fibers, AX and BG, are crucial for plants and humans. In plants, these fibers contribute to the cell wall structure (Marc et al., 2024). For humans, AX and BG are important dietary fibers that promote digestive health, lower cholesterol levels, and help regulate blood sugar (Fuller et al., 2016). However, some factors can influence the biosynthesis and accumulation of these dietary fibers (Dickin et al., 2011). The influence of weather conditions, cropping systems, and cereal-legume intercropping on cereal grains' AX and BG content, including barley, wheat, and oats, was analyzed. It may help to provide insightful information to explore the potential for enhancing dietary fiber content through agricultural practices.

6.1.1. Effect of weather on wheat, barley, and oat dietary fibers

This study found that weather conditions significantly influenced spring barley and winter wheat grains' AX content compared to the cropping system and fertilizer treatments. It has previously been shown that year (i.e., weather conditions) has an effect on AX formation (Finnie et al., 2006; Marconi et al., 2020; Selga et al., 2023) via changing the conditions during kernel growth and ripening (Buksa et al., 2014). Previous studies also suggested that drought has a direct relationship with wheat AX content (Coles et al., 1997; Selga et al., 2023), but this present study found that air temperature significantly influences the AX content in grains. As the prediction of the future climatic models suggests, the highest warming rate may occur in northern Europe (Mitchell et al., 2019; Perkins-Kirkpatrick and Lewis, 2020; Patterson, 2023), and higher AX content in grains could also be achieved. The factor in this thesis project that determined the AX content was the temperature at two developmental stages for the crops: temperature during the tillering/stem elongation stage and temperature in the grain filling period. The tillering stage of spring barley and winter wheat occurred in May under the Estonian condition (the tillering/stem elongation period continued

till the second part of May, while the regrowth of winter wheat after hibernation started at the end of April).

The development of spring barley primordia was affected by temperatures during the tillering/elongation stage. The barley plant probably forms more seed primordia at the higher air temperature, leading to a lower 1000 kernel weight and a higher number of grains for each spike. The kernel/seed coat ratio was affected by 1000 kernel weight, with smaller seeds having a larger AX proportion (Lempereur et al., 1997). Results revealed that the AX content of studied cereals has a positive association with temperature in the grain filling period (Fig. 2 Article I). The field experiment showed that the AX content of the examined crops was less at lower temperatures during grain filling (Table 3 Article I).

In the 2022 and 2023 experimental years, differences in AX values in barley and oat were absent. The AX content in grains relied on the temperatures of the plant growth period, preferring higher temperatures. In 2022 and 2023, the temperature in July was similar (indicating 18.1 and 16.9 ^{0}C, respectively); therefore, AX values were also stable.

For BG, the current experiment revealed that the year (weather) conditions, including air temperature and precipitation, influenced BG content (Table 4 Article II). The BG result revealed a negative association between air temperature in May and the BG content of barley grains. Conditions that are suitable for endosperm development raise the accumulation of BG in the grain, but BG content may decline under moist growing conditions and high temperatures in this period (Goudar et al., 2020). Barley is in the tillering/elongation stage in May under Estonia's climate. Therefore, lower temperatures in May (9 –10 °C) lengthen the tillering and stem elongation phase together with stronger rooting, a larger number of shoots, and the formation of higher above-ground biomass. We found that barley 'Anni' BG was positively correlated with 1,000 kernel weight (Figure 4 Article II), and also test weight. This finding is similar to Yalçin et al. (2007), who found a positive relationship between BG content and 1,000 kernel weight in hull-less barleys grown in Turkey. Their findings revealed that dense and large grains have larger endosperm content than smaller grains, leading to a higher content of BG.

In my experiment, test weight (TW), which is known as an indicator of grain density and yield potential, had a positive association with BG content in barley grains (Table 4 Article II). The 1000 KW is directly proportional to TW. As a result, the BG content of larger seeds is higher. However, similar relationships were not significant for wheat. This could be because BG is biosynthesized mainly in the endosperm of barley grains, whereas in wheat grains, it is biosynthesized in the aleurone layer (Sapirstein, 2016). An association between the BG content of barley and wheat grains and precipitation in June and July shows that precipitation during the grain-filling period decreases the BG content (Table 4 Article II). Present results are in line with Ehrenbergerová et al. (2008), who found that higher precipitation during grain filling and barley flowering decreases grain BG content, contrary to the results of Choi et al. (2020) that show a positive influence of rainfall on grain BG content. The stress resulting from temperature and precipitation that causes the end of early grain development also reduces BG concentration because BG accumulates in the late stage of grain development (Zhang et al., 2001; Tiwari and Cummins, 2009). The timing of water availability affects the synthesis and deposition of main storage substances and their relative proportions in the grain (Havrlentová et al., 2023).

The content of BG in wheat grains was constant in all studied years except for 2014. The low content of BG in the year 2014 may be due to the high precipitation in June, July, and August (until harvesting). The lowest BG content of barley grains was found for the year 2016, where the total amount of precipitation for June and July was the highest among all studied years (2014–2021) and the highest for the year 2021, when we had the lowest precipitation in June and July compared to all other studied years. In Estonia's climate, barley is in flowering and grain-filling stages during June and July. Therefore, higher precipitation during this period can lead to a dilution effect, which raises the water content in the grains, hence inducing a reduction in BG content (Aastrup, 1979; Güler, 2003a, b). Contrarily, precipitation during the flowering time can cause a reduction of the yield by disturbing the pollination process and reducing the quality of grains, including their BG content (Aastrup, 1979).

In the 2022 and 2023 experimental years, weather significantly influenced the BG content of cereals in mixed plots but did not alter the content of BG in pure stands of barley and oats. The BG content in

the barley and oat grown in a mixed cropping system was significantly lower in the year 2022 as compared against the year 2023. As grain's BG content is negatively related to precipitation, much higher precipitation in 2022 was caused by heavy rain in July more than in 2023, which may have led to the lower content of BG in barley and oat grains in mixes in 2022. The decrease in grain BG concentration and BG accumulation could be attributed to the stress from weather conditions that interrupt grain development (Zhang et al., 2001; Tiwari and Cummins, 2009). BG content also showed the same, though insignificant, trend in oat and barley pure stands between 2022 and 2023.

6.1.2. Effect of cropping system and nitrogen fertilization on wheat, barley, and oat dietary fibers

Findings revealing that AX yield in grains can be attained irrespective of the fertilization regime help to achieve the goals of the European agricultural policy that encourages organic production with less fertilizer. The effect of different cropping systems on the AX content of cereals has received less attention in research so far. Past studies have demonstrated a minor influence of fertilization on AX content in grains (Coles et al., 1997; Lempereur et al., 1997). Since N availability from mineral fertilizers in the cereals' first phase of development stages is superior to the availability of N from organic manure (Alaru et al., 2014; Efretuei et al., 2016), the conventional system grain yield is much greater (Keres et al., 2020). Our findings indicated up to 45% smaller winter wheat grain yield from organic treatments, winter wheat AX content and grain yield were negatively related, and winter wheat grains cultivated in the conventional system had lesser AX content. Our experiment involved measuring AX from whole-grain flour without separating different flour fractions. Using mineral N fertilizer twice during the growing season may have helped to establish an extended duration of green leaves, including the flag leaf. This led to a longer grain filling period and resulted in up to 5% higher test weight in spring barley grains obtained from conventional treatments ($P < 0.05$) (Hafsi et al., 2013). Therefore, it can be deduced that the positive relationship between test weight and AX content reflects the rise of AX content in the grain endosperm.

The study hypothesized that the content of BG rises with higher mineral nitrogen rates than organic fertilizers. Earlier, Güler (2003a) indicated that the largest BG content in their two-year experiment was observed

when barley was fertilized with 80 kg N ha^{-1}, which was the maximum N rate in their experiment. That study indicated that BG content in barley differs depending on nitrogen, cultivar, and irrigation treatments as well as between the two-year study period. In contrast, our findings suggest that, in barley and wheat, fertilization up to 150 kg N ha^{-1} did not significantly affect BG content across the entire eight years (see Table 4 Article II). Previous study based on the same field experiment showed that genes involved in the biosynthesis of BG in barley had similar expression trends under different treatments (Esmaeilzadeh-Salestani et al, 2023). It has been observed that Güler (2003a) used only two-year periods from 1998 – 2000 for their study and utilized three two-rowed barley cultivars, 'Tokak 157/37', 'Bülbül 89' and 'Obruk 86' for their experiment in Turkey. Current research extended the study period to eight years and, in addition, analyzed spring barley 'Anni' and winter wheat 'Fredis.'

Similar to our findings, Habiyaremye et al. (2021) did not report any effect of N fertilizers on barley BG content in their three-year experiment, with N rates varying from 0 to 162 kg ha^{-1}. This result suggests that the crops can yield high BG content without adding high mineral fertilizers into the soil, though the BG content of wheat grains is significantly less than barley grains.

Cropping type has no effect on barley and oat AX content, as it was similar between pure stand and intercropping and was not connected with grain quality parameters. Similarly, there was no variance in BG content for oats, whether the grains were grown in a pure stand or a mixture. As the formation of peas in the sward was weak, the oat likely did not perceive stress from co-cultivation, as shown also by the higher 1000 KW in the mixed stand than pure and similar TW values for treatments. The unstable production of peas was caused by their very differing formation, which caused gaps in the sward and high differences in production between replicates (Alaru et al., 2024). Oat average yield in the pure-sown system was close to the national average (2.1 t ha^{-1}), and the lower yield in 2022 can be explained by late sowing. Above-average yield in 2023 shows high drought-tolerance of the used variety 'Symphony'. It has been shown to be a well-performing variety for organic production, so further experiments can prove its suitability for climatic conditions with increasing drought. Lower yield in the intercropped system was expected due to a lower seeding rate. Oat yield and 1000 KW correlation

were negative since yield increased mainly due to the higher number of grains per plant (data not shown). There was no correlation between oat yield, 1000 KW, and BG. The difference could be explained by the fact that in oats, BG is found mainly in the outer layer of the endosperm, which is a marked difference from barley, which contains BG uniformly throughout the endosperm (Kale et al. 2014).

Contrarily, cropping type had an effect on barley BG content; against our hypothesis, barley grains grown in a mixture with vetch had a lower overall concentration of BG when compared to that in barley pure stand. Barley is generally regarded as one of the least competitive cereals in mixtures (Hauggaard-Nielsen and Jensen 2005). As vetch in the mixture was largely aggressive, this led to lower quality parameters of barley (Alaru et al., 2024), including BG content.

The production of BG in barley and oat grains manifests in the endosperm, as indicated by Guleria et al. (2015). The grain quality factors such as TW and 1000 KW, which measure the weight and density of the grains, are associated with the endosperm in the barley and oat. Therefore, a larger and denser endosperm will occur in heavier and denser grains, leading to higher TW and 1000 KW values. In our experiment, TW and 1000 KW were positively associated with barley BG, showing that higher TW and 1000 KW are linked with higher BG content in barley. Barley yield and BG had a positive relationship. As 1000 KW and yield were positively correlated, it can be assumed that higher 1000 KW also increased yield and BG. The average yield of barley (1.94 t ha^{-1}) was less than the national average over the years (3.0 t ha^{-1}). The extreme drought in 2023, specifically during May and June, had a negative impact on barley yield, and even the national average was only 2.2 t ha^{-1}. The total sum of precipitation from May to August was only 177 mm (Fig. 2). However, given the zero-fertilizer system, this yield outcome can be considered acceptable or good on agronomic grounds.

Barley is considered to be proficient at tillering (Lepajõe 1986), but this experiment had fewer tillers in the intercrop. One reason for this could have been that barley has weak competitiveness compared to other cereals (Hauggaard-Nielsen et al. 2001). It had fewer grains in the ear when grown in an intercrop with vetch. My finding is supported by Yalçın et al. (2007), who reported a positive correlation between grain quality and BG content in barley.

In this experiment, the mixture's TW and 1000 KW of barley were also lower than in a pure stand. As they were in a strong positive relationship, TW and 1000 KW are affected by stress on plant growth. However, it could be reasoned that cereal plants in mixtures were experiencing detrimental growing conditions. Further, Yalcin et al. (2007) indicate a lesser 1000 KW for barley + vetch in a ratio of 60:40 when compared to pure stand. The study also reported lower NDF (i.e., neutral detergent fiber) in the mixture than in the pure stand. As NDF also represents hemicelluloses, their results could be similar to ours, suggesting that intercropping could lower the fiber value of mixed crops. Further research is required to estimate the value of intercropped systems for animal feed.

6.2. Effect of genotype on wheat, barley, and oat dietary fibers

The cereal species and cultivar influenced grain AX content (Lempereur et al., 1997; Finnie et al., 2006; Li et al., 2009; Saeed et al., 2014). Winter wheat species are superior to AX sources compared to spring barley grains (Knudsen, 2014), and oats have a higher AX content than barley. This fact must be regarded when selecting species for cultivation with the target of producing high AX-content grains. Generally, although genotype had some effects on the presently studied cereal grains, their AX content was still primarily determined by the cereal species itself. Winter wheat 'Fredis' and spring barley 'Anni' that were studied are high in AX content and could be effective candidates for crop breeders who are choosing cultivars oriented to give high AX production. The AX contents of all wheat cultivars as well as a few barley cultivars ('Anneli', 'Breustar' and 'Anni') ranges between 4 and 6 g 100 g^{-1}, while for the majority of spring barley cultivars, the AX content stayed below 4 g per 100 g^{-1} of whole grain flour.

Cereal species also influence BG content, I inconsistent with Sinkovicˇ et al. (2023), we found that BG content is higher in barley grains than in wheat. There was also a higher concentration of BG in barley grains than in oat grains, as mentioned above (Lazaridou and Biliaderis, 2007).

BG content in cereal grains is affected by genotype (Choi et al., 2020). Furthermore, genotype positively affects the quality properties of grains, such as the weight of 1,000 kernels (Yalçin et al., 2007). This study's experiment shows that barley 'Anni' had the largest content of BG compared to cultivars 'Maali', 'Grace', 'Planet', 'Feedway', 'Tuuli',

and ‘Breustar’. This revealed that the propensity of variant genotypes for developing grains with larger BG content is different. Similarly, the content of BG in wheat was affected by genotype, and cultivar ‘Ada’ had the smallest BG content compared to all other cultivars.

In summary, the differences in BG content are likely a result of a combination of genetic factors controlling BG synthesis, physiological traits like cell wall structure, and, potentially, breeding goals. It is possible that ‘Anni’ is more genetically predisposed to adapt to environmental conditions. Therefore, as indicated by Choi et al. (2020), variances in BG content may result from a genotype-environment interaction, which is the major factor considering grain end-use. Future research should consider genotype and management interactions in changing climate conditions in more detail.

7. CONCLUSIONS

Investigating the effect of weather conditions, cropping systems, cereal-legume intercropping, and genotype on the AX and BG content of cereal grains, including barley, wheat, and oats showed that:

- The research results from an eight-year period confirmed the hypothesis that weather conditions influence cereal grains' AX and BG content.

- Air temperature during the tillering/ stem elongation stage and temperature in the grain filling period significantly influence spring barley and winter wheat grains' AX content.

- As 1000 KW depends on temperatures at the beginning of plant growth, higher temperatures increase spring barley biomass formation, decrease 1000 KW, and form smaller seeds with higher AX content. Lower temperatures during tillering and stem elongation phase increase barley BG content, forming larger grains with larger endosperm containing more BG.

- Higher temperatures during the grain filling period significantly increased the AX content in winter wheat and spring barley.

- While grain's AX content does not depend on precipitation, precipitation during flowering and the grain filling period negatively impacts the BG content in barley and wheat grains due to the stress inhibiting grain development.

- Contrarily to the hypothesis, nitrogen fertilization had a limited impact on AX and BG content in cereal grains, and fibers yields remained consistent regardless of fertilization levels.

- Only at lower temperatures during the grain filling period can higher AX content in winter wheat in the organic system be achieved compared to conventional system farming.

- Unlike the established hypothesis, intercropping can decrease BG content in barley while not affecting the content of AX in barley and

oats. weak competitiveness of a crop in the mixture can also alter its grain quality parameters.

- The impact of genotype on the dietary fiber content of cereals is less significant than the impact of the cereal species itself.

These findings can guide farmers and policymakers in adopting practices that enhance the nutritional quality of cereal grains while promoting sustainable agriculture. Future research should be conducted on different cereal-legume intercropping combinations to identify the most effective pairings for increasing AX and BG content without compromising yield. This research could focus on understanding the competitive dynamics between different species and optimizing planting densities and ratios. One necessary study for the future is to perform an economic analysis to assess the cost-effectiveness and market potential of producing high-fiber cereal genotypes. This study could help farmers and policymakers understand the financial benefits of adopting practices that enhance dietary fiber content. Future research should also closely examine the environmental effects by conducting simultaneous studies in both field and controlled conditions.

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SUMMARY IN ESTONIAN

KOKKUVÕTE EESTI KEELES

Teraviljade kiudainesisalduse sõltuvus ilmastikust, väetamisest ning viljelusviisist

Sissejuhatus

Teraviljad, sealhulgas nisu, oder ja kaer, sisaldavad vajalikke toitaineid ja on rikkad kiudainete nagu arabinoksülaanid (AX) ja beetaglükaanid (BG) poolest. Need kiudained mängivad olulist rolli inimeste tervise toetamisel, vähendades krooniliste haiguste riski, parandades seedimist ja suurendades üldist toitumise kvaliteeti. Siiski on kiudainete tarbimine endiselt ebapiisav, mis rõhutab vajadust optimeerida teraviljatootmist, et rahuldada toitainevajadusi. See on eriti oluline globaalse rahvastiku kasvu kontekstis, mis suurendab nõudlust jätkusuutlike ja toitainerikaste toiduallikate järele.

