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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\`).

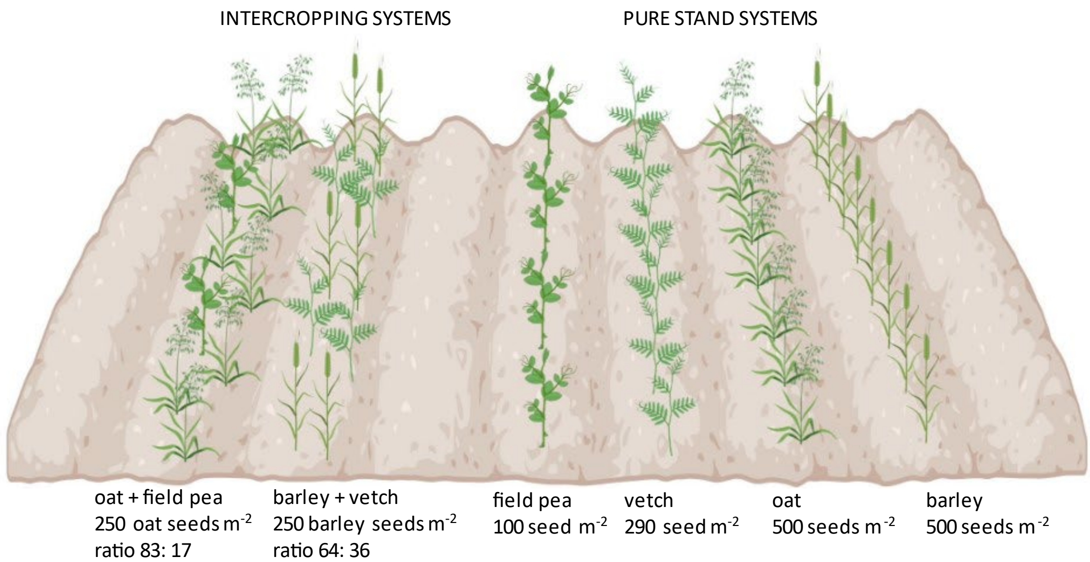

Figure 1. Experimental scheme and seeding ratios.

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.

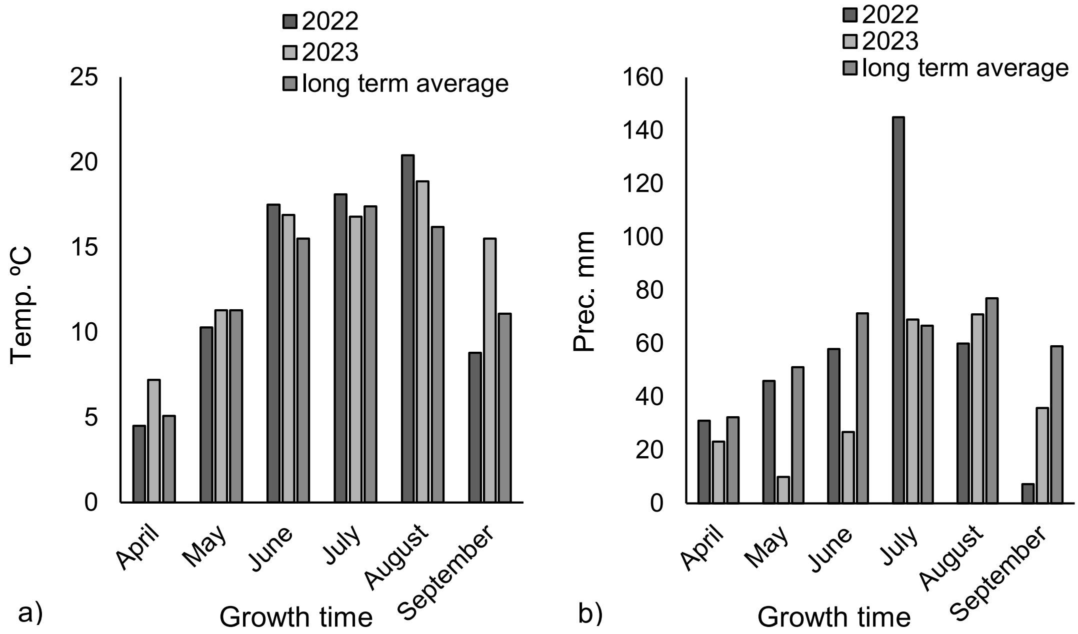

Figure 2. (a) Average air temperatures °C and (b) monthly precipitation mm during experimental period and their long-term (60 years) averages.

