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Mixed cropping with lentils increases grain protein of wheat and barley

Mischanbau mit Linsen steigert den Rohproteingehalt in Weizen und Gerste

Abstract

Spring wheat (*Triticum aestivum*) and spring barley (*Hordeum vulgare*) were mixed cropped with lentils (*Lens culinaris*) in five seeding ratios (100:0, 75:25, 50:50, 25:75, 0:100) at the Experimental Station for Organic Farming Kleinhohenheim, SW Germany in the years 2009 and 2010. Aim of the study was to test the effect of different mixing ratios on seed quality (crude protein: CP, thousand kernel mass: TKM) of cereals and lentils. Seed crude protein of cereals increased significantly when their proportion was reduced in the mixture with their companion crop lentil. Wheat crude protein increased from 10.3% DM (2009) and 11.0% DM (2010) in monocropping to 11.5% DM (2009) and 15.1% DM (2010) in mixed cropping with 75% lentils. Barley crude protein increased in the same way from 13.7% DM in monocropping to 15.8% DM in mixed cropping with 75% lentils. The percentage of CP in lentils, however, did not differ significantly across all mixing ratios. The TKM of cereals and lentils increased significantly when their share in the mixture was decreased. Generally, the total crude protein yield in mixtures (one cereal crop plus lentils) was significantly higher than that in cereal or lentil monocropping. Mixed cropping with lentils can thus be an option to obtain a high protein content of wheat which is important for a suitable bread-making quality, particularly in organic farming. If barley is used for feed or food, a high protein content in mixed cropping with lentils is also welcome. On the other hand, malting barley seems not a suitable partner for a mixed cropping system with lentils as the protein content might be too high.

Key words: Mixing ratio, *Lens culinaris*, *Hordeum vulgare*, *Triticum aestivum*, bread-making quality, crude protein

Zusammenfassung

Sommerweizen (*Triticum aestivum*) und Sommergerste (*Hordeum vulgare*) wurden im Gemenge mit Linsen (*Lens culinaris*) in fünf Mischungsverhältnissen (100:0, 75:25, 50:50, 25:75, 0:100) auf der Versuchsstation für Ökologischen Landbau Kleinhohenheim (SW-Deutschland) in den Jahren 2009 und 2010 angebaut. Ziel der Studie war zu prüfen, welchen Einfluss der Mischanbau auf den Rohproteingehalt im Korn und die Tausendkornmasse hat. Der Rohproteingehalt im Getreidekorn stieg signifikant mit steigendem Linsenanteil in der Mischung. Bei Weizen nahm der Rohproteingehalt von der Reinsaat (10,3% in 2009, 11,0% in 2010) bis zur Mischsaat mit hohem Linsenanteil auf 11,5% (2009) bzw. 15,1% (2010) zu. In ähnlicher Weise stieg der Rohproteingehalt der Gerste von 13,7% auf 15,8%, während der Rohproteingehalt der Linsen bei allen Mischungspartnern und -verhältnissen unbeeinflusst blieb. Die Tausendkornmasse aller Kulturarten stieg, je geringer ihr jeweiliger Anteil an der Mischung war. Im Mischanbau war der gesamte Proteinertrag je Fläche generell höher als bei den Reinsaaten. Der Mischanbau mit Linsen kann besonders im Ökologischen Landbau eine Möglichkeit darstellen, den Proteingehalt von Weizen zu steigern und hohe Backqualitäten zu erzielen. Auch für Futtergerste ist dieser Effekt gewünscht und möglich; Braugerste scheint allerdings ungeeignet für den Mischanbau mit Linsen, und

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möglicherweise auch nicht mit anderen Leguminosen, da hier vergleichsweise niedrige Rohproteingehalte gefordert sind.

Stichwörter: Mischungsverhältnis, *Lens culinaris*, *Hordeum vulgare*, *Triticum aestivum*, Backqualität, Rohprotein

Introduction

High crude protein contents in wheat (*Triticum aestivum*) are wanted for food processing, e.g. for a good bread-making quality, or for a high nutritional value in general. Unlike wheat, which is mainly used as a staple food, barley (*Hordeum vulgare* L.) with high protein content is often used for animal feed, and barley with low protein content is wanted as malting barley for beer making. A limited crude protein content of malting barley is crucial for the malting process, the fermentation period, beer foaming ability, taste and other characteristics (Jones, 2005). Additionally, the uniformity of the barley grains and the grain size and shape are also important. The optimal crude protein in malting barley should be 9–11% (Líšková et al., 2011), or < 11.5% respectively (Aufhammer, 2003).