Jätkusuutlikud põllumajandustavad on üliolulised kasvava nõudluse rahuldamiseks ja samal ajal keskkonna säästmisel. Vaja on kohaneda kliimamuutustega, vältida mulla degradeerumist. Euroopa Liidu roheleppe strateegia „Talust taldrikule" rõhutab jätkusuutliku toidutootmise tähtsust, püüdes suurendada mahepõllumajanduse levikut ja vähendada sõltuvust sünteetilistest väetistest. Uurides, kuidas ilmastikutingimused, väetamispraktikad ja põllukultuuride kasvatamise süsteemid mõjutavad teraviljade kiudainesisaldust, on võimalik välja töötada strateegiaid, mis suurendavad teraviljatootmise toiteväärtust ja jätkusuutlikkust.

Käesolev doktoritöö uurib erinevate tegurite nagu ilmastikutingimuste, lämmastikväetamise ja segakülvide mõju AX ja BG sisaldusele Eestis kasvatatud nisus, odras ja kaeras. Viies põllumajandustavad vastavusse tootmise jätkusuutlikkusega, selgitab uurimus võimalusi toitainerikaste ja tervislike toiduallikate tootmiseks, samas aidates kaasa rahvatervise parandamisele ja keskkonna vastupanuvõime suurendamisele.

Doktoritöö eesmärk oli määrata kahe peamise kiudaine, AX ja BG, sisalduse muutusi teraviljades järgmiste tegurite mõjul:

1. Viljelusviisid lämmastikväetise erinevatel tasemetel (Artiklid **I**, **II**).

2. Ilmastikutingimused (õhutemperatuur ja sademed) taimede kasvuperioodil (Artiklid **I**, **II**, **III**).

3. Teraviljade genotüüp (Artiklid **I**, **II**).

4. Teravilja ja liblikõieliste segakülvid (Artikkel **III**).

Hüpoteesid:

1. AX sisaldus on suurem mahedalt kasvatatud teraviljades võrreldes tavaviljelusega ning sõltub ilmastikutingimustest, eriti põuast (Artikkel **I**).

2. BG sisaldus on suurem kõrgema väetustasemega teraviljades ning sõltub ilmastikutingimustest spetsiifilistes kasvufaasides (Artikkel **II**).

3. BG ja AX sisaldus on suurem odras ja kaeras, mis on kasvatatud koos viki ja põldhernega, võrreldes puhaskultuuridega (Artikkel **III**).

Metoodika ja materjal

Käesolev doktoritöö põhineb kolme põldkatse andmetel. Esimene ja kolmas katse viidi läbi Eesti Maaülikooli põldudel Tartu maakonnas (Eerika) (58°21′53.2″N, 26°39′57.7″E), mille mullatüüp oli pruun kahkjas (näivleetunud) liivasavimuld (I, II, III). Teine katse viidi läbi Eesti Taimekasvatuse Instituudi põldudel (58°45’56.1”N 26°24’26.5”E) Jõgeva maakonnas (Jõgeva põld), mis asus 50 km kaugusel esimesest katsest (I, II). Sealne mullatüüp oli leostunud muld.

Esimese põldkatse andmed saadi, analüüsides talinisu ‘Fredis’ ja suviodra ‘Anni’ teraviljaproove, mis koguti aastatel 2014–2021 pikaajalise viieväljalise külvikorra katse raames (I, II). Külvikord hõlmas järgmisi kultuure: suvioder (*Hordeum vulgare L.*) punase ristiku allakülviga,

punane ristik (*Trifolium pratense L.*), talinisu (*Triticum aestivum L.*), hernes (*Pisum sativum L.*) ja kartul (*Solanum tuberosum L.*). Katse rajati neljas korduses. Tava- ja maheviljeluse katselappidel (60 m²) kasutati vastavalt mineraalset lämmastikväetist (N) (N0, N1, N2, N3) ja orgaanilist väetist (Org0, Org1, Org2).

Tavasüsteemi väetusvariandid olid kontroll N0 ($N_0P_0K_0$), mineraalväetis (NH_4NO_3) N1 ($N_{40\text{-}50}P_{25}K_{95}$), N2 ($N_{80\text{-}100}P_{25}K_{95}$) ja N3 ($N_{120\text{-}150}P_{25}K_{95}$). Suviotra väetati madalama lämmastiku normiga (N_{40}, N_{80}, N_{120}) ja talinisu kõrgema lämmastiku normiga (N_{50}, N_{100}, N_{150}). Mahekasvatuse väetusvariandid olid mahe kontroll (Org0), vahekultuurid haljasväetisena suviodra, herne ja kartuli ees (Org1), vahekultuurid koos täielikult komposteerunud sõnnikuga (10 t ha^{-1}; Org2). Sõltuvalt sõnniku kuivainesisaldusest jäi sõnnikus sisalduv lämmastik suviodra ja talinisu korral 44–54 kg ha^{-1} vahele.

Sortide geneetilise mõju hindamiseks AX ja BG sisaldusele koguti andmeid teise põldkatse raames 2021. aastal. Mõju hinnati samade sortide (talinisu 'Fredis' ja suvioder 'Anni') ning kuue talinisu sordi ('Ada', 'Creator', 'Perenaise', 'Julie', 'Širvinta', 'Kallas') ja üheksa suviodra sordi ('Maali', 'Grace', 'Planet', 'Feedway', 'Tuuli', 'Breustar', 'Irina', 'Quench' ja 'Anneli') võrdlusel. Suvioder sai mineraalväetist $N_{90}P_{24}K_{54}$ ja talinisu $N_{152}P_{36}K_{75}$. Kõik uuritud odrasordid olid sõkalteralised kaherealised sordid, mis on kantud Eesti sordilehele. Kõik talinisusordid peale 'Širvinta' (Eesti pärandsort) olid samuti Eesti sordilehel.

Kolmandal põldkatsel, mis viidi läbi aastatel 2022 ja 2023, hinnati kahte erinevat kasvatamissüsteemi: puhas- ja segukülvi (joonis 1) (III). Põldkatses kasutati süsteemset blokkdisaini, kus iga katselapi pindala oli 2 × 10 m ja kordusi oli 4. Segukülvis oli kaks varianti: oder + vikk (seemnete suhe külvi ajal 64:36) ja kaer + põldhernes (suhe 83:17). Katses ei kasutatud mineraal- ega orgaanilisi väetisi, ega taimekaitsevahendeid.

2022. aastal külvati põllukultuurid 25. mail ja koristati 29. augustil, välja arvatud vikk ja oder segukülvis, mis koristati 29. septembril. 2023. aastal külvati põllukultuurid 12. mail ja koristati 6. septembril. Kuid segukülvi oder + vikk 2023. aastal ei koristatud, sest vikk ei olnud selleks ajaks koristusküps. Saagid koristati väikese katsekombainiga Sampo ning seemed segukülvides eraldati pärast koristust käsitsi. Kaera ja odra

saagikus määrati 20 m² katselapi kohta tonnides hektari kohta (kombaini heedri laius oli 2 m).

AX sisaldus jahuproovides määrati ensümaatiliselt, kasutades D-ksüloosi testi mikrotiiterplaadile, ja BG sisaldus mõõdeti Mixed-linkage BG analüüsikomplekti abil. Ilmaandmed, st õhutemperatuur ja sademed, registreeriti kasvuperioodidel aastatel 2014–2023. Andmete statistilisel analüüsil kasutati ühe- ja kahefaktorilist dispersioonanalüüsi (ANOVA). Lineaarsete seoste tugevuse hindamiseks arvutati Pearsoni korrelatsioonikordajad r.

Tulemused ja kokkuvõte

Uuring näitas, et ilmastikutingimused mõjutasid märkimisväärselt mõõdetud teraviljakiudude AX ja BG sisaldust. Mõlema puhul avaldas suurimat mõju katseaasta ilmastik, mis moodustas faktorite kogumõjust 79% AX ning 90% BG puhul. Samas kui uuritud teine katsefaktor väetamine mõjutas mõlema kiudaine varieeruvust vaid 2%.

Talinisu AX sisaldus varieerus aastate lõikes oluliselt, kusjuures madalaimad väärtused registreeriti 2017. ja 2020. aastal (vastavalt 3.24 ja 2.84 g 100 g^{-1}) ja kõrgeimad 2016. aastal (6.68 g 100 g^{-1}). Sarnane varieeruvus ilmnes ka suviodra AX sisalduses, mis oli madalaim 2020. aastal (2.79 g 100 g^{-1}) ja kõrgeim 2016. aastal (5.59 g 100 g^{-1}). Need tulemused on kooskõlas varasemate uuringutega, mis kinnitavad, et kasvuperioodi temperatuur ja sademed mõjutavad oluliselt teraviljade kiudainesisaldust. Kõrgemad õhutemperatuurid odra kasvu alguses (võrsumis- ja kõrsumisfaasis) põhjustavad väiksema tuhande tera massiga terade moodustamise, mille AX protsentuaalne sisaldus on suurem, samas madalamad temperatuurid tera täitumisperioodil pärsivad AX akumuleerumist terasse. Need tulemused viitavad, et kliimamuutused, mis on seotud temperatuuride tõusuga, võivad potentsiaalselt suurendada AX sisaldust teraviljades. Siiski võivad äärmuslikud ilmastikutingimused avaldada terade AX sisaldusele negatiivset mõju.

Terade BG sisaldus sõltus samuti õhutemperatuurist, olles kõrgem aastatel 2018-2021 (5.12 ja 0.56 g 100 g^{-1}, vastavalt odral ja nisul) võrreldes jahedama perioodiga aastatega 2014-2017 (4.41 ja 0.49 g 100 g^{-1}, vastavalt odral ja nisul). Jahedam võrsumisperiood, mis soodustab juurdumist, võrsete arengut ja biomassi moodustumist, suurendab ka

odra 1000 tera massi. Kuna suuremate terade endospermi osakaal on suurem, on ka nende terade BG sisaldus kõrgem. Tera täitumisaegsed sademed, pidurdades toitainete liikumist teradesse vähendavad nende BG sisaldust.

Uuritud viljelusviisidel ja lämmastikväetise tasemetel mõju teraviljade BG sisaldusele puudus ning AX sisaldusele oli minimaalne. Koosmõjus terade täitumisperioodi madalama temperatuuriga oli AX sisaldus maheviljelusüsteemide talinisu terades kõrgem võrreldes tavaviljelussüsteemiga. Suviodra terades sama efekt ei ilmnenud. Talinisu ja suviodra AX sisaldus varieerus erinevates viljelusviisides vastavalt 4.54-5.12 g 100 g^{-1} ja 4.00-4.51 g 100 g^{-1}ning BG puhul 0.51-0.56 g 100 g^{-1} ja 4.55-4.80 g 100 g^{-1}.

Viljelussüsteemide vähene mõju AX ja BG sisaldusele viitab sellele, et nii mahe- kui ka tavapõllumajanduses on võimalik toota sarnase kiudainesisaldusega teravilju. See avastus on oluline jätkusuutlike põllumajandustavade edendamiseks, kuna näitab, et keskkonnasõbralik mahepõllumajandus ei halvenda teraviljade toiteväärtust.

Uuringus käsitleti ka segukülvi mõju teraviljade kiudainesisaldusele. Tulemused näitasid, et oder segus vikiga ja kaer segus põldhernega ei avaldanud märkimisväärset mõju AX sisaldusele. Kuid vikiga segus külvatud odra BG sisaldus vähenes, mis kinnitab varasemaid uuringuid, et oder segukülvides on võrreldes teiste teraviljadega väiksema konkurentsivõimega. Kaera BG sisaldus jäi stabiilseks, olenemata sellest, kas see kasvas puhas- või segukülvis. BG sisalduse vähenemine vikiga segus külvatud odras võib olla tingitud konkurentsist ressursside suhtes, mis võib piirata toitainete kättesaadavust BG biosünteesiks. Need tulemused rõhutavad vajadust hoolikalt valida kultuurliikide kombinatsioone segukülvisüsteemides, et optimeerida nii saagikust kui ka toiteväärtust.

Geneetiline varieeruvus mängis samuti olulist rolli teraviljade kiudainesisalduse kujunemisel. Uuring näitas, et talinisus oli üldiselt kõrgem AX sisaldus võrreldes suviodraga ning kaeras oli see kõrgem võrreldes odraga. Talinisu sort 'Fredis' ja suviodra sort 'Anni' olid mõlemad kõrge AX sisaldusega. Uuritud talinisu sortide AX sisaldus varieerus vahemikus 4 and 6 g 100 g^{-1}, enamusel odrasortidel jäi see alla 4 g 100 g^{-1}. BG sisaldus varieerus samuti oluliselt erinevate sortide vahel,

kusjuures odras oli BG sisaldus kõrgem kui nisus ja kaeras. Suviodra sort 'Anni' näitas uuritud odrasortide seas üht kõrgeimat BG sisaldust. Erinevad tulemused BG sisalduses terades on ilmselt tingitud geneetilistest faktoritest, mis kontrollivad BG sünteesi, tera füsioloogilisi omadusi ja seeläbi ka aretuseesmärke. Olulised geneetilise varieeruvuse tulemused rõhutavad sobivate teraviljasortide valiku tähtsust aretusprogrammides, et parandada teraviljade toiteväärtust.

Kokkuvõtteks: kõrgemad õhutemperatuurid terade täitumise faasis suurendasid AX sisaldust, samas kui suurem sademete hulk mõjutas BG sisaldust negatiivselt. Uuringu tulemused näitasid, et mahepõllumajanduslikud viljelussüsteemid ei kahanda teraviljade kiudainelist väärtust, pakkudes olulist panust inimeste tervise ja keskkonna vastupanuvõime parandamisse. Segukülvid, mis on jätkusuutliku põllumajanduse oluline osa, võivad vähendada teraviljade BG sisaldust. Seetõttu on teraviljade ja liblikõieliste segude planeerimisel oluline arvestada segusse valitavate kutluuride eripärasid, nende omavahelist konkurentsivõimet ja üheaegset seemnete valmimist. Kiudainete sisaldust saagis saab oluliselt mõjutada teravilja liigi valikuga, samas kui sordivalikul on sellele mõju väiksem.

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I

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The influence of cropping system, weather conditions and genotype on arabinoxylan content in wheat and barley grains

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ABSTRACT

Due to the increasing role of organic farming in cereal production the accumulation of arabinoxylan, a key dietary fibre component with many health benefits, was analyzed in winter wheat and spring barley grains cultivated in organic and conventional conditions during an eight-year period. The long-term experiment was conducted at the Estonian University of Life Sciences from 2014 to 2021. The content of arabinoxylan in different spring barley and winter wheat varieties was investigated in grains collected from the Estonian Crop Research Institute in 2021. Results showed that the arabinoxylan content of winter wheat and spring barley grains were influenced by weather conditions, especially by temperature during the grain filling period. Higher temperature increased arabinoxylan content in grains. Cropping system and fertilizer treatments affected the level of the crop yield and through this altered winter wheat grain arabinoxylan content, which was higher in organic cropping system in years with cooler grain filling period temperatures. Cereal species had a greater impact on winter wheat and spring barley arabinoxylan content than variety, with wheat having higher arabinoxylan content. These results suggest that arabinoxylan from wheat and barley grains can be a valuable resource for cereal valorization via nutritional enhancement.

1. Introduction

Cereal grains are composed of many layers containing different substances in smaller and larger scale. Dietary fibers are components that grains contain in large amounts. They are carbohydrates which are not digested or absorbed in the small intestine, but pass intact to the colon, where they undergo complete or partial bacterial fermentation (Ibba et al., 2021). Arabinoxylans (AX) are the main nonstarch polysaccharide components in the grain followed by levels of cellulose and beta-glucan (Knudsen, 2014). There they serve as major structural components of endosperm cell walls, aleurone layer, bran, and husk (Comino et al., 2013) but may also have metabolic activity (Guillon and Champ, 2000).

It is known that these fibers have important characteristics, which can affect positively the properties of food and consequently human health. Arabinoxylan influences biotechnological processes such as bread baking, brewing and production of animal feeds (Van Craeyveld, 2009). When consumed in sufficient quantity AX can reduce blood cholesterol, improve metabolic syndrome, regulate blood sugar levels and intestinal microorganisms (Sun et al., 2022). These impacts have been studied widely throughout the latest decades. But at the same time, we are still facing a problem of inadequate human daily intake of dietary fiber (Jones, 2014). This problem could be alleviated by using arabinoxylan supplements or enriched functional foods, but also by choosing high AX content species and varieties for cultivation as food crops and by creating growth conditions needed for achieving the highest possible AX content in grains harvested.

Although AX is a minor component of the caryopsis, it is a main structural polysaccharide of the cell walls and therefore a major portion of the dietary fibre in flour (Skendi et al., 2011). All cereal species (wheat, barley, oat, rye) grown in northern Europe contain AX. While the grains of rye, triticale and wheat are considered as the main sources of AX, grains of barley and oats, in contrast, have a high level of beta-glucan (Knudsen, 2014). It has been found that arabinoxylans content in barley and wheat is in the range of 4–8% (Han and Schwarz, 1996; Izydorczyk, Biliaderis, 2007; Pritchard et al., 2011), being lower than in rye (8–12%), but higher than in oats (3–3.5%) (Izydorczyk,

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Abbreviations

AX	arabinoxylan
N	nitrogen
SB	spring barley
WW	winter wheat

Biliaderis,2007).