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).

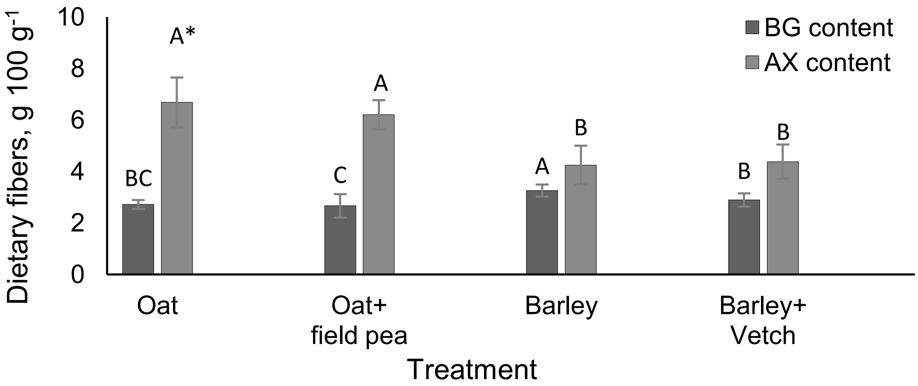

Figure 3. Average BG and AX contents (g 100 g^{-1}) in all treatments.

*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.

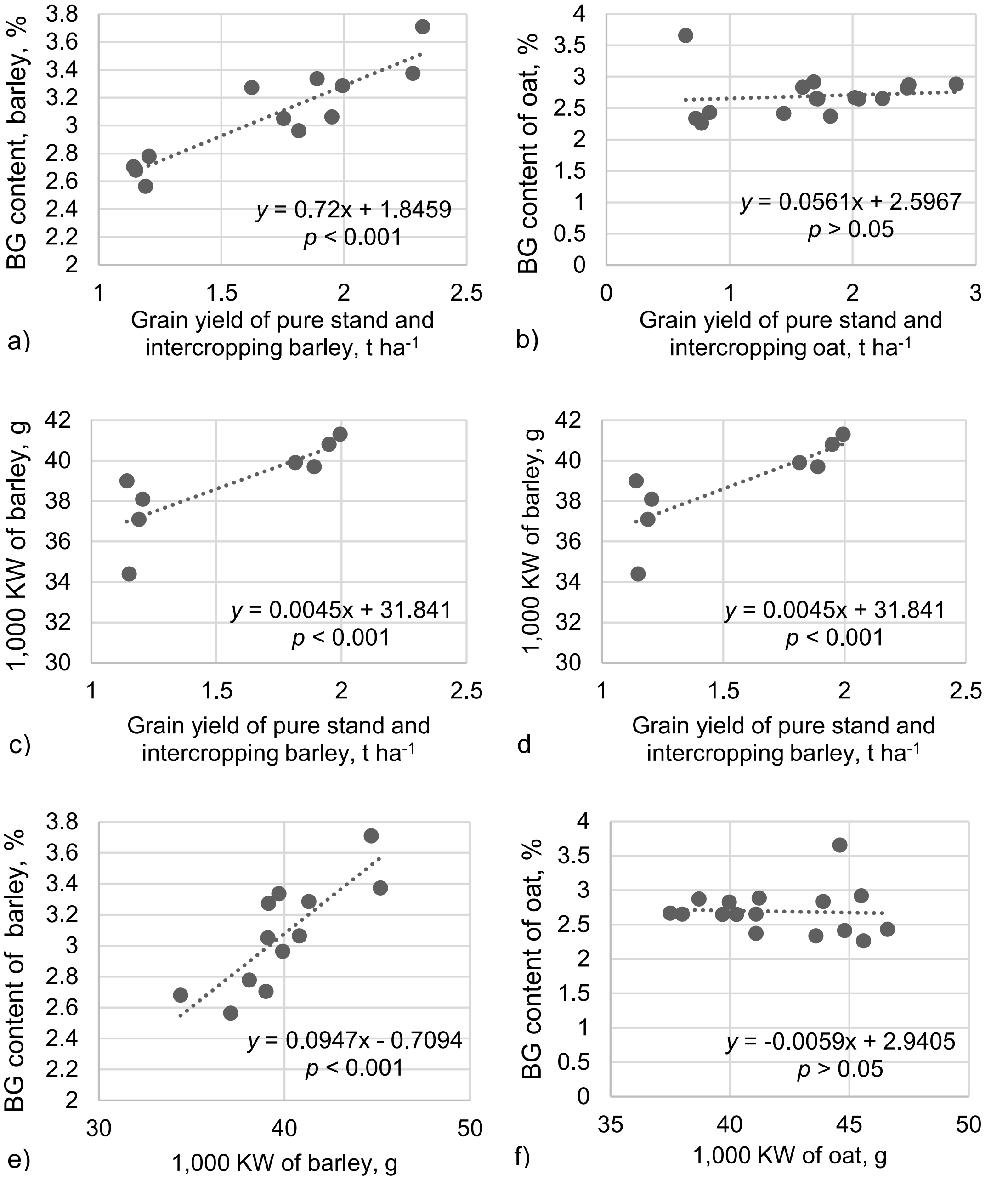

Figure 4. BG content in barley (a) and oat (b) depending on grain yields; 1,000 kernel weight of barley(c) and oat (d) depending on grain yields; BG content of barley (e) and oat (f) seeds depending on 1,000 kernel weight.