The seed protein content of many crops depends on the variety (Mosse and Baudet, 1983; Guarda et al., 2004), but can also be highly influenced by environmental conditions and agronomy practices (Erekul and Köhn, 2006). It is generally a challenge in organic farming to increase the grain protein of cereals because readily available, chemical-synthetical fertilizers are not permitted and thus a targeted, late application of nitrogen (e.g. at the stage of heading) is not possible. Hence, it is necessary to find alternative solutions, such as mixed cropping of cereals with legumes. Lentils (*Lens culinaris* L.) with high nutritional value are a traditional and popular food in Europe (Horneburg, 2006). The crop was neglected in farming systems particularly in Germany for many years, but has now a renaissance with increasing acreage. As lentils need a companion crop to avoid lodging under Central European conditions, cereals (mainly barley) are often intercropped with lentil. The objective of the study was to evaluate whether different mixing ratios of lentils and wheat or barley affect the seed crude protein content and thousand kernel mass of the crops.

Materials and Methods

Location, climate and soil

A two-year field experiment was carried out at the organic research station Kleinhohenheim (48° 43' N, 9° 11' E, and 435 m above sea level) of the University of Hohenheim, Southwest Germany, in 2009 and 2010. The long-term (1961–2010) annual average rainfall is 710 mm, with about 377 mm between April and August (Tab. 1). Luvisols and Cambisols are the dominant soil types of the location, with loess to sandy loamy clay textures. Characteristics of topsoil (0–20 cm) were: pH 7.0, P_2O_5 24 mg, K_2O 19 mg, and MgO 10 mg/100 g soil in 2008. Soil mineral nitrogen (NH_4-N + NO_3-N) was determined before sowing in March and was about 9 kg ha^{-1} in both 0–30 cm and 30–60 cm soil layers in 2009, or 8 and 6 kg ha^{-1} in 2010, respectively.

The research station has been managed according to the organic standards of Bioland and Naturland since 1993, and an 8-year crop rotation of grass/clover (two years), winter wheat (*T. aestivum*), oat (*Avena sativa* L.), faba bean (*Vicia faba* L.), spelt (*Triticum spelta* L.), maize (*Zea mays* L.)/potatoes (*Solanum tuberosum* L.), and triticale (× *Triticosecale* Wittm. ex A. Camus.) was performed. The current experiment with lentils was integrated in the faba bean field, therefore the preceding crop was oat in both years.

Treatments, crop management and measurements

Lentil (L, cv. Anicia) was intercropped with spring wheat (W, cv. Triso; quality class E according to the German classification system: highest bread-making quality, pro-

Tab. 1. Rainfall and average temperature during the growing season of mixed cropping systems of lentils, spring wheat and spring barley at the experimental station Kleinhohenheim, SW Germany

Niederschlag und mittlere Temperatur während der Vegetationsperiode von Linsen im Mischanbau mit Sommerweizen und Sommergerste; Versuchsstation Kleinhohenheim, SW Deutschland

Month	Rainfall (mm)			Temperature (°C)		
	2009	2010	1961–2010	2009	2010	1961–2010
April	32.2	7.4	51.3	12.6	10.1	8.9
May	162.4	83.8	82.3	15.0	11.4	13.3
June	95.0	70.4	89.2	16.2	17.5	16.4
July	215.6	99.0	79.2	18.5	20.8	18.3
August	95.4	100.9	75.3	19.3	17.2	17.8
April-August	601	362	377	16.3	15.4	14.9
Annual			710			9.2

tein content > 14%) or spring naked barley (B, cv. Hora) in five ratios: 100% L, 75% L + 25% W (or B), 50% L + 50% W (or B), 25% L + 75% W (or B), 100% W (or B). The target crop density for all cropping systems was 240 plants m^{-2}. The current experiment originated from a field trial (Wang et al., 2012) in which lentil was mixed cropped with five spring-sown companion crops: naked-barley, wheat, oat, linseed (*Linum usitatissimum* L.) and buckwheat (*Fagopyrum esculentum* L.) in five proportions (see above). To test whether different mixing ratios had effects on seed crude protein, we focused on two mixtures lentil-barley and lentil-wheat in this study. The original experiment was a Graeco-Latin Square design with three replicates.

The individual plot was 4.2 m in length, with 16 rows in an inter-row distance of 15 cm. The crops were sown uniformly with 3 cm sowing depth using a plot drill on 23 April 2009 and 20 April 2010. There was no further crop management (e.g. mechanical weed control or fertilizer application) performed in the field. Hand harvesting was done by pulling up the plants of the sample area 1 m^2 (2 × 0.5 m^2) per plot on 18–21 August 2009 and 16–20 August 2010 when the majority of lentil pods had turned to brown and began to open. After the separation of lentils and wheat (or barley) plants, roots of all crops were cut off and only the aboveground parts were used for further analysis. The samples were oven-dried at 80°C to a constant weight (over three days) and then were threshed indoors by a small experimental threshing machine. To determine the seed crude protein, the whole grains of dry samples were milled on a Cyclotec 1093 centrifugal mill (Tecator AB, Hoganas, Sweden). The seed nitrogen content (N% in dry matter; DM) was determined in grain samples using a Vario Max CNS analyzer (Elementar, Hanau, Germany) according to the Dumas Method (Dumas, 1962). Seed crude protein content was calculated by the N content multiplied with the factor 5.7 for wheat (Teller, 1932) while the classical 6.25 factor was used for barley and lentils.