However, the content of AX in cereal grains depends on several factors from agronomic, environmental and genetical aspects (Takač et al., 2022). Increasing organic farming in the EU is favored by the Farm to Fork strategy under the European Green Deal initiatives, which aims to ensure sustainable food production and increase European Union agricultural land under organic farming to 25% by 2030 (European Commission, 2020). Thus, it is important to know how organic farming with lower yields may affect the AX content of cereals. From the restricted number of studies on the matter higher total dietary fibre values have been found in organically grown spelt wheat (Kwiatkowski et al., 2015), oat and barley (Menkovska et al., 2017). More specifically how AX content in grains depends on cropping type has received little attention. Results have shown higher common wheat AX values in conventional farming compared to organic and vice versa for spelt wheat (Takač et al., 2022). These results imply that dietary fiber production could be successful also in systems with lower inputs. It has been found that N fertilization has no impact on AX content in durum wheat grains (Lempereur et al., 1997), but also that N application during grain filling increases AX content considerably (Coles et al., 1997). Among environmental factors drought has been found to have increasing impact on AX content in wheat grains (Coles et al., 1997). Genetic studies have reported higher AX levels in common wheat compared to those of spelt wheat (Takač et al., 2022) and in spring wheat genotypes compared to winter wheat (Li et al., 2009), but also no differences in AX values between spring and winter wheat (Selga et al., 2023). All abovementioned studies have gathered data from rather short periods from one to three consecutive growing years, but due to the high variability in weather conditions their conclusions can be limited. Long-term field experiments can provide more profound insight.

The novelty of the current study is the evaluation of an eight-year period of winter wheat and spring barley growing in crop rotation experiment. Previously we have studied the impact of cropping system and weather on the size of starch granules in winter wheat (Keres et al., 2020) as well as on dough quality (Keres et al., 2021). The present article continues this research focused on overall grain quality, moving from starch to non-starch polysaccharides and investigates the effect of organic and conventional cropping system with different N inputs and rates on the content of AX in winter wheat and spring barley grains in different environmental conditions during an eight - year period. Eight years was postulated to be a long enough time to capture sufficiently different environmental stresses to affect phenotype. Our hypothesis was that AX content in organic grains is higher than in conventional ones and its content depends on weather conditions, more specifically on drought. In addition, we aimed to characterize AX content in different genotypes i.e. varieties of wheat and barley.

2. Material and methods

2.1. Experimental setup

The grain samples were collected from the long-term field crop rotation experiment located at the Estonian University of Life Sciences in Tartu County (58°21′53.2″N, 26°39′57.7″E) established in 2008. The data were obtained by analyzing winter wheat (WW) ('Fredis') and spring barley (SB) ('Anni') grain samples from the period of 2014–2021. Crop rotation consisted of SB (*Hordeum vulgare* L.) with undersown red clover, red clover (*Trifolium pratense* L.), WW (*Triticum aestivum* L.), pea (*Pisum sativum* L.) and potato (*Solanum tuberosum* L.) following each other on *Stagnic Luvisol* soil in four replications. Organic and conventional cropping systems were fertilized respectively with organic (Org0, Org1, Org2) and mineral nitrogen (N) (N0, N1, N2, N3) fertilizers, as described in detail earlier by Alaru et al. (2014) and Keres et al. (2021). Organic treatments were organic control (Org0), off-season cover crops as green manure (Org1) and off-season cover crops with fully composted cattle manure (10 t ha^{-1} for SB + 10 t ha^{-1} for WW and 20 t ha^{-1} for potato; Org2). Nitrogen amounts for SB and WW depended on manure dry matter content and ranged in trial years between 44 and 54 kg ha^{-1}. Conventional treatments were control N0 ($N_0P_0K_0$), minerally (NH_4NO_3) fertilized N1 ($N_{40–50}P_{25}K_{95}$), N2 ($N_{80–100}P_{25}K_{95}$) and N3 ($N_{120–150}P_{25}K_{95}$). Spring barley undersown with red clover was fertilized with lower N rates (N_{40}, N_{80}, N_{120}) and WW with higher rates (N_{50}, N_{100}, N_{150}).

The same varieties, WW 'Fredis' and SB 'Anni' were grown in 2021 in comparison with six WW and nine SB varieties to evaluate the genotype effect. Field experiment was conducted on *Luvisol* soil in Estonian Crop Research Institute in Jõgeva County (50 km north of the first field trial). Spring barley received mineral fertilize at a rate $N_{90}P_{24}K_{54}$ and WW at rate of $N_{152}P_{36}K_{75}$.

Content of AX in milled samples (Perten Laboratory Mill 3100 (Perten Instruments AB, Sweden)) of WW (N = 179) and SB (N = 221) grains from the crop rotation experiment and SB (N = 10) and WW (N = 7) grains obtained from Estonian Crop Research Institute was determined enzymatically with the microplate assay procedure for d-xylose (Megazyme International, Ireland Ltd) in triplicate. Clariostar (BMG Labtech, Germany) absorbance microplate-reader reported the absorbance of the samples at a wavelength of 340 nm. The d-xylose percentage used to calculate AX content of samples were literature-based values of 0.62% for WW and 0.75% for SB on a conservative assumption so as not to exaggerate the results, but rather provide lower than higher results possible.

2.2. Weather conditions during 2014–2021 growing periods

Meteorological data of the period 2014–2021 were collected from a weather station approximately 2 km from the trial site. Air temperatures and precipitation during these growing periods of eight years were variable (Table 1). Table 1 shows the indicators from April to August; this time is the post-winter period of WW and the vegetative period of SB in Estonia. The period from 2014 to 2017 can be described as cooler and wetter with an average temperature of 13.1 °C similar to the long-term average (1964–2021). While 2018–2021 was mostly warmer (14.5 °C as period average). Also, more precipitation occurred between 2014 and 2017 (average 322 mm) compared to 2018–2021 (average 276 mm). The most exceptional years were 2018 and 2021, with 2.6 and 1.7 °C higher mean temperatures during the growing period. 2021 was a year also with the highest sum of precipitation, exceeding that of the long-term average by 85 mm due to the rainy months of May and August. Also 2014 had similarly higher precipitation caused by rain in May, June and July. The coolest years were 2015 and 2017, with May, June and July being unusually cold. The driest months during growing period appeared in 2018 and 2019, with approximately 100 mm less rain than that of long-term average. The year's most similar to the long-term average growing period temperature and precipitation were 2016 and 2020.

2.3. Statistical analysis

All AX measurements were performed in triplicate. Results were expressed as means ± standard error. Results were analyzed using one-way and factorial analysis of variance (ANOVA), with statistical software package Statistica version 13 (TIBCO Software Inc, USA). When

Table 1
Average air temperatures and monthly precipitation during experimental period and their long-term averages.

Month	Average air temperature per month, °C								1964–2021 average temperature, °C
	2014	2015	2016	2017	2018	2019	2020	2021	
April	6.5	5.4	6.1	3.4	7.2	7.7	4.8	5.3	5.0
May	12.0	10.3	14.0	10.4	16.0	11.4	9.5	10.9	11.4
June	13.4	14.2	15.9	14.0	15.9	18.6	18.4	19.8	15.4
July	19.4	15.7	17.9	16.0	20.8	16.4	16.3	22.2	17.4
August	16.7	17.0	16.1	16.7	18.8	16.7	16.8	15.8	16.0
Mean of April–May	13.6	12.5	14.0	12.1	15.7	14.1	13.2	14.8	13.1
	Sum of precipitation per month, mm								1964–2021 average precipitation, mm
April	13	69	52	52	28	3	50	29	33
May	84	62	2	16	8	60	32	108	53
June	103	39	12	94	61	51	117	19	72
July	71	61	82	61	14	41	69	17	67
August	113	41	42	106	59	58	64	217	80
Sum of April–August	385	273	301	328	170	213	332	390	305

significant ($P < .05$) differences were found, Fisher's least significant difference test (LSD *post hoc* test) was used to determine the differences among means.

3. Results

Long term two-factor (first factor: weather conditions, i.e. air temperature and precipitation; second factor: cropping systems with different N treatments) experiment results showed that the AX content of WW and SB grains were significantly influenced by weather conditions, especially by temperature. Whereas the proportion of variance was 79% for weather conditions, 2% for N treatment and 18% for weather and N treatment interactions.

The greatest source of variation for AX was year, i.e. weather conditions ($P < .001$) (Fig. 1) compared with N treatments and species grown. In WW grains AX content as an average of N treatments was the lowest in 2017 and 2020 (3.24 and 2.84 g 100 g^{-1}) and the highest in 2016 (6.68 g 100 g^{-1}). Its content stayed between 4.31 and 5.75 g 100 g^{-1} in other years. The AX content of SB was the lowest in 2020 (2.79 g 100 g^{-1}), the highest in 2016 (5.59 g 100 g^{-1}) and between 4.09 and 5.19 g 100 g^{-1} in other years.

Variability in results between years was much higher for both species compared to the variability between fertilizer treatments. The range of mean AX values for WW and SB between treatments was 0.58 and 0.50 g 100 g^{-1} respectively, while between years it was 3.85 and 2.41 g 100 g^{-1} respectively. As an average of all experimental years and N treatments, the AX content of WW was in the range 4.54–5.12 g 100 g^{-1} and SB values ranged from 4.00 to 4.51 g 100 g^{-1}. Values of AX were slightly higher in WW grains compared to those in SB, but as an average AX value of N treatments in WW grains (4.74 g 100 g^{-1}) was ($P = .025$) higher than that of SB (4.23 g 100 g^{-1}).

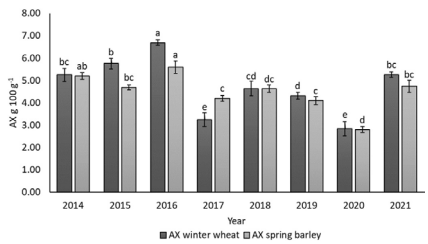

Fig. 1. The AX content as an average of N treatments in winter wheat and spring barley flours in different years, g 100 g^{-1}
n: letters on bars refer to comparison between different cropping years of the same species; bars with the same letter are not significantly different ($P < .05$).

When considering the air temperatures of plants development stages, the AX content in grains was influenced by the temperatures of May (tillering/elongation stage) and July (grain filling period) (Fig. 2). In years when the average air temperature in grain filling period of WW was 15.7–16.4 °C, the content of AX in wheat grains varied between 2.84 and 5.75 g 100 g^{-1}, when the July's temperature was higher, between 18 and 22.4 °C, then AX fluctuated between 5.25 and 6.68 g 100 g^{-1}. The same tendency was observed for the SB grain filling period (starting 2–3 weeks later), where at lower temperature between 16.1 and 17.2 °C AX content varied between 2.74 and 3.87 g 100 g^{-1}. Higher temperature (19.9–23 °C) caused an increase in AX content, which ranged between 4.21 and 5.14 g 100 g^{-1}. The amount of precipitation did not significantly affect the AX content in grains.

The temperature in May, in combination with the cropping method and N fertilization, affected the yield of WW and SB indirectly and through this, also the AX content in grains. The results showed that the cropping system and fertilizer treatments primarily affected the level of the crop yield (for WW R = 0.43, $P < .001$ and R = 0.51, $P < .001$, respectively; for SB R = 0.56, $P < .001$ and R = 0.65, $p < .001$, respectively). Mean grain yield of WW over trial years varied in fertilized treatments of conventional system (i.e., N1–N3) between 4.66 and 4.98 t ha^{-1}, which was up to 45% higher than that in organic treatments (i.e., Org0–Org2). The same data for SB grain yield were 3.32–3.80 t ha^{-1} in conventional system, which was up to 57% higher than that in organic treatments. Grain yield of WW was negatively correlated with AX content of grains (R = − 0.27, $P < .05$), but grain yield of SB was not significantly correlated with AX values (Table 2). Arabinoxylan content in SB was negatively correlated with 1000 kernel weight and positively with test weight values (R = − 0.50, $P < .001$ and R = 0.29, $P < .05$, respectively), whereas mean 1000 kernel weight of SB was negatively correlated with air temperature of grain filling period (R = − 0.38, $P < .01$).

The combined effect of grain filling period temperature, cropping system and N fertilization on the AX content of SB and WW is shown in Table 3. At the lower temperature of grain filling period, the mean AX content of both crops was lower than that at higher temperatures. The influence of cropping systems on AX values was significant only for WW grains at the lower grain filling temperature, when mean AX value in organic treatments was significantly higher compared to conventional treatments (Table 3). Arabinoxylan content in SB grains was not significantly influenced by cropping system.

When analysing the AX content of WW variety 'Fredis' and spring barley 'Anni' grown in the same year in different location and fertilized at similar N rate (N150 and N80 respectively) then the results were comparable, being 5.04 and 5.91 g 100 g^{-1} for WW ($P = .28$) and 2.82 and 4.20 g 100 g^{-1} for SB ($P = .07$) in Eerika and Jõgeva fields

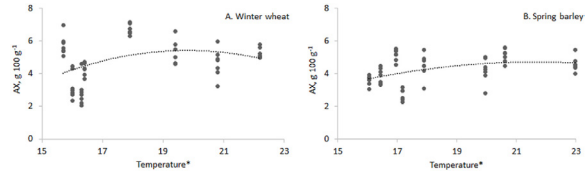

Fig. 2. AX content in winter wheat (A) and spring barley (B) depending on air temperatures during grain filling period; A. winter wheat: $y = -0.0827x^2 + 3.2708x - 26.936$; $R = 0.36$ $P < .05$; B. spring barley: $y = -0.033x^2 + 1.43x - 10.752$; $R = 0.42$; $P < .01$; *air temperatures during grain filling period.

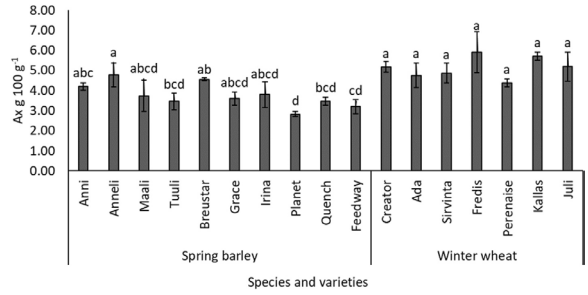

Fig. 3. Average content of AX in different spring barley and winter wheat varieties, g 100 g^{-1}
n: letters on bars refer to comparison between varieties of the same species; bars with the same letter are not significantly different ($P < .05$).

Table 2
Arabinoxylan correlations with cereal yields, its quality indicators and environmental factors.

Variable	Mean ± Std. Err.	AX g 100 g^{-1} 2014–2021
Winter wheat		
Yield t ha^{-1}	3.66 ± 0.22	−0.27
1000 grain weight, g	45.4 ± 0.68	ns
Test weight, kg hl^{-1}	779 ± 4.31	0.22
Temperature, °C	13.8 ± 1.15	0.24
Precipitation, mm	299 ± 10	ns
Spring barley		
Yield t ha^{-1}	2.55 ± 0.17	ns
1000 grain weight, g	44.1 ± 0.72	−0.5
Test weight, hl kg^{-1}	629 ± 5.39	0.29
Temperature, °C	13.8 ± 1.15	ns
Precipitation, mm	299 ± 10	ns

n: marked correlations are significant at $P < .05$; ns - not significant; temperature and precipitation are mean of months April–August of 2014–2021.

respectively. Arabinoxylan contents among all studied WW varieties were overall similar ($P = .663$), ranging from 4.37 g 100 g^{-1} in 'Perenaise' to 5.91 g 100 g^{-1} in 'Fredis' (Fig. 3). Genotype did not have a significant ($P = .089$) effect also on AX content of SB, which was between 2.83 and 4.77 g 100 g^{-1}. The highest values were in varieties 'Anneli', 'Breustar' and 'Anni' (4.77, 4.56 and 4.20 g 100 g^{-1} respectively).

All wheat varieties and in addition a few barley varieties ('Anneli', 'Breustar' and 'Anni') had AX contents between 4 and 6 g 100 g^{-1}, while for most SB varieties the AX content remained below 4 g per 100 g^{-1} of whole grain flour. Varietal mean AX content was 3.76 and 5.18 g 100 g^{-1} for SB and WW respectively, with barley varietal mean AX content being lower ($P < .001$) (more than 25%) compared to wheat varieties.

Table 3
The interaction of grain filling period temperatures and N treatment on winter wheat and spring barley grains AX content (g 100 g^{-1}).

Treatment	Winter wheat			Spring barley		
	Temperature in grain filling stage			Temperature in grain filling stage		
	16.3 °C	20.8 °C	22.2 °C	16.1 °C	17.9 °C	20.6 °C
AX in organic systems						
Org 0	4.3 ± 0.28 a	5.4 ± 0.12 ab	5.6 ± 0.09 a	3.8 ± 0.18 a	4.6 ± 0.16 bc	5.3 ± 0.11 a
Org 1	2.9 ± 0.08 ab	5.9 ± 0.07 a	5.1 ± 0.05 b	3.3 ± 0.07 a	4.9 ± 0.11 ab	4.3 ± 0.16 ab
Org 2	2.9 ± 0.12 ab	4.7 ± 0.08 b	5.5 ± 0.13 a	3.7 ± 0.16 a	4.2 ± 0.03 c	5.1 ± 0.10 ab
Average	3.3 ± 0.16 B	5.3 ± 0.09 A	5.4 ± 0.09 A	3.6 ± 0.14 B	4.6 ± 0.09 A	5.3 ± 0.12 A
AX in conventional systems						
N0	1.9 ± 0.10 c	4.2 ± 0.2 bc	4.7 ± 0.77 abc	3.9 ± 0.14 a	5.5 ± 0.09 a	4.4 ± 0.05 ab
N1	2.4 ± 0.09 cb	4.7 ± 0.22 bc	5.2 ± 0.35 ab	3.8 ± 0.05 a	3.2 ± 0.14 d	4.5 ± 0.11 b
N2	2.2 ± 0.09 cb	3.4 ± 0.16 c	4.3 ± 0.46 c	3.5 ± 0.11 a	4.5 ± 0.14 bc	4.5 ± 0.11 ab
N3	2.4 ± 0.08 cb	5.4 ± 0.15 ab	4.8 ± 0.12 b	3.1 ± 0.19 a	4.8 ± 0.06 b	5.6 ± 0.07 a
Average	2.3 ± 0.09 B	4.4 ± 0.18 A	4.8 ± 0.43 A	3.6 ± 0.12 B	4.5 ± 0.11 A	5.2 ± 0.09 A

n: different upper and lowercase letters indicate significant differences in rows and columns, respectively; values in the same row with the same letter are not significantly different ($P < .05$).