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.

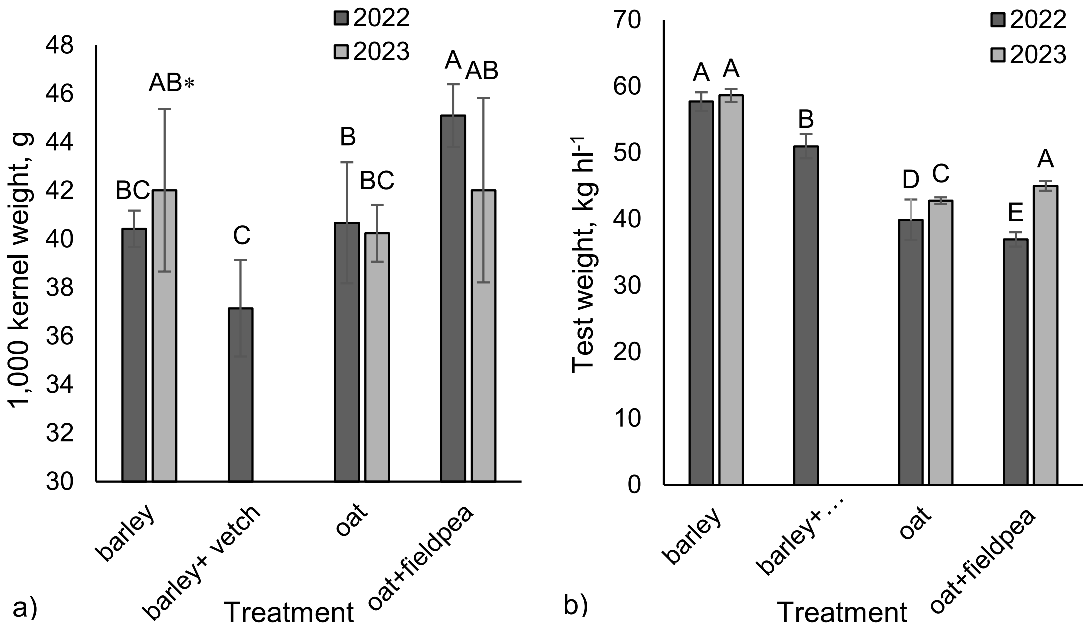

Figure 5. Comparison between (a) 1,000 kernel weight (g) and (b) test weight (kg hl^{-1}) in each treatment in experimental years.

*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.

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.

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.

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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