4. Discussion

Dietary fiber AX forms a considerable part of the cereal grain and determines also the grain health promoting properties. Therefore, it is important to know the agricultural possibilities to increase that value. The effect of different cropping systems on AX content of cereals has been little studied so far. Previous studies have shown minor effect from fertilization on AX content in grains (Coles et al., 1997; Lempereur et al., 1997). The results of this field experiment showed that weather conditions had greater effect on WW and SB grains AX content than fertilizer treatments and cropping system. Result that AX yield in grains can be achieved regardless of the fertilization regime suggests helpful opportunity to achieve the goals of the European agricultural policy, which promotes organic production with reduced fertilizers use.

It has been reported that year i.e. weather conditions affects AX formation (Finnie et al., 2006; Marconi et al., 2020; Selga et al., 2023) through changing the conditions during kernel growth and ripening (Buksa et al., 2014). Previously drought has been found to have a positive relationship with wheat AX content (Coles et al., 1997; Selga et al., 2023), but in this current study air temperature had a significant effect on the AX content in grains. As future climatic models are predicting greatest warming rates in northern Europe (Zhao et al., 2022), this could mean that also higher AX content in grains could be achieved.

The determining factor for AX content was the temperature of two developmental stages for both crops – temperature during the tillering/stem elongation stage and in grain filling period. Under Estonian conditions the tillering stage of SB and WW is in May (the regrowth of WW after hibernation starts at the end of April and tillering/stem elongation period lasts till the second part of May). The temperature in May, in combination with the cropping system, affects the formation of the above-ground biomass and grain yield (number of shoots per plant, number of grains per head and 1000 kernel weight) (Evans 1983; Ryan et al., 1997). Since N availability from mineral fertilizers in early development stages of cereals is better than availability of N from organic manure (Alaru et al., 2014; Efretuei et al., 2016), the grain yield in conventional system is much higher (Keres et al., 2020). Our results showed up to 45% lower WW grain yield from organic treatments, WW AX content and grain yield were negatively correlated, and WW grains grown in the conventional system had lower AX content. However, high dietary fiber content can potentially increase the value of organic wheat in bioeconomy value chain. Economic assessments would help in this context to ascertain the value of cereal crops, which have lower yields with higher AX content from agronomic, soil heath and population health perspectives.

In this experiment the 1000 kernel weight was negatively correlated with AX content in SB ($R = -0.50$, $P < .001$; Table 2), whereas 1000 kernel weight in turn was negatively correlated with temperature in May ($R = -0.58$, $P < .001$). Temperatures during tillering/elongation stage of SB effects the development of SB primordia. At the higher air temperature, the barley plant forms probably more seed primordia, which in turn led to a higher number of grains per spike and a lower 1000 kernel weight. 1000 kernel weight affects the kernel/seedcoat ration with smaller seeds having higher AX proportion (Lempereur et al., 1997).

Results showed that AX content of studied cereals positively correlated with temperature in the grain filling period ($P < .05$ for WW and $P < .01$ for SB (Fig. 2). This result is similar to our previous work with starch granules, showing that starch granules, similarly to dietary fiber component AX, are impacted by the temperature of grain filling period (Keres et al., 2020). In this field experiment, the AX content of both investigated crops was less at lower temperatures during the grain filling (Table 3), while the differences in AX content between fertilizer treatments and cropping systems decreased at higher temperatures. AX content of WW grains was less in conventional system at lower temperature of grain filling period. Also, temperature during this time was positively correlated with test weight of SB ($P < .01$), which in turn was positively correlated with AX content in SB grains ($P < .05$; Table 2). In our experiment, AX was measured from whole grain flour, without separating different flour fractions. The use of mineral N fertilizer twice during the growing season ensured a longer duration of green leaves including the flag leaf (Hafsi et al., 2013), which in turn led to a longer grain filling period and resulted in up to 5% higher test weight in SB grains obtained from conventional treatments ($P < .05$). Since the AX content of different flour fractions was not determined separately, it can be assumed that the positive correlation between test weight and AX content reflects the increase of AX content in the grain endosperm.

The content of AX in our experiment was lower (Coles et al., 1997) or similar to AX values measured earlier for wheat (Pritchard et al., 2011; Saeed et al., 2014) and lower than that found for barley grains (Han and Schwarz, 1996; Marconi et al., 2020). Arabinoxylans, also referred to as pentosans, due to the fact that they consist predominantly of the pentoses, arabinose and xylose (Wang and Zhang, 2010) may have very variable arabinose to xylose (A/X) ratios, which is an indicator of the AX structure, that varies between grain layers as well as between the same tissues from different grains (Knudsen, 2014). The A/X ratio has been shown to be higher for barley (0.6–0.76 (Han and Schwarz, 1996; Knudsen, 2014; Marconi et al., 2020), than for wheat (0.53–0.62 (Knudsen, 2014; Selga et al., 2023). The fact that we used literature-based A/X ratios in our AX calculations must therefore also be considered, when making implications of the research results, as they may have somewhat differed from measured A/X values used.

Both cereal species and cultivar affect grain AX content (Lempereur et al., 1997; Finnie et al., 2006; Li et al., 2009; Saeed et al., 2014). Based on AX content comparison between the same varieties grown on different fields, it can be stated that AX content is an inherent trait to a certain variety, which does not depend much on the growing location if the other factors (weather, fertilization etc.) are similar. Still this assumption needs to be tested further. Both studied varieties, WW 'Fredis' and SB 'Anni', are high in AX content and could be suitable candidates for the crop breeders in the selection of varieties oriented to give high AX yields. Experiment shows that WW species are better AX sources compared to SB grains, as it has been stated before (Knudsen, 2014). This fact must be considered when choosing species for cultivation with the aim to produce high AX content grains. Overall, although variety had some impact on the currently studied cereal grains, their AX content was still mainly determined by the cereal species itself.

5. Conclusions

The above results support the idea that AX from wheat and barley grains can be a valuable resource for cereal valorization. This study supported hypothesis that cereal grains from organic crop production have higher AX content only partially; higher AX content was obtained from WW grains grown in organic cropping system in years with cooler temperatures during grain filling period. We hypothesized that AX content in grains depends on weather conditions and it was supported by our results, but instead of drought, as suggested by short-term experiment, the air temperature during the plants' grain filling period had the greatest influence on the AX content. While the effect of fertilizer treatments and cropping systems was more indirect and was expressed through their effect on grain yield, 1000 kernel weight and test weight. In addition to environment, also genotype had an impact on the AX content of cereal grains. Variety had less impact on the AX content when compared to the effect of cereal choice, with wheat having higher AX content in these experiments when compared to that in barley grains. Future AX studies should include winter barley, a cereal species whose cultivation is gaining momentum, and also spring wheats as a comparator.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcs.2023.103650.

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Barley and wheat beta-glucan content influenced by weather, fertilization, and genotype

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Introduction: Beta-glucan is one of the most important dietary fibers in cereal grains, having a great impact on human health and food quality. Wheat and barley are strategic crops and their fibers contribute toward their nutraceutical impact. However, the health of the environment is also important to ensure sustainable crop production. Hence the European decision makers have agreed to move toward organic cropping and to reduce the use of mineral fertilizers. Environment and agricultural management have a vast impact on the content of β-glucan in cereal grains. To exploit the valuable properties of β-glucan, the knowledge of factors increasing the amount of this component is crucial. We hypothesized that annual weather conditions, nitrogen fertilization, and cropping system affect the β-glucan amount in wheat and barley.

Methods: For this purpose, spring barley and winter wheat flour samples from an 8-year-long period (2014-2021) were characterized. The experiment included conventional and organic cropping systems with different nitrogen rates between 0 and 150 kg ha^{-1}. In addition, the variation of β-glucan content in different varieties was analyzed.

Results and discussion: The results showed that the content of β-glucan in barley and wheat grains was affected mainly by weather conditions not by fertilization. The latter finding means that the regulation of crop nutrition is not going to impact dietary fiber content in our everyday food. Lower temperatures during tillering and higher 1,000 kernel weight and test weight showed a positive correlation with β-glucan content in barley grains, while precipitation during the grain filling period had a negative correlation. Our findings suggest that β-glucan can be obtained from low-input and organic systems as efficiently as from fertilized treatments. However, there is a need for adaptation strategies in industry, as β-glucan content can vary from year to year.

KEYWORDS

beta-glucan, nitrogen, dietary fiber, cropping system, organic, conventional, genotype

1 Introduction

Cereals are the most widely produced crops in the world, with multiple forms of use. They are considered the major source of calories and protein in the diets of both humans and livestock (Lafiandra et al., 2014). In Europe, in countries such as Germany, Poland, and the Baltic countries, the routine diet is based on cereal grains, and the use of cereal products is exclusively high (Lachman et al., 2012; Nogala-Kałucka et al., 2020). At the same time as wheat has become the most important source of food, barley on the

other hand has been mostly grown for animal feed and brewing purposes. However, in recent years, the importance of barley grains as a nutraceutical ingredient has increased because of their high content of soluble fibers, especially as a rich source of β-glucan (beta-glucan; Kayal and Nirmala, 2014). But when considering that zero-waste production systems are what we are aiming for in the near future we should try to valorize cereal crops beyond their nutraceutical value. Considering barley the valorization of its hulls has been a focus of much research (Martínez-Subirà et al., 2021), but in addition the properties of cereal grain's dietary fibers have been studied extensively through the decades (Herforth et al., 2019). Cereal crops are important sources of dietary fiber. Arabinoxylans and β-glucans are primary components, accounting for ∼70 and 20% of the total dietary fiber content of whole grain wheat, respectively, and 20–70% of the total dietary fiber content in barley grains, respectively (Prasadi and Joye, 2020). Paying more attention to the content of β-glucan in cereal crops could help to increase human dietary fiber consumption and alleviate the problems related to their deficiency.

Cereal β-glucans are linear homopolysaccharides of D-glucopyranosyl residues linked via β- (1–3) and β- (1–4) linkages (Lazaridou and Biliaderis, 2007), mainly found in the endosperm (75%) and aleurone layer (26%; Tiwari and Cummins, 2009; Mejía et al., 2020). Among cereals, β-glucans can be found in barley, oat, rye, and wheat (Maheshwari et al., 2017) with numeric values ranging from 2.5 to 11.3% in barley, 2.2–7.8% in oat, 1.2–2.0% in rye, and 0.4–1.4% in wheat (Lazaridou and Biliaderis, 2007). In barley, β-glucans are located throughout the starchy endosperm whereas in wheat the highest concentration is in the subaleurone layer with little in the rest of the endosperm (Izydorczyk and MacGregor, 2000; Lazaridou and Biliaderis, 2007).

Consumption of β-glucan revealed various positive effects on human health including regulation of blood glucose levels, and reduction of serum cholesterol, hypertension, and obesity (Lazaridou and Biliaderis, 2007; El Khoury et al., 2012). In the food industry, β-glucans are used as thickeners, texture enhancers, stabilizers, and fat substitutes (Mejía et al., 2020). Therefore, foods with dietary fibers such as β-glucans are considered healthier foods with lower calories and are fat-free (Elleuch et al., 2011). The importance of β-glucans for human health is supported also by the health claim authorized by the European Commission stating that barley β-glucans have been shown to lower blood cholesterol with a daily intake of 3 g of barley β-glucans (European Commission, 2012).

Content of β-glucan in grains is affected by different cropping systems (Tiwari and Cummins, 2009; Dickin et al., 2011). Nitrogen (N) availability in the soil is the main factor influencing β-glucan content in cereals (Güler, 2003b; Dvončová et al., 2010). It was reported that mineral N fertilizers increase the β-glucan content (Güler, 2003a; Noworolnik et al., 2014; Habiyaremye et al., 2021), particularly when they are fertilized with 100 kg N ha^{-1} (for wheat) and 80 kg N ha^{-1} (for barley; Güler, 2003a) regardless of the fact that β-glucan location in the grain among compared cereal grains is different (mostly in aleurone layer for wheat and endosperm for barley; Izydorczyk and MacGregor, 2000; Lazaridou and Biliaderis, 2007). On the other hand, β-glucan content of rye, wheat, barley, and oat obtained from organic farming was comparable with those from conventional farming (Menkovska et al., 2017), indicating that β-glucan content can be associated with grain size and yield (Güler, 2003b).

Weather and environmental conditions including temperature and precipitation are other factors affecting the content of β-glucans (Andersson and Börjesdotter, 2011; Dickin et al., 2011). Menkovska et al. (2017) stated that β-glucan content in oat grains depended on precipitation during the grain filling period. Drought stress decreases β-glucan concentration in barley grain (Coles et al., 1991), while Güler (2003a,b) demonstrated also a negative effect of increased levels of irrigation on barley β-glucan content. It has been found that rainy and cool weather during the period of grain establishment and flowering negatively affects β-glucan content in grain (Ehrenbergerová et al., 2008), while dry and warm growing conditions have a positive effect (Lazaridou and Biliaderis, 2007).

Genotype is another factor influencing the β-glucan content in cereal grains (Güler, 2003a; Dickin et al., 2011; Noworolnik et al., 2014; Choi et al., 2020; Habiyaremye et al., 2021). Waxy (high amylopectin; no or low amylose) barley genotypes as well as hull-less varieties have higher β-glucan content compared to genotypes with high amylose starch and hulled varieties (Ehrenbergerová et al., 2003; Izydorczyk and Dexter, 2008). Fan et al. (2009) found in the pot experiment with oat no genotype effect on grain β-glucan content, while Saastamoinen et al. (2004) stated that oat grain β-glucan depends significantly on cultivar. Tiwari and Cummins (2009) suggested that genotype is the most important factor in determining β-glucan content and found that initial levels in the grain genotype also play an important role in β-glucan levels at grain harvest. Choi et al. (2020) has found genotypic differences for barley β-glucan in interactions with year and location.

It is important to know whether β-glucan content in grains can be changed through agronomic management and varietal choice in field conditions and thereby develop action plans for plant breeding programs toward increased β-glucan content in grains. This study aimed to evaluate the effect of organic and mineral fertilizers on β-glucan content in winter wheat and spring barley grains, as well as to determine the impact of temperature and precipitation on β-glucan over 8 years. We hypothesized that the content of β-glucan in grains collected from intensively fertilized treatments is greater than in grains from organic treatments and it depends on weather conditions in specific growth stages. In addition, we characterized the content of β-glucan with respect to genotype variation.

2 Materials and methods

2.1 Experimental setup

In this study, being a part of a larger field experiment evaluating cropping systems' effect on crop production, two cereal grains, winter wheat and spring barley, were assessed based on their β-glucan content. Winter wheat "Fredis" variety and spring barley "Anni" variety grains were collected from 2014 to 2021 from the long-term field crop rotation experiment located at the Estonian University of Life Sciences in Tartu County (Eerika field). Experimental crop rotation consisting of barley (*Hordeum vulgare* L.) with under-sown red clover, red clover (*Trifolium pratense* L.), wheat (*Triticum aestivum* L.), pea (*Pisum sativum* L.), and potato (*Solanum tuberosum* L.) in four replications was established on

Stagnic Luvisol soil. All species were cultivated organically (Org0, Org1, and Org2) and conventionally (N0, N1, N2, and N3) in parallel, as described in detail earlier by Alaru et al. (2014) and Keres et al. (2021). Organic treatments were organic control (Org0), off-season cover crops as green manure (Org1), and off-season cover crops with fully composted cattle manure (10 t ha^{-1} for barley + 10 t ha^{-1} for wheat and 20 t ha^{-1} for potato; Org2). Nitrogen amounts for barley and wheat in trial years were between 44 and 54 kg ha^{-1} depending on manure dry matter content. Conventional treatments were control N0 ($N_0P_0K_0$), minerally (NH_4NO_3) fertilized N1 ($N_{40-50}P_{25}K_{95}$), N2 ($N_{80-100}P_{25}K_{95}$), and N3 ($N_{120-150}P_{25}K_{95}$). Lower N rates (N_{40}, N_{80}, N_{120}) were used for barley undersown with red clover, and higher rates (N_{50}, N_{100}, N_{150}) for wheat.

Samples for the genotype analysis were collected in 2021 from the Estonian Crop Research Institute in Jõgeva County (50 km north of Tartu; Jõgeva field), the breeding institution of cereal grains. The soil type was *Luvisol* and fertilizer rates were $N_{90}P_{24}K_{54}$ for spring barley and $N_{151}P_{36}K_{75}$ for winter wheat. All studied spring barley varieties (Maali, Grace, Planet, Feedway, Tuuli, Breustar, Irina, Quench, Anneli, and Anni) were hulled two-row grains listed on Estonian Plant Variety Register. Most winter wheat varieties (Ada, Creator, Perenaise, Julie, Fredis, Kallas) are listed on Estonian Plant Variety Register (except for "Širvinta," which is an Estonian heritage wheat grain).

After collection, all samples were milled [Perten Laboratory Mill 3100 (Perten Instruments AB, Sweden)]. The level of β-glucan in wheat ($N = 179$) and barley ($N = 221$) grains from the crop rotation experiment and barley ($N = 10$) and wheat ($N = 7$) varieties were determined using Mixed-linkage β-glucan assay kit (Megazyme, Ireland) based on the method published by Limberger-Bayer et al. (2014). This method is accepted by the Association of Official Analytical Chemists International (AOACI; Method 995.16) and the American Association of Cereal Chemists International (AACCI; Method 32-23). In brief, samples (100 mg) were suspended and hydrated in a sodium phosphate buffer (pH 6.5), incubated with purified lichenase enzyme, and an aliquot of filtrate was reacted with purified β-glucosidase enzymes. The glucose product was assayed using an oxidase/peroxidase reagent. The measurement of the content of β-glucan was performed in wheat and barley parallel assays, respectively, using a Helios Omega UV-Vis Spectrophotometer (Thermo Scientific) at 510 nm. The β-glucan content was calculated in dry weights and values of each sample were presented as mean ± standard deviation.

2.2 Weather conditions during the experiment

Air temperatures and precipitation during the study growing periods, which were collected from a weather station near the trial site, were fluctuating (Tables 1, 2). Tables represents the data from January to December from 2014 to 2021. The period between 2014 and 2017 was relatively colder (with an average temperature of 13.1°C) whereas the period between 2018 and 2021 was warmer (with an average temperature of 14.5°C). Precipitation was higher in the period of 2014–2017 (an average of 322 mm) when compared with the period of 2018–2021 (an average of 276 mm).

2.3 Statistical analysis

All β-glucan measurements were performed in two replicates and the results were expressed as means ± standard error. Results were analyzed using one-way and factorial analysis of variance (ANOVA), where factors were treated as fixed effects with the statistical software package Statistica version 13 (TIBCO Software Inc, USA). In case of significant ($P < 0.05$) differences, Fisher's least significant difference test (LSD *post-hoc* test) determined the differences among means. Pearson's correlation coefficient R was calculated for measuring the strength of a linear association between variables studied.

3 Results

3.1 Factors affecting the β-glucan content in barley and wheat kernels

3.1.1 Year conditions

Of the two factors investigated in the Eerika field experiment, only the year (weather) factor significantly influenced the β-glucan content of barley and wheat grains ($R = 0.64$, $p < 0.001$; $R = 0.62$, $p < 0.001$, respectively; Figures 1, 2). The proportion of variance was 90% for year and 2% for N treatment.

Mean β-glucan content of spring barley "Anni" over N treatments in different trial years ranged between 3.74 and 5.60 g 100 g^{-1}, whereas the same data for winter wheat "Fredis" were 0.40–0.57 100 g^{-1}, respectively. It should be taken into account that at this particular time there were two crop rotations, the first in 2014–2017 and the second in 2018–2021. In general, the second rotation was much warmer and the β-glucan content in the grains was much higher for barley (4.41 and 5.12 g 100 g^{-1} for 2014–2017 and 2018–2021, respectively) and also for wheat (0.49 and 0.56 g 100 g^{-1} for 2014–2017 and 2018–2021, respectively).

The content of β-glucan of wheat correlated negatively with the sum of precipitation in June and July (i.e., in the flowering and grain filling period of wheat; Figure 3A) and positively with air temperature in grain filling period ($R = -0.23$, $p < 0.05$; $R = -0.35$, $p < 0.01$ and $R = 0.28$, $p < 0.05$, respectively). Barley "Anni" β-glucan content correlated negatively with the sum of precipitation in June (flowering stage of barley; $R = 0.67$, $p < 0.001$) and July (grain filling period, $R = 0.85$, $p < 0.001$; Figure 3B). β-glucan content of barley correlated also negatively with air temperature in May (tillering and stem elongation stage of barley; $R = 0.48$, $p < 0.01$; Table 4).

3.1.2 Crop production system and N treatment

Data analysis of 8 years of results showed that the grain β-glucan content of barley and wheat was not significantly influenced by either crop production system or N treatments. Mean β-glucan content of barley grains over trial years fluctuated in different N treatments between 4.55 and 4.80 g 100 g^{-1} and in wheat from 0.51 to 0.56 g 100g^{-1}.

TABLE 1 Average air temperatures during the experimental period and their long-term averages.

Month	Average air temperature per month, °C								
	2014	2015	2016	2017	2018	2019	2020	2021	1964–2021 average, °C
January	−8.1	−1.9	−9.2	−3.4	−2.3	−6	2.5	−4.2	−5.4
February	−0.3	−1	0.3	−2.9	−7.6	−0.3	1.1	−6.9	−5.3
March	2.2	2.6	0	1.4	−3.4	1.4	2.3	0.1	−1.2
April	6.5	5.4	6.1	3.4	7.2	7.7	4.8	5.3	5.0
May	12.0	10.3	14.0	10.4	16.0	11.4	9.5	10.9	11.4
June	13.4	14.2	15.9	14.0	15.9	18.6	18.4	19.8	15.4
July	19.4	15.7	17.9	16.0	20.8	16.4	16.3	22.2	17.4
August	16.7	17.0	16.1	16.7	18.8	16.7	16.8	15.8	16.0
September	12.1	12.6	12.3	12.2	14.3	11.8	13.8	10	11.1
October	5.2	4.6	4.1	5.2	7.4	7.1	8.9	7.6	5.8
November	1.4	3.6	−1	2.4	2.4	2.6	4.2	2.4	0.7
December	−1.6	2.4	−0.3	0.2	−2.7	1.8	−0.7	−6.5	−3.1

TABLE 2 Monthly precipitation during the experimental period and their long-term averages.

Month	Sum of precipitation per month, mm								
	2014	2015	2016	2017	2018	2019	2020	2021	1964–2021, mm
January	25.0	29.6	34.0	27.4	20.4	16.4	19.8	19.0	32.6
February	12.4	8.4	55.8	22.4	11.7	36.0	64.0	16.6	27.2
March	9.0	12.0	23.3	17.0	12.9	35.2	33.6	23.2	26.7
April	13.4	69.0	51.6	51.5	28.1	3.2	49.6	29.4	32.5
May	83.8	62.0	1.6	15.5	7.8	59.8	32.2	107.8	53.3
June	103.4	39.4	124.6	94.3	60.8	50.8	117.4	18.5	72.4
July	71.4	61.4	81.6	60.7	14.0	40.8	68.8	17.4	67.2
August	113.0	41.2	42.0	106.2	59.3	58.0	64.2	216.6	79.6
September	22.2	59.0	15.4	83.4	72.1	75.2	45.2	58.2	59.6
October	35.8	10.8	33.2	75.3	55.3	82.4	50.2	43.0	57.5
November	10.4	53.8	45.5	26.1	18.9	59.8	36.8	59.2	48.7
December	41.6	46.3	30.6	51.8	36.9	32.8	31.5	23.0	40.5

3.1.3 Genotype

β-glucan content of barley "Anni" measured in the Eerika field experiment varied between 3.53 and 5.85 g 100 g^{-1}. Such a large variation in the results was caused by different weather conditions and crop production systems (fertilizing with organic or mineral fertilizers) during the experimental period. Genotype of barley influenced the content of β-glucan in the Jõgeva field significantly, whereas the lowest value was obtained from "Maali" kernels and the highest from "Anni" kernels (Table 3). Differences between varieties reached up to 1.51 g 100 g^{-1}.

β-glucan content in wheat kernels measured was ∼10 times lower than that of barley values. β-glucan content of wheat "Fredis" in the Eerika field fluctuated during eight trial years between 0.32 and 0.63 g 100 g^{-1}. As with barley, genotype also affected wheat β-glucan values in the Jõgeva field experiment. Differences between wheat varieties reached up to 0.14 g 100 g^{-1}, which was a relatively small difference, but still significant nonetheless.

The experiment showed that the effect of location on the β-glucan content of barley variety "Anni" was significant ($p < 0.001$; Table 3). We compared the β-glucan content of "Anni" variety obtained from the Eerika field with the data from the Jõgeva field (β-glucan values in 2021 at the same level of N quantities – 100 kg ha^{-1}). Mean β-glucan values over repetitions were 5.85 ± 0.366 and 4.51 ± 0.192 g 100 g^{-1} in Eerika and Jõgeva fields, respectively. Higher 1,000 kernel weight values of barley resulted in higher β-glucan values (Figure 4).

The 1,000 kernel weight (TKW) values of barley and winter wheat cultivars were not significantly influenced by genotype. The TKW values of barley cultivars ranged between 37.6 ("Anneli") and 43.6 ("Tuuli") g, and for winter wheat cultivars between

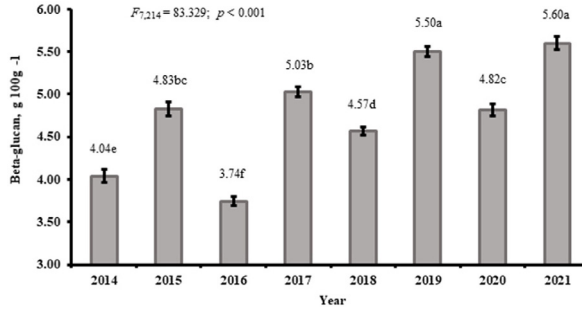

FIGURE 1
β-glucan content as an average of N treatments in spring barley "Anni" from Eerika field in different experimental years, g 100 g^{-1}. Lower-case letter on the bars refer to significant differences ($P < 0.05$).

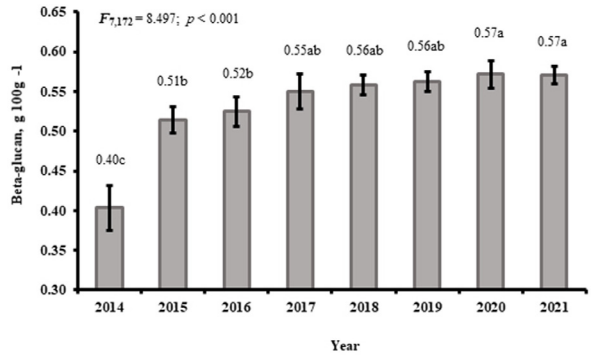

FIGURE 2
β-glucan content as an average of N treatments in winter wheat "Fredis" from Eerika field in different experimental years, g 100 g^{-1}. Lower-case letter on the bars refer to significant differences ($P < 0.05$).

37.6 ("Perenaise") and 45.2 ("Julie") g. Test weight of different spring barley and winter wheat cultivars also was not influenced by genotype; it ranged between 62 ("Anneli") and 68 ("Grace") and 75 ("Creator") and 83 ("Ada") kg hl^{-1}, respectively. One thousand kernel weight values of barley "Anni" from Jõgeva (treatment N90) and Eerika (treatment N100) experimental field in 2021 were 40.3 ± 0.22 g and 44.0 ± 0.32 g, respectively. The β-glucan content of barley grains was positively correlated with grain TKW (Figure 4) and test weight values ($R = 0.64$, $R = 0.54$, respectively, $p < 0.001$, for both values), while the β-glucan content of winter wheat "Fredis" was not affected by either TKW or test weight (4).

4 Discussion

The quantity and quality of β-glucans are affected by fertilization, genotypes and their interaction with environment, including year and location (Güler, 2003b; Dvončová et al., 2010; Choi et al., 2020; Habiyaremye et al., 2021). Realization of β-glucan benefits requires an understanding of how those factors affect β-glucan concentration and how this variation affects the biological activity of cereals (Dickin et al., 2011). In order to optimize the quality of cereal grain, we should not only consider the traditional quality but also β-glucan concentration that may greatly benefit people facing high levels of blood-serum cholesterol.

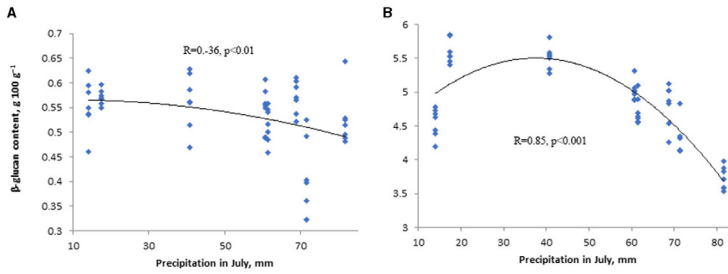

FIGURE 3
The influence of precipitation in grain filling period on β-glucan content of winter wheat "Fredis" **(A)** and spring barley "Anni" **(B)** of Eerika field.

TABLE 3 Content of β-glucan (g 100 g^{-1}) in spring barley and winter wheat genotypes in 2021 from Jõgeva field and Eerika field.

Barley genotype	β-glucan (g 100 g^{-1})	Wheat genotype	β-glucan (g 100 g^{-1})
Maali	3.14 ± 0.03 d*	Ada	0.47 ± 0.03 b
Grace	3.26 ± 0.21 d	Creator	0.53 ± 0.05 a
Planet	3.36 ± 0.17 cd	Perenaise	0.55 ± 0.01 a
Feedway	3.37 ± 0.13 cd	Širvinta	0.55 ± 0.01 a
Tuuli	3.46 ± 0.07 cd	Julie	0.55 ± 0.00 a
Breustar	3.49 ± 0.13 cd	Fredis	0.55 ± 0.05 a
Irina	3.78 ± 0.18 bcd	Kallas	0.55 ± 0.03 a
Quench	3.52 ± 0.15 bc	Fredis (Eerika field)	0.55 ± 0.02 a
Anneli	3.92 ± 0.18 bc		
Anni	4.51 ± 0.19 b		
Anni (Eerika field)	5.85± 0.29 a		

*Mean values (averaged from replicates in the same field and same year) are presented with ±SE.
n: different lowercase letters in the columns indicate significant differences, values with the same letter are not significantly different ($P < 0.05$).

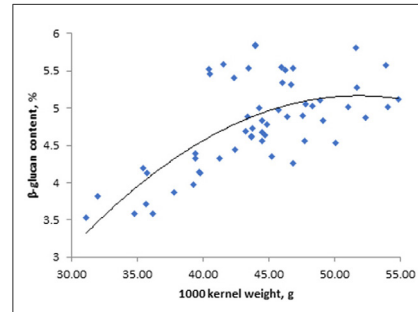

FIGURE 4
Relationship between 1,000 kernel weight and β-glucan content in spring barley "Anni" from Eerika field; R = 0.64, $p < 0.001$; $n = 55$.

In the present experiment, β-glucan content was influenced by year (weather) conditions including air temperature and precipitation (Table 4). Previously, we showed that weather conditions had a greater impact on the content of arabinoxylan, another important polysaccharide, in barley and wheat than fertilization and cropping systems within the same experimental field (Korge et al., 2023). Our current result showed that there was a negative correlation between air temperature in May and the β-glucan content of barley grains. Conditions favorable to endosperm development would increase the accumulation of β-glucan in the grain, but β-glucan content may decrease under moist growing conditions and high temperatures in this period (Goudar et al., 2020). In Estonia's climate, barley is in the tillering/elongation stage in May, therefore, lower temperatures in May (9–10°C) extend the tillering and stem elongation phase accompanied by stronger rooting, higher number of shoots and formation of larger above ground biomass. Barley plants with higher above-ground biomass take up more nutrients to transport to the head during the grain filling period, resulting in higher 1,000 kernel weight and test weight. There was a positive correlation between barley "Anni" β-glucan and 1,000 kernel weight (Figure 4) as well as test weight ($R = 0.64$, $p < 0.001$ and $R = 0.50$, $p < 0.01$, respectively). Similarly, Yalçin et al. (2007) found a positive correlation between β-glucan content and 1,000 kernel weight in hull-less barleys grown in Turkey. They showed that dense and large grains have higher endosperm content compared to smaller grains, leading to a higher content of β-glucan. Test weight (TW), which is considered an indicator of grain density and properties, had a positive correlation with β-glucan content in barley grains in our experiment (Table 4). Since grains with a higher 1,000 kernel weight have a higher test weight, the β-glucan content of larger seeds is higher. The same correlations were not

TABLE 4 Spring barley and winter wheat correlation coefficients (R) between β-glucan content and grains quality factors and environmental indicators (for Eerika field trial).

Indicator/factor	β-glucan of spring barley "Anni"	β-glucan of winter wheat "Fredis"
Trial year	0.64***	0.62***
N treatment	Ns	Ns
Temperature in tillering stage, °C	−0.48***	Ns
Precipitation in grain filling period, mm	−0.57***	−0.35**
TKW, g	0.64***	Ns
TW, g l^{-1}	0.54***	Ns

TKW, thousand kernel weight; TW, test weight.
**$p < 0.01$; ***$p < 0.001$. Ns, not significant.

significant for wheat. This may be due to the fact that β-glucan is biosynthesized mainly in the endosperm of barley grains whereas in wheat grains, it is biosynthesized in the aleurone layer (Sapirstein, 2016).

A negative correlation between the β-glucan content of barley and wheat grains and precipitation in June and July (Table 4) indicates that precipitation during the grain-filling period decreases the β-glucan content. While Choi et al. (2020) demonstrated a positive effect of rainfall on grain β-glucan content, current results are in accordance with Ehrenbergerová et al. (2008), who found that higher precipitation during barley flowering and grain filling decrease grain β-glucan content. Since β-glucan accumulates in the late stage of grain development, stress resulting from temperature and precipitation, which causes the end of early grain development, also reduces β-glucan concentration (Zhang et al., 2001; Tiwari and Cummins, 2009). The timing of water availability influences synthesis and deposition of main storage substances and their relative proportions in the grain (Havrlentová et al., 2023). The content of β-glucan in wheat grains was comparable in all studied years, except for 2014. High precipitation in June, July, and August (until harvesting) may be the reason for the low content of β-glucan in the year 2014. The lowest β-glucan content of barley grains was found for the year 2016, where the sum of precipitation for June and July was the highest among all studied years (2014–2021) and the highest for the year 2021, when we had the lowest precipitation in June and July compared to all other studied years. In Estonia's climate, barley is in flowering and grain-filling stages in June and July, respectively. Therefore, higher precipitation during this period can induce a dilution effect, which increases the water content in the grains and reduces the viscosity of the acid flour extract, leading to a decrease in β-glucan content (Aastrup, 1979; Güler, 2003a,b). In contrast, precipitation during the flowering time can lead to a reduction of the yield by disturbing the pollination process and decreasing the quality of grains including their β-glucan content (Aastrup, 1979).

We hypothesized that the content of β-glucan increases with higher mineral nitrogen rates compared to organic fertilizers. Previously, Güler (2003a) showed that the highest β-glucan content in their two-year experiment was observed when barley was fertilized with 80 kg N ha^{-1}, which was the maximum N rate in their experiment. Our result showed that, in barley and wheat, fertilization up to 150 kg N ha^{-1} did not have a significant effect on β-glucan content throughout the 8 years (Table 4). Similarly, to our result Habiyaremye et al. (2021) did not find any effect of N fertilizers on barley β-glucan content in their three-year experiment with N rates varying from 0 to 162 kg ha^{-1}. Although the β-glucan content of wheat grains is significantly less than barley grains, this result suggests that both crops can produce high β-glucan content without adding high mineral fertilizers into the soil. These results provide the opportunity for achieving strategies of the European Commission for increasing sustainability through organic farming.

However, the content of β-glucan can be influenced by locations and growing conditions rather than fertilization (Aastrup, 1979; Hesselman et al., 1981; Zhang et al., 2001). In our experiment, this was true for the barley variety "Anni," in which β-glucan content was significantly different between the two locations (Table 3). In the crop rotation experiment, we had barley undersown with clover, which can affect the absorption of N fertilizer and make it easier and more accessible for the plant. Overall, undersowing legumes can be a beneficial practice for improving the absorption of N fertilizer and increasing the yield and quality of cereal grains (Lagerquist et al., 2022).

Genotype is another factor which significantly influences the content of β-glucan in grains of cereal (Choi et al., 2020). One reason can be that different genotypes have variations in sequence and transcript for genes engaged in the biosynthesis of β-glucan, which leads to a genotype-dependent production of β-glucan at grain development (Garcia-Gimenez et al., 2019). In addition, genotype directly affects the quality properties of grains such as 1,000 kernel weight (Yalçin et al., 2007). Similarly to Sinkovič et al. (2023) we found that β-glucan content is higher in barley grains compared to wheat and genotype has an impact on grain β-glucan content. This result shows that barley could have a greater role in human food as it can improve its physiological effect and provide health benefits (Izydorczyk and Dexter, 2008). In our experiment barley "Anni" had the highest content of β-glucan compared to varieties "Maali," "Grace," "Planet," "Feedway," "Tuuli," and "Breustar." This result indicates that the propensity of different genotypes for developing grains with higher content of β-glucan is different. Similarly, in wheat, the content of β-glucan was influenced by genotype, and variety Ada had the lowest β-glucan content compared to all other varieties. Unlike barley, we did not find a correlation between β-glucan content and 1,000 kernel weight of wheat grains in our experiment (Table 4), therefore, differences in β-glucan content may be the result of a genotype-environment interaction, which is the main factor deciding grain end use, as shown by Choi et al. (2020). Future studies should take a more detailed look on genotype and management interactions in conditions of changing climate.

5 Conclusions

The content of β-glucan in barley and wheat grains is affected mainly by year (weather) conditions in different plant growth stages, which supports our hypothesis. Lower temperatures during

tillering and stem elongation and higher temperatures during grain filling period result in higher β-glucan content in barley grains. High amounts of precipitation during flowering and grain filling period reduce the content of β-glucan in grains. Future research could use this data for model development, to provide early information to industry for adoption. As there is a positive correlation between 1,000 kernel weight and β-glucan content then the conditions favoring 1,000 kernel weight increase also the β-glucan content in barley grains. This study did not support the idea that higher mineral fertilizer rates increase grain β-glucan content. The latter means that the regulation of crop nutrition is not going to impact dietary fiber content in our everyday food. These findings suggest that β-glucan can be obtained efficiently from systems managed organically or with low inputs. Genotype and growing location were other factors influencing β-glucan amount in our experiment. The content of β-glucan was 0.6–1.4 g 100 g^{-1} higher in "Anni" compared to other barley varieties, whereas variety "Ada" had the lowest β-glucan content in wheat. However, achieving cereal grains with higher quality including higher β-glucan content requires consideration of different factors, and different strategies may be needed for different crops and varieties.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

BK: Data curation, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. KE-S: Writing – review & editing. MKo: Data curation, Formal analysis, Methodology, Supervision, Writing – review & editing. MA: Formal analysis, Writing – review & editing. KM: Methodology, Resources, Writing – review & editing. RV: Writing – review & editing. RK: Resources, Writing – review & editing. ÜT: Resources, Writing – review & editing. MKu: Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. IA: Supervision, Writing – review & editing. EL: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing.

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Dedication

This year is the 110th Anniversary of the birth of Estonia's world-famous cereal biochemist. This cereal chemistry manuscript is dedicated to Commemorate the 110 years of Perten Instruments' Founder, Harald Perten.

Conflict of interest

MKu was employed by BASF UAB.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Can intercropping alter cereal grains dietary fiber composition?

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Abstract. Barley and oats are essential cereal grains that contribute to satisfying the consumer's need for dietary fibers, namely arabinoxylans (AX) and beta-glucans (BG). Due to the increasing popularity of intercropping as an environmentally friendly and low-cost approach to sustainable intensification of agriculture and crop production, it is important to understand the relationship between mixed cropping on cereal grains' dietary fiber content. Therefore, the effect of intercropping barley and oat with vetch and field pea was studied compared to pure sown barley and oat on grain AX and BG content in two successive years (2022–2023). Our result showed that oat had consistently higher AX content while barley demonstrated greater BG content. Intercropping had an influence only on barley grains' BG content, but contrary to our hypothesis, intercropping with vetch decreased the overall concentration of BG when compared to barley pure stand, as vetch was a too competitive partner. We found that grain quality parameters, such as thousand kernel weight and test weight, were positively associated with higher BG content in barley grains. Over a two-year period, weather conditions had an impact only on BG content in intercropped treatments. Increased precipitation decreased BG content in oat and barley in 2022 compared to 2023. Both grains AX content was not affected by intercropping or annual growing conditions.

Key words: barley, oat, arabinoxylan, beta-glucan, intercropping.

INTRODUCTION

Cereals are of prime importance because of their rich nutritional benefits and potential for food production, making them the primary sources of many diets worldwide (Garutti et al., 2022). They have a high content of carbohydrates, fibers, proteins, minerals, phytochemicals, and vitamins; their consumption is linked with numerous positive effects on health (Zhu & Sang, 2017; Tieri et al., 2020). Barley and oat, two essential cereal grains, are known for their high soluble dietary fiber content, which decreases cholesterol and blood glucose levels and prevents type 2 diabetes (Lafiandra et al., 2014; Prasadi & Joye, 2020).

Arabinoxylan (AX) and beta-glucan (BG) are central dietary fibers and non-starch polysaccharides in the cell walls of barley, oat, wheat, and other cereals (Izydorczyk & Dexter, 2008). Among cereals, the highest amount of BG can be found in barley (2.5 to 11.3%) and oat (2.2 to 7.8%) (Lazaridou & Biliaderis, 2007; Maheshwari et al., 2017). Arabinoxylan content is ranged from 4 to 8% in barley and 1.6 to 6% in oat (Izydorczyk and Dexter, 2008; Zambrano et al., 2023). Recent approaches aim to increase BG and AX content in cereal to improve their nutritional values and beneficial effects on human health and well-being (Shvachko et al., 2021; Barjuan Grau et al., 2023).

The content of BG and AX in grains is influenced by different factors, including genotype (Tiwari & Cummins, 2009; Dickin et al., 2011; Takač et al., 2022), weather and environmental conditions (Lazaridou & Biliaderis, 2007; Ehrenbergerová et al., 2008; Andersson & Börjesdotter, 2011), and fertilization (Tiwari & Cummins, 2009; Dickin et al., 2011; Takač et al., 2022). Although several studies showed the mineral nitrogen fertilizer as the main factor for increasing the content of BG and AX in cereal grains (Coles et al., 1997; Noworolnik et al., 2014; Habiyaremye et al., 2021), our recent findings indicated that weather, including temperature and precipitation during the grain filling, has more significant impact than genotype and cropping systems on the content of these components (Korge et al., 2023; Khaleghdoust et al., 2024). In addition, we showed that high BG and AX content can be efficiently obtained from low-input systems.

Since the EU regulations, including the Farm to Fork strategy under the European Green Deal initiatives, have prioritized sustainable consumption and production of agricultural crops (European-Commission, 2020), the impact of cropping systems on dietary fiber should also be looked at, including BG and AX. Intercropping is an environmentally friendly and low-cost approach to sustainable agriculture and crop production intensification under the current climate change scenario (Tilman et al., 2011; Raza et al., 2021). Intercropping systems maintain or increase crop productivity through the improvement of crop diversity. This, in turn, enhances natural ecosystem services and reduces the need for agrochemical applications such as pesticides and fertilizers (Brooker et al., 2015; Iqbal et al., 2019).

Intercropping systems' effectiveness is shaped by species identity, richness, functional diversity, and interspecific competition (Mahaut et al., 2020). Therefore, selecting suitable crops and species used for intercropping systems to utilize different space, time, or form resources is crucial for decreasing interspecific competition and increasing complementarity interactions (Hauggaard-Nielsen & Jensen, 2005; Stomph et al., 2020). In intercropping systems, integrating cereals with legumes efficiently combines available resources, owing to facilitation and complementary processes (Singh et al., 2019; Engbersen et al., 2021; Li et al., 2023). Intercropped legumes increase atmospheric nitrogen fixation to support their growth and development, fostering complementary interactions for nitrogen utilization by cereals in intercropping systems (Hauggaard-Nielsen & Jensen, 2005; Raza et al., 2023).

It has been shown that intercropped cereals with legumes (e.g., wheat/beans, wheat/ winter pea, maize/soybean) increased cereals biomass nitrogen and grain protein content compared with those cultivated as sole crops (Naudin et al., 2010; Chapagain & Riseman, 2014; Raza et al., 2022). However, the effect of intercropping and crop combinations on nutrient uptake and its contribution to the crop's quality is inadequately understood. There is evidence that intercropping systems improve cereal grain quality

parameters, like 1,000 kernel weight compared to sole crops, due to the presence of legumes (Benider et al., 2021). Therefore, this study aimed to compare the content of AX and BG in pure sown cereals and intercropped cereal-legume systems. We hypothesized that the content of BG and AX is higher in barley and oat intercropped with vetch and field peas compared to pure stands of barley and oat.

MATERIALS AND METHODS

Experimental design

The experiment was set up in a systematic block design with four replicates of each treatment at the experimental field of the Estonian University of Life Sciences (58° 22′N, 26° 40′E). The soil type was Stagnic Luvisol (sandy loam) (World Reference Base for Soil Resources, 2014). Soil samples were collected in April in 2022 and the chemical composition was C 1.32%, N 0.11%, plant available P 116 mg kg^{-1}, plant available K 150 mg kg^{-1}, pH 6.0). The pre-crop was spring barley. The experiment included two different cropping systems (Fig. 1). In the pure-stand system, four field crops were grown, including barley (*Hordeum vulgare* `Laureate`), oat (*Avena sativa* `Symphony`), field pea (*Pisum sativum* `Casablanca`), and vetch (*Vicia sativa* `Hanka`).

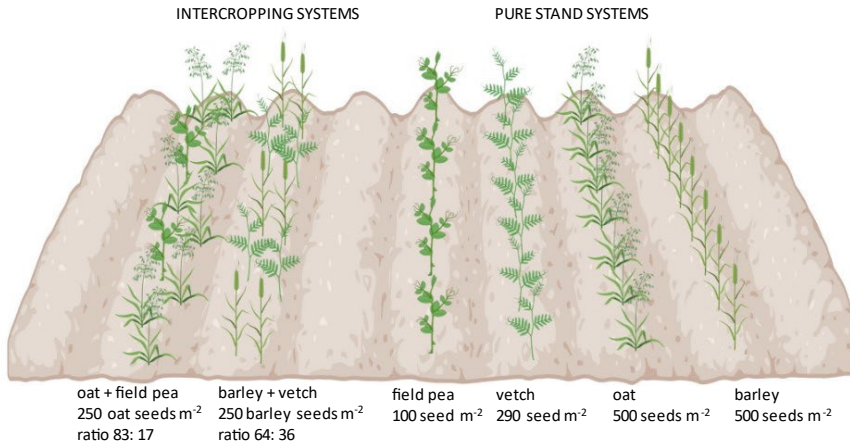

Figure 1. Experimental scheme and seeding ratios.

Sowing rates in the pure stand were 500 seeds per m^{-2} for barley and oat, 100 seeds per m^{-2} for field pea, and 290 seeds per m^{-2} for vetch. The intercropping system had two treatments, oat + field pea (in ratio of 83: 17) and barley + vetch (in ratio of 64: 36). Each plot was 10 m in length and 2 m in width. Treatments received no fertilizers (neither mineral nor organic) and no plant protection. Seeding was done with plot-seeder Wintersteiger A-4910 (Austria) in one sowing, using previously mixed seeds, with 12.5 cm row spacing. Legumes were not inoculated, as it was assumed that rhizobia was already present in the soil. In 2022, crops were sown on May 25 and harvested on August 29, except for vetch and barley in the intercropping system, which was harvested

on September 29. In 2023, crops were sown on May 12 and crops were harvested on September 6. However, intercropped barley+ vetch was not harvested due to the continuous growth of vetch. Seeds were harvested with a small experimental harvester Sampo, and seeds from intercropped systems were separated manually after harvest. Oat and barley yields were determined on the basis of a 20 m^{-2} test plot per t ha^{-1} (header width of the combine was 2 m).

Fiber and grain quality measurements

Barley and oat grains were milled using a Perten Laboratory Mill 3100 (Perten Instruments AB, Sweden). The content of BG in barley (N = 16) and oat (N = 6) was determined using a Mixed-linkage BG assay kit (Megazyme, Ireland) according to the method published by (Limberger-Bayer et al., 2014), which is accepted by the Association of Official Analytical Chemists International (AOACI; Method 995.16) and the American Association of Cereal Chemists International (AACCI; Method 32-23). Briefly, 100 mg of samples were suspended and hydrated in a sodium phosphate buffer (pH 6.5), incubated with purified lichenase enzyme, and an aliquot of filtrate was reacted with purified beta-glucosidase enzymes. The glucose product was assayed using an oxidase/peroxidase reagent. The BG content was measured in barley and oat parallel assays using a Clariostar (BMG Labtech, Germany) absorbance microplate reader at a wavelength of 510 nm. The BG content was calculated in dry weights, and values of each sample were presented as mean ± standard deviation. The content of AX in milled samples was determined enzymatically with the microplate assay procedure for d-xylose (Megazyme International, Ireland Ltd) in triplicate using the same microplate reader at a wavelength of 340 nm. The d-xylose percentage used to calculate the AX content of samples was according to literature-based values of 1% for oat and 0.75% for barley, which is a conservative assumption to avoid exaggerating the results and provide lower than the highest possible results.

Grain quality parameters, such as 1,000 kernel weight and test weight, were determined using seed counter Contador (Pfeuffer, Germany) and test weight meter Perten Aquamatic 5200 (PerkinElmer, Inc., USA), respectively.

Weather during the experiment

During crop growth periods in our study, air temperatures and precipitation were monitored at a nearby (2 km from the experiment) weather station iMetos 3.3 (Pessl Instruments, Austria), and the data was presented in Fig. 2. The figure shows the collected data from April to September 2022 and 2023. The average temperatures for these two years were relatively similar (14.1 °C in 2022 and 14.2 °C in 2023) and higher than the average for the long term (13.1 °C). When the first months of the growing periods were cooler in 2022 (April 4.5 °C and May 10.3 °C) compared to 2023 (April 7.2 °C and May 11.4 °C), then months from June to August were warmer in 2022 (June 17.5 °C, July 18.1 °C and August 20.1 °C) compared with 2023 (June 16.9 °C, July 16.9 °C and August 18.9 °C). The sum of precipitation in 2022 and 2023 was 340 and 200 mm, respectively. Precipitation in May and June was much lower in 2023 (10 mm and 27 mm) compared to 2022 (46 mm and 58 mm) and 60 years average 52 mm and 71 mm). Precipitation in July of 2022 was extremely high (145 mm) due to heavy rain on July 16th (83 mm) and similar in 2023 with long-term averages (69 mm and 69 mm, respectively). September of 2022 was cooler compared to long-term average and it was

atypically dry month. In the second year (2023) we had atypically warm September (temperature was comparable to August) with close to average amount of precipitation.

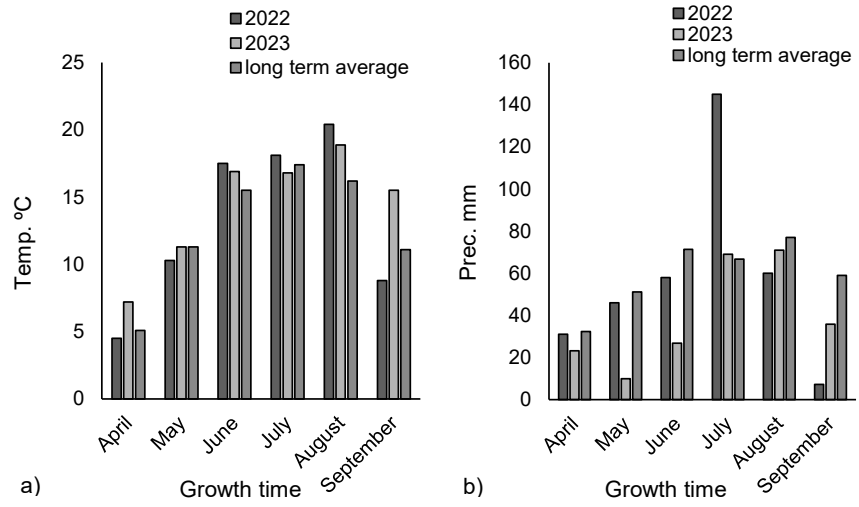

Figure 2. (a) Average air temperatures °C and (b) monthly precipitation mm during experimental period and their long-term (60 years) averages.

Statistical analysis

BG and AX were measured in three replicates, and results were presented as means ± standard deviation. Statistical analysis was performed using one-way and factorial analysis of variance (ANOVA) using the software package Statistica version 13 (TIBCO Software Inc, USA). Fisher's least significant difference test (*LSD post-hoc test*) was used to determine the differences among means ($P < 0.05$). Pearson's correlation coefficient (R) was calculated to evaluate the strength of the linear association between the variables studied.

RESULTS

Crops and cropping type impact on BG and AX in barley and oat

The AX content was significantly different based on the crop type, with oat showing a considerably higher content of AX (5.00–8.08 g 100 g^{-1}) than barley (3.14–5.20 g 100 g^{-1}) (Fig. 3), regardless of pure stand or intercropping. The difference between oat and barley in their BG concentration was less (12% on average) than between their AX values (33% on average). Barley showed significantly higher BG content compared to both oat treatments. At the same time, the BG of barley intercropped with vetch was similar to the oat treatment. Barley BG content (2.56–3.74 g 100 g^{-1}) in the intercropping system was similar to oat (2.26–3.66 g 100 g^{-1}) in the same system. Cropping type did not affect the AX concentration of cereal grains. Similarly, to AX, oat BG content did not differ between its pure stand and intercropping treatment, but at the

same time, barley from pure stand showed significantly higher (3.25 g 100 g^{-1}) BG concentration compared to barley from mixed cropping (2.90 g 100 g^{-1}); proportion of variance for barley cropping type was 38% (Fig. 3).

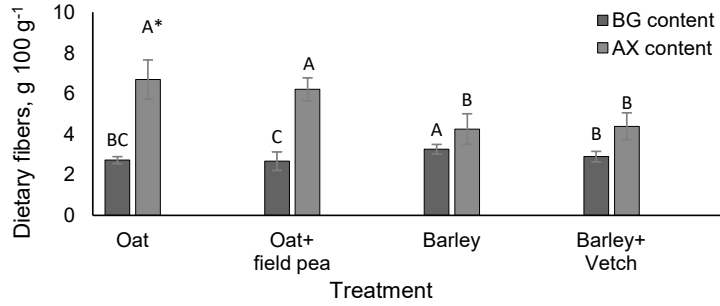

Figure 3. Average BG and AX contents (g 100 g^{-1}) in all treatments.
*Different letters on the vertical bars denote significant differences between treatments of the same fiber.

Effect of the year on BG and AX in barley and oat

Both grain's AX content was similar in both years in both cropping types (Table 1). Also, the BG content was similar in both years in pure stands, but it differed significantly between intercropped treatments. In 2022, the barley in a mixture with vetch showed a significantly lower amount of BG (2.68 g 100 g^{-1}) compared to the same treatment in 2023 (3.10 g 100 g^{-1}). The oat in mixture with field pea in 2023 showed a significantly higher amount of BG (2.66 g 100 g^{-1}) compared to 2022 (2.34 g 100 g^{-1}).

In 2022, the grain yield for barley was similar to 2023 (Table 1). In contrast, oat showed an opposite trend, with a yield of 1.71 t ha^{-1} in 2022 increasing significantly ($p < 0.05$) to 2.50 t ha^{-1} in 2023. Oat yield in mixture was also higher in 2023 being statistically significant. Average yield of barley was 1.94, which is less than Estonian average (3.0 t ha^{-1}), while pure-sown oat average yield was 2.10 t ha^{-1}, which is equal to Estonian average. However, the intercropped oat average yield was 1.39 t ha^{-1}, which was 43% less compared to pure-sown system.

Table 1. Grain yields and their BG and AX g 100 g^{-1} content in treatments in both experimental years

Cropping type	Year	Grain yield, t ha^{-1}	BG	AX
Barley	2022	1.99 ± 0.18B**	3.16 ± 0.09^{a*}	4.65 ± 0.27^{b}
Barley	2023	1.89 ± 0.09B	3.35 ± 0.14^{a}	3.84 ± 0.38^{b}
Barley + vetch	2022	1.21 ± 0.06b	2.68 ± 0.04^{b}	4.18 ± 0.12^{b}
Barley+ vetch	2023	-	3.10 ± 0.09^{a}	4.58 ± 0.46^{b}
Oat	2022	1.71 ± 0.05B	2.62 ±.0.10^{b}	6.71 ± 0.49^{a}
Oat	2023	2.50 ± 0.13A	2.80 ± 0.05^{b}	6.65 ± 0.55^{a}
Oat+ field pea	2022	0.98 ± 0.04b	2.34 ±0.33^{c}	6.44 ± 0.16^{a}
Oat+ field pea	2023	1.80 ± 0.15a	2.66 ±0.10^{b}	5.96 ± 0.35^{a}

*Different letters in columns denote significant differences between treatments; **Significant differences in grain yield of pure stand and intercropping systems are indicated by uppercase and lowercase letters, respectively.

Additionally, barley BG showed a strong positive correlation ($R = 0.83$; $p < 0.001$) with TW (test weight), 1,000 KW (1,000 kernel weight) ($R = 0.82$; $p < 0.001$), yield ($R = 0.63$; $p < 0.001$), and moderate correlation with the year ($R = 0.52$; $p < 0.05$) (Fig. 4). There was no correlation between oat BG and 1,000 KW nor between oat BG and yield. Only barley 1,000 KW correlated with its yield ($R = 0.50$, $p < 0.001$). Barley AX and oat fibers did not correlate with the parameters measured.

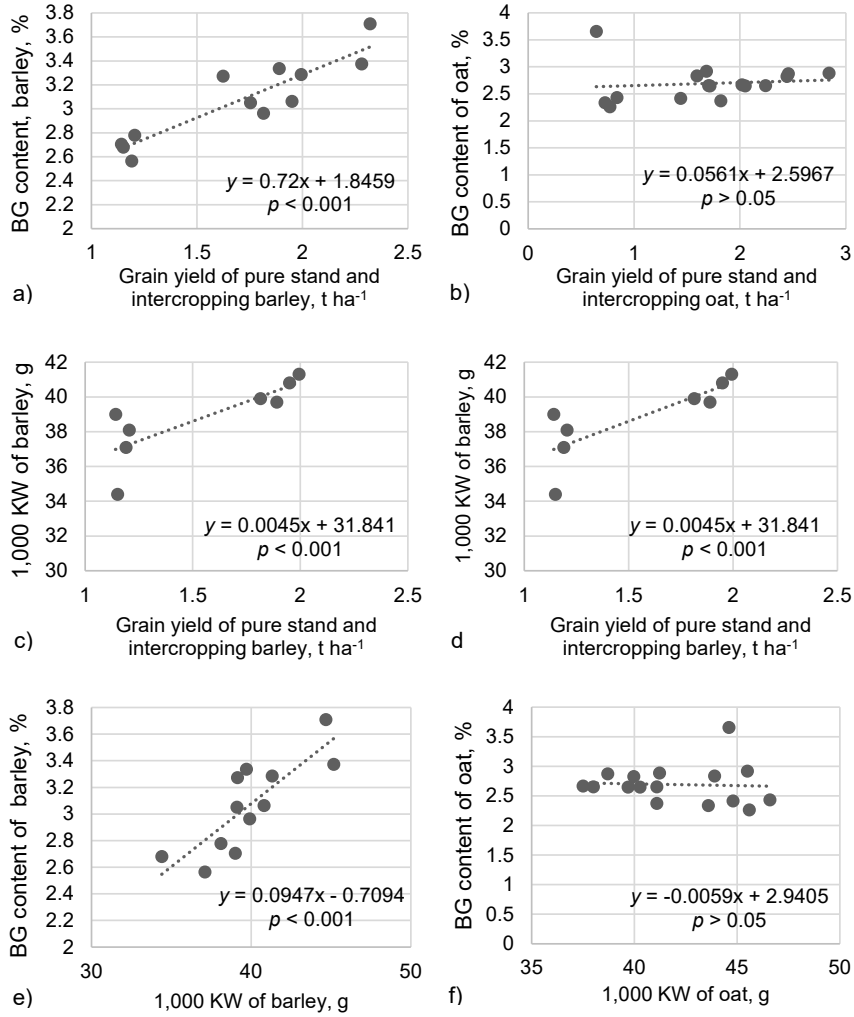

Figure 4. BG content in barley (a) and oat (b) depending on grain yields; 1,000 kernel weight of barley(c) and oat (d) depending on grain yields; BG content of barley (e) and oat (f) seeds depending on 1,000 kernel weight.

In two-year average 1,000 KW (41.2 and 37.2 g respectively) and TW (58.2 and 51.0 kg hl^{-1}, respectively) of barley in pure stand and intercropping system were significantly different, as was also 1,000 KW between oat and oat+ field pea treatments (40.5 and 43.6 g respectively) (Fig. 5). When comparing these values between years then, 1,000 KW was similar in all treatments (barley +vetch 1,000 KW was not measured in 2023).TW was significantly higher in oat pure stand and oat+ field pea in 2023 compared to 2022, while for barley pure stand TW was similar.

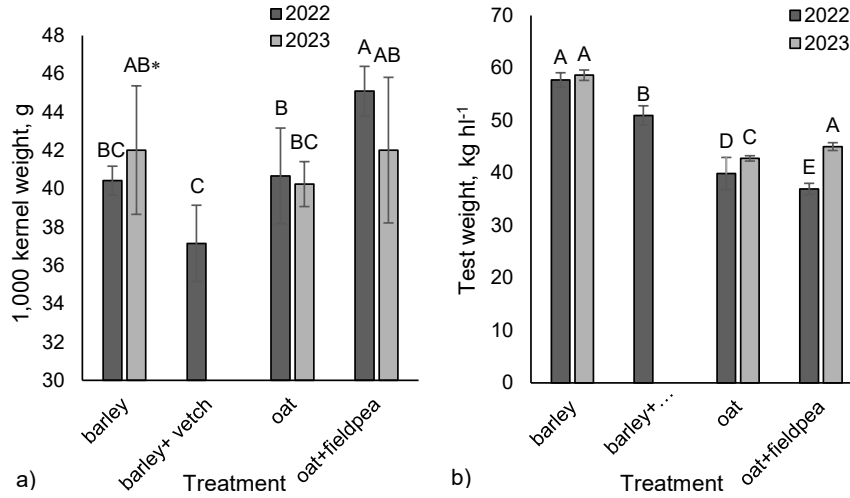

Figure 5. Comparison between (a) 1,000 kernel weight (g) and (b) test weight (kg hl^{-1}) in each treatment in experimental years.

*Different letters on the vertical bars denote significant differences between treatments of the same parameter measured.

DISCUSSION

In addition to expected differences in BG content between crops, where there was a higher concentration of BG in barley grains compared to oat grains, as has been stated before (Lazaridou & Biliaderis, 2007), there appeared also the effect of cropping type on the grain's BG content. Contrary to our hypothesis, barley grains grown in a mixture with vetch had a lower overall concentration of BG when compared to that in barley pure stand. Barley is generally considered to be one of the least competitive cereals in mixtures (Hauggaard-Nielsen and Jensen, 2005), and thus the aggressive growth of vetch in the intercrop resulted in lower quality parameters of barley (Alaru et al., 2024), including BG content.

The production of BG in barley and oat grains occurs in the endosperm, as noted by Guleria et al. (2015). The grain quality factors such as yield, 1,000 KW, and TW, which measure the weight and density of the grains, are related to the endosperm in both crops. Therefore, a larger and denser endosperm will result in heavier and denser grains, leading to higher yield, 1,000 KW, and TW values. In our experiment, barley yield,

1,000 KW, and TW were positively correlated with barley BG, indicating that higher yield, 1,000 KW, and TW are associated with higher BG content in barley. Barley yield and BG had a positive relationship. As 1,000 KW and yield were positively correlated, it can be assumed that higher 1,000 KW also increased yield and BG. The average yield of barley (1.94 t ha^{-1}) was less than national average over the years (3.0 t ha^{-1}). The extreme drought in 2023 May and June had negative impact on barley yield and even the national average was only 2.2 t ha^{-1}. Total sum of precipitation from May to August was only 177 mm (Fig. 2). However, given the zero-fertilizer system, this yield outcome can be considered good.

Barley is considered to be good at tillering (Lepajõe, 1986), but in this experiment, it had fewer tillers in the intercrop. One reason for this could have been that barley has weak competitiveness compared to other cereals (Hauggaard-Nielsen et al., 2001), and it had fewer grains in the ear when grown in a intercrop with vetch. Our finding is supported by Yalçın et al. (2007), who reported a positive correlation between grain quality and BG content in barley. In our experiment, the 1,000 KW and TW of intercropped barley were lower than the pure stand. As they were in a strong positive correlation and 1,000 KW and TW are influenced by stress on plant growth, it could be reasoned that cereal plants in intercrop were experiencing adverse growing conditions. Also Yalçın et al. (2007) showed lower barley 1,000 KW in barley+ vetch (ratio 60:40) treatment when compared to pure stand. They also found lower NDF (neutral detergent fiber) in the mixture compared to the pure stand. As NDF also represents hemicelluloses, their finding could be related to ours, stating that intercropping could lower the fiber value of cereals in intercropped systems. Further research is required to estimate the value of intercropped systems for animal feed.

In addition, AX content in grains depended on crop species, with oat having a higher AX content compared to barley. Grain AX content was not affected by cropping type, as its values were similar between pure stand and intercropping, and it was not connected with grain quality parameters. In this trial, the establishment of pea in the sward was weak, thus it is likely that the oat did not perceive stress (competition, space to grow) from co-cultivation, as also demonstrated by the higher 1,000 KW in mixed stand compared to pure and similar TW values for treatments. The unstable yield of peas was caused by its very uneven establishment (data not shown), which caused gaps in the sward and high variability in yield between replicates (Alaru et al., 2024). Oat average yield in pure-sown system was close to national average (2.1 t ha^{-1}) and lower yield in 2022 can be explained by late sowing. Above-average yield in 2023 shows high drought-tolerance of used variety `Symphony`. It has been shown to be a great variety for organic production, so further experiments can prove its suitability to climatic conditions with increasing drought. Lower yield in intercropped system was expected due to lower seeding rate. Oat yield and 1,000 KW correlation were negative due to the fact that yield increased mainly due to the higher number of grains per plant (data not shown). There was no correlation between oat yield, 1,000 KW, and BG. The difference could be explained by the fact that in oat, BG is found mainly in the outer layer of the endosperm, which is a marked difference from barley, which contains BG uniformly throughout the endosperm (Kale et al., 2014).

While annual weather did not change the content of BG in pure stands of barley and oat, it had significant influence on BG content of cereals in intercropping. The BG content in both crops grown in a mixed cropping system was significantly lower in the

year 2022 compared to 2023. As grain's BG content is in a negative relationship with precipitation (Khaleghdoust et al., 2024), much higher precipitation in 2022 caused by heavy rain in July (grain filling period) when compared to 2023 probably caused the lower content of BG in barley and oat grains in intercropping in 2022. Stress from weather conditions can interrupt grain development and BG accumulation and reduce grains' BG concentration (Zhang et al., 2001; Tiwari & Cummins, 2009). Although not significantly different, BG content showed a similar trend in oat and barley pure stands between the years 2022 and 2023. Previously, we have shown that BG content is influenced by air temperatures at the beginning of the growing period (Khaleghdoust et al., 2024), but as temperature in May (period of tillering and stem elongation of cereals) of 2022 and 2023 was quite similar (10.3 and 11.4 °C), this relationship did not appear.

There were also no differences in AX value between the two experimental years. As we have found earlier (Korge et al., 2023), the AX content in barley grains depends on the temperature during grain filling period, preferring higher temperatures. In the years 2022 and 2023, temperature in July was similar, (18.1 and 16.9 °C, respectively) and therefore, AX values were also stable.

Table 2. Ranking of factors affecting BG and AX content as correlation coefficients

Factor	Barley		Oat	
	BG	AX	BG	AX
TW*, kg ha^{-1}	0.83***	ns	Ns	ns
1,000 KW, g	0.82***	ns	Ns	ns
Yield, t ha^{-1}	0.63*	ns	Ns	ns
Intercropping	-0.62*	ns	Ns	ns
Temperature in filling period, July, C°	-0.52*	ns	Ns	ns
Precipitation in filling period, July, mm	-0.52*	ns	Ns	ns

*TW – test weight; 1,000 KW – thousand kernel weight; *** and * indicate the plausibility at 99% and 95% probability; ns – not significant.

Taking the results together we can say that intercropping had the effect only on BG content in barley (Table 2). Grain quality parameters, such as thousand kernel and test weight, were positively associated with higher BG content in barley grains. Intercropping and July weather had negative effect. Both grain's AX content was unaffected by intercropping and annual growing conditions. This is good news when considering the potential of cereal valorization via dietary fibers.

CONCLUSIONS

Our pilot study of intercropping uncovered differences in AX and BG contents within barley and oat kernels grown in pure stand and mixed with vetch and field pea, respectively. Oat consistently showed higher AX concentrations, regardless of cropping system or year. Barley had higher BG content, and intercropping affected it negatively. We found that grain quality parameters, such as thousand kernels and test weights, were positively associated with higher BG content in barley grains. Both grain's AX content was unaffected by intercropping or annual growing conditions. The most critical period impacting grains fiber content is the period of grain filling (July), where plant stress from increased precipitation can have great decreasing effect on grains BG content. Based on our results, finding a suitable partner for barley in intercropping is much more difficult than for oats, because barley is a crop with relatively weak competitiveness. Future studies can test different combinations, as well as later sowing time or lower sowing rate to find suitable means for growing barley in intercropping systems.

Overall, these findings provide valuable insight that intercropping can decrease BG content in barley, while being neutral for AX content in barley and oats alike, which is offering guidance for future agronomic strategies to improve the nutritional and functional qualities of cereal grains.

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ELULOOKIRJELDUS

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Töökohad ja ametid

11.03.2019–31.12.2023	Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, Taimekasvatuse ja taimebioloogia õppetool, nooremteadur (0,50)
01.03.2023–31.08.2023	Tartu Ülikool, Meditsiiniteaduste valdkond, bio- ja siirdemeditsiini instituut, spetsialist (0,35)
02.05.2024–14.10.2024	Maaelu Teadmuskeskus (METK), Vanemassistent (1,00)
01.03.2024–31.08.2024	Tartu Ülikool, Loodus- ja täppisteaduste valdkond, tehnoloogiainstituut, mudel- ja põllutaimede ökofüsioloogia spetsialist (0,70)
09.09.2023–29.02.2024	Tartu Ülikool, Loodus- ja täppisteaduste valdkond, tehnoloogiainstituut, mudel- ja põllutaimede ökofüsioloogia, Assistent (1,00)
11.03.2019–31.12.2020	Eesti Maaülikool, Nooremteadur (1,00)
11.03.2019–31.12.2023	Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, Taimekasvatuse ja taimebioloogia õppetool, nooremteadur (0,50)
01.03.2023–31.08.2023	Tartu Ülikool, Meditsiiniteaduste valdkond, bio- ja siirdemeditsiini instituut, spetsialist (0,35)

Teaduskraadid

Banafsheh Khaleghdoust, magistrikraad ärijuhtimises turunduse erialal, (juh) Fereydoun Abd Sabour, Teenuse kvaliteedi mõju kliendirahulolule, kordusostu kavatsusele ja suusõnalisele suhtlusele Rashti suuremates toidupoodides. Islami Azadi Ülikool, Iraan.

Haridustee

2019–2024	Doktorant, Eesti Maaülikool, Eesti.
2013–2015	Magistrikraad ärijuhtimises, turunduse eriala, Islami Azadi Ülikool, Iraan.
2005–2010	Bakalaureusekraad agronoomias ja taimearetuses, Guilani Ülikool, Iraan.

Jooksvad projektid

- PRG1949 "Saagi ja kvaliteedi stabiilsus ja jätkusuutlikkus pikaajalises külvikorrakatses" (01.01.2023–31.12.2027); Vastutav täitja: Evelin Loit-Harro; Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut; Finantseerija: Sihtasutus Eesti Teadusagentuur; Eraldatud summa: 577 200 EUR.
- PRG1620 "Õhulõhede VPD-toimeline signalisatsioon ja gaasivahetustunnuste aretuspotentsiaal saagi suurendamiseks" (01.01.2022–31.12.2026); Vastutav täitja: Ebe Merilo; Tartu Ülikool, Loodus- ja täppisteaduste valdkond, tehnoloogiainstituut; Finantseerija: Sihtasutus Eesti Teadusagentuur; Eraldatud summa: 568 050 EUR.

Lõppenud projektid

- RESTA28 "Teravilja ja õlikultuuride väärindamine" (01.02.2021–31.07.2023); Vastutav täitja: Evelin Loit-Harro, Ivi Jõudu; Eesti Maaülikool (koordinaator); Finantseerija: Sihtasutus Eesti Teadusagentuur; Eraldatud summa: 458 100 EUR.
- L180052PKTT (RITA1/01-18-17) "Lisandväärtuse tõstmine ja toorme tõhusam kasutamine Eesti biomajanduses ja selle sektorites" (01.03.2018–30.10.2021); Vastutav täitja: Evelin Loit-Harro; Eesti Maaülikool (partner); Finantseerija: Sihtasutus Eesti Teadusagentuur; Eraldatud summa: 62 709 EUR.

Juhendatud väitekirjad

- Max Kurg, magistrikraad, 2024, (juh) Evelin Loit-Harro; Banafsheh Khaleghdoust; Mailiis Korge, Beetaglükaani sisaldus suviodra terades sõltuvalt lämmastikuga väetamisest ja ilmastikust, Eesti Maaülikool

CURRICULUM VITAE

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Institutions and positions

11.03.2019–31.12.2023	Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Chair of Crop Science and Plant Biology, Junior Research Fellow (0,50)
01.03.2023–31.08.2023	University of Tartu, Faculty of Medicine, Institute of Biomedicine and Translational Medicine, Specialist (0,35)
02.05.2024–14.10.2024	Estonian Rural Knowledge Center METK, Swnior Technician (1,00)
01.03.2024–31.08.2024	University of Tartu, Faculty of Science and Technology, Institute of Technology, Specialist in Model & Crop Plant Ecophysiology (0,70)
09.09.2023–29.02.2024	Faculty of Science and Technnology, Institute of Technology, Model and Crop Plant Ecophysiology, Laboratory Assisant (1,00)
11.03.2019–31.12.2020	Estonian University of Life Sciences, Junior Researcher (1,00)
11.03.2019–31.12.2023	Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Chair of Crop Science and Plant Biology, Junior Research Fellow (0,50)

Academic degrees

Banafsheh Khaleghdoust, Master's Degree in Business Administration Marketing, (sup) Fereydoun Abd Sabour, Impact of service quality on customer satisfaction, intention to repurchase and word of mouth communication in major food stores of Rasht. Islamic Azad University, Iran.

Education

2019–2025	PhD, Estonian University of Life Sciences, Estonia.
2013–2015	M.Sc., Business Administration Marketing, Islamic Azad University, Iran.
2005–2010	B.S., Agronomy and Plant Breeding, University of Guilan, Iran.

Projects in progress

- PRG1949 "Yield and quality stability and sustainability in long-term cropping systems" (01.01.2023–31.12.2027); Principal Investigator: Evelin Loit-Harro; Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences; Financier: Estonian Research Council; Financing: 577 200 EUR.
- PRG1620 "Stomatal VPD signaling and the breeding potential of gas exchange traits to increase crop yield" (01.01.2022–31.12.2026); Principal Investigator: Ebe Merilo; University of Tartu, Faculty of Science and Technology, Institute of Technology; Financier: Estonian Research Council; Financing: 568 050 EUR.

Completed projects

- RESTA28 "Valorization of cereal and oil seed crops" (01.02.2021–31.07.2023); Principal Investigator: Evelin Loit-Harro, Ivi Jõudu; Estonian University of Life Sciences (coordinator); Financier: Estonian Research Council; Financing: 458 100 EUR.
- L180052PKTT (RITA1/01-18-17) "Maximising added value and efficient use of raw materials in bioeconomy and its sectors in Estonia" (01.03.2018–30.10.2021); Principal Investigator: Evelin Loit-Harro; Estonian University of Life Sciences (partner); Financier: Estonian Research Council; Financing: 62 709 EUR.

Supervised dissertations

- Max Kurg, Master's Degree, 2024, (sup) Evelin Loit-Harro; Banafsheh Khaleghdoust; Mailiis Korge, Beetaglükaani sisaldus suviodra terades sõltuvalt lämmastikuga väetamisest ja ilmastikust (Content of beta-glucan in spring barley grains depending on nitrogen fertilization and weather), Estonian University of Life Sciences

PUBLICATIONS

1.1. Scholarly articles indexed by Web of Science

Khaleghdoust, Banafsheh; Esmaeilzadeh-Salestani, Keyvan; Korge, Mailiis; Alaru, Maarika; Möll, Kaidi; Värnik, Rando; Koppel, Reine; Tamm, Ülle; Kurg, Max; Altosaar, Illimar; Loit, Evelin (2024). Barley and wheat beta-glucan content influenced by weather, fertilization, and genotype. Frontiers in Sustainable Food Systems, 7. DOI: 10.3389/fsufs.2023.1326716.

Alaru, Maarika; Kaasik, Riinu; Khaleghdoust, Banafsheh; Korge, Mailiis; Keres, Indrek; Möll, Kaidi; Talgre, Liina; Loit, Evelin (2024). The combined effects of weather conditions and fertilization on wheat fiber to protein ratio and on dough quality. Journal of Cereal Science, 117, 103923. DOI: 10.1016/j.jcs.2024.103923.

Samandari-Bahraseman, Mohammad R.; Esmaeilzadeh-Salestani, Keyvan; Dogani, Manijeh; Khaleghdoust, Banafsheh; Hatami, Nima; Esmaeili-Mahani, Saeed; Elyasi, Leila; Loit, Evelin; Harro, Jaanus (2024). Antidepressant- and Anxiolytic-Like Effect of the Froriepia subpinnata Extract in the Rat: Neurochemical Correlates. Brain and Behavior, 14 (11). DOI: 10.1002/brb3.70171.

Esmaeilzadeh-Salestani, Keyvan; Samandari_Bahraseman, Mohammad Rasoul; Tohidfar, Masoud; Khaleghdoust, Banafsheh; Keres, Indrek; Mõttus, Aleks; Loit, Evelin (2023). Expression of AMT1;1 and AMT2;1 is stimulated by mineral nitrogen and reproductive growth stage in barley under field conditions. Journal of Plant Nutrition, 46 (7), 1246–1258. DOI: 10.1080/01904167.2022.2067764.

Esmaeilzadeh-Salestani, Keyvan; Tohidfar, Masoud; Ghanbari Moheb Seraj, Rahele; Khaleghdoust, Banafsheh; Keres, Indrek; Marawne, Hashem; Loit, Evelin (2023). Transcriptome profiling of barley in response to mineral and organic fertilizers. BMC Plant Biology, 23, 261. DOI: 10.1186/s12870-023-04263-2.

Korge, Mailiis; Alaru, Maarika; Keres, Indrek; Khaleghdoust, Banafsheh; Moll, Kaidi; Altosaar, Illimar; Loit, Evelin (2023). The influence of cropping system, weather conditions and genotype on arabinoxylan content in wheat and barley grains. Journal of Cereal Science, 110, ARTN 103650. DOI: 10.1016/j.jcs.2023.103650.

Samandari Bahraseman, Mohammad Rasoul; Khorsand, Babak; Esmaeilzadeh-Salestani, Keyvan; Sarhadi, Solmaz; Hatami, Nima; Khaleghdoust, Banafsheh; Loit, Evelin (2022). The use of integrated text mining and protein-protein interaction approach to evaluate the effects of combined chemotherapeutic and chemopreventive agents in cancer therapy. PLoS ONE, 17 (11), e0276458. DOI: 10.1371/journal.pone.0276458.

Esmaeilzadeh-Salestani, Keyvan; Bahram, Mohammad; Ghanbari Moheb Seraj, Rahele; Gohar, Daniyal; Tohidfar, Masoud; Eremeev, Viacheslav; Talgre, Liina; Khaleghdoust, Banafsheh; Mirmajlessi, Seyed Mahyar; Luik, Anne; Loit, Evelin (2021). Cropping systems with higher organic carbon promote soil microbial diversity. Agriculture Ecosystems & Environment, 319, 107521. DOI: 10.1016/j.agee.2021.107521.

Esmaeilzadeh-Salestani, Keyvan; Riahi-Madvar, Ali; Maziyar, Mohammad Ali; Khaleghdoust, Banafsheh; Loit, Evelin (2021). COPPER ION INDUCED PRODUCTION OF ROSMARINIC ACID IN LEMON BALM (Melissa officinalis L.) SEEDLINGS. Agricultural Biology. A series of "Plant Biology" / Сельскохозяйственная биология. Серия "Биология р, 56 (3), 578–590. DOI: 10.15389/agrobiology.2021.3.578eng.

3.4. Articles/presentations published in conference proceedings

Alaru Maarika; Keres Indrek; Khaleghdoust Banafsheh; Möll Kaidi; Loit Evelin (2023). Teraviljade arabinoksülaani sisaldus sõltuvalt viljelusviisit, ilmastikust ja sordist. Agronoomia 2023. (91–102). Rebellis.

Korge, Mailiis; Khalegh Doust, Banafsheh; Alaru, Maarika; Keres,Indrek; Kurg, Max; Loit, Evelin (2022). Beetaglükaani sisaldus odra ja nisu terades sõltuvalt lämmastikväetise normist ja viljelusviisist. Maarika Alaru (Toim.). Agronoomia 2022. (224–230). Tartu: Vali Press.

5.2. Conference abstracts

Khaleghdoust, Banafsheh; Esmaeilzadeh Salestani, Keyvan; Korge, Mailiis; Kurg, Max; Varnik, Rando; Loit, Evelin (2022). Valorization of cereals: Impact of year and fertilization on Beta-glucan content in wheat and barley. European Society for Agronomy kongress, 29.08 - 2.09.2022, Potsdam, Saksamaa. Leibniz Centre for Agricultural Landscape Research (ZALF), 241.

Khaleghdoust, Banafsheh; Esmaeilzadeh Salestani, Keyvan; Korge, Mailiis; Kurg, Max; Varnik, Rando; Loit, Evelin (2022). Content of beta-glutan and arabinoxylan in barley and wheat grains depending on N fertilization rate and cropping system. 4th International Scientific Conference AgroEco2022, 26-27.10.2022, Kaunas, Leedu. Vytautas Magnus University.

Esmaeilzadeh-Salestani, Keyvan; Eremeev, Viacheslav; Talgre, Liina; Khaleghdoust, Banafsheh; Loit, Evelin (2020). Crop Rotation Outperform Organic and Conventional Cropping Systems in Structuring Soil Microbial Communities. 3rd International Scientific Virtual Conference of Agroecosystem Sustainability, Aleksandras Stulginskis University, Lithuania, Dec 2-3. AgroEco2020, 80.

VIIS VIIMAST KAITSMIST

PEETER LÄÄNISTE
RISTÕIELISTE KULTUURIDE SOBIVUS VAHEKULTUURINA, NENDE VASTUPIDAVUS HAIGUSTELE NING OPTIMEERITUD KASVATUSTEHNOLOOGIATE MÕJU TALVITUMISELE JA SAAGIKUSELE
SUITABILITY OF CRUCIFEROUS OILSEEDS AS COVER CROPS, THEIR DISEASE RESISTANCE AND OPTIMIZED CULTIVATION TECHNOLOGIES FOR IMPROVED OVERWINTERING AND YIELD
Dotsent Eve Runno-Paurson, professor Ülo Niinemets
31. detsember 2024

GIOVANNI MARCONE
LAMMASTE HEAOLU VÕRDLEV HINDAMINE ERINEVATES PIDAMISSÜSTEEMIDES
COMPARATIVE WELFARE EVALUATION OF SHEEP PRODUCTION SYSTEMS
Professor David Arney, lektor Peep Piirsalu
9. jaanuar 2025

DANIELA GHIA
INVASIIVSETE VÄHILIIKIDE KÄITUMISOMADUSTE JA ÖKOLOOGILISE MÕJU HINDAMINE MAGEVEEKOGUDES
ASSESSING BEHAVIOURAL TRAITS AND ECOLOGICAL IMPACTS OF INVASIVE CRAYFISH SPECIES IN FRESHWATER ECOSYSTEMS
Teadur Fabio Ercoli, professor Roberto Sacchi
6. veebruar 2025

SIGMAR NAUDI
JÄTKUSUUTLIKKU MESINDUST MÕJUTAVAD FAKTORID: PATOGEENIDE LEVIK JA DIAGNOSTIKA, MESILASEMADE ARETUS
FACTORS AFFECTING BEEKEEPING SUSTAINABILITY: PATHOGEN SPREAD, DIAGNOSTICS AND QUEEN BREEDING
Professor Marika Mänd, kaasprofessor Reet Karise
11. veebruar 2025

BRITT PUIDET
KARTULI-LEHEMÄDANIKU TEKITAJA *PHYTOPHTHORA INFESTANS* EUROOPA POPULATSIOONIDE MUUTUSI MÕJUTAVAD FENOTÜÜBILISED TUNNUSED
PHENOTYPIC TRAITS INFLUENCING CHANGES IN POTATO LATE BLIGHT PATHOGEN *PHYTOPHTHORA INFESTANS* POPULATIONS IN EUROPE
Professor Marika Mänd, professor Mati Koppel, Riinu Kiiker
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