Statistical analysis

Analysis of variance with the factors ratio (R) and year (Y) was performed using the MIXED procedure of SAS version 9.2 (SAS Institute, 2009). Two cereals (wheat and barley) crude protein content and other traits (e.g. seed N content and TKM) were analyzed separately, without regard to the original experimental design. Data were log transformed to get a normal distribution and homogeneity of variance. For letter description, a multiple *t*-test was made only if the *F*-test was significant.

Results

Grain yields of all crops increased when their proportion increased in the mixture. Barley yield increased from 1.1 to 1.6 t dry matter (DM) ha^{-1} when the proportion in the mixture was raised from 25% to 100% (sole cropping), wheat yield increased from 2.1 to 3.3, and lentil yield from 0.6 to 1.5 t ha^{-1} (Wang et al., 2012). Seed crude protein (CP) of either wheat or barley increased significantly when their ratios in mixed cropping with lentils were reduced. The wheat crude protein content in mixed cropping ranged from 10.9 to 11.5% DM (2009) and 11.6 to 15.1% DM (2010) compared to 10.3% DM (2009) and 11.0% DM (2010) in monocropping (Tab. 2). Barley crude protein was 14.5–15.8% DM in mixed cropping compared to 13.7% DM in monocropping (two years average). Generally, mixtures with the lowest proportion of wheat or barley (25%) resulted in highest crude protein content in the grains, with an increase of 25% (wheat) or 15% (barley) compared to monocropping. The protein content of lentil was not affected by the mixing ratio and varied from 27.2% to 27.7% DM. All crops (cereals and lentils) obtained significantly higher seed N content and crude protein in the year 2010 compared to 2009. The mixing ratio also significantly affected the thousand kernel mass (TKM) of cereals and lentils: the lower the proportion of a crop in the mixture, the higher was the TKM.

Total crude protein yield (TCP, lentils plus one cereal crop) in each cropping system differed significantly with the cropping partner (lentil-wheat or lentil-barley), mixing ratio, ratio × cropping partner interactions, and experimental year (Tab. 3). The mixture of 75% lentil + 25% cereals obtained the highest TCP among five ratios, with the value of 55.9 g m^{-2} in lentil-wheat and 46.0 g m^{-2} in lentil-barley cropping systems, compared with the lowest TCP content in cereal crops monocropping (35.0 g m^{-2} and 21.6 g m^{-2}, respectively). Generally, the TCP in three mixtures were significantly higher than that in cereals or lentil monocropping.

Discussion

High crude protein content of wheat or barley could be obtained when the proportion of the cereals in the mixture with lentils was low (25%). The results agreed with the study of Pflaum et al. (2011) who reported that malting barley showed higher crude protein (13.8%) in mixed cropping with lentils at a ratio of 3:1 (lentil:barley) than in an 1:1 ratio (12.5%) or in monocropping (11.2%). Similar results were found in mixtures of winter wheat and grain legumes (pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.); Hof et al., 2006) and barley-narbon vetch (*Vicia narbonensis* L.) mixed cropping (Azizi et al., 2011). A reduction of the ratio of cereals in the mixture means a decrease of the cereal crop density, similar to the system of “wide rows” which is sometimes used to increase the protein content of wheat in organic farming (Neumann et al., 2006). In that study, the cultivation of winter wheat with a wide row spacing resulted in higher grain crude protein of up to + 0.8%, compared with the common row distance (12 cm). Similarly, Hiltbrunner et al. (2005) showed that grain protein of winter wheat increased from 11.7% to 12.7%, and the TKM increased from 42.6 g to 43.5 g by a wider row spacing (37.50 cm) compared to the narrow row spacing (18.75 cm). Different

Tab. 2. Seed N content (N%), crude protein (CP, % dry matter) and thousand kernel mass (TKM, g) of crops in lentil-wheat and lentil-barley cropping systems with different mixing ratios over two years (2009–2010) in organic farming
N-Gehalt im Samen (N%), Rohproteingehalt (CP, % i. TM) im Samen und Tausendkornmasse (TKM, g) von Linsen, Sommerweizen und Sommergerste beim Mischanbau in unterschiedlichen Anteilen 2009–2010, Ökologischer Landbau

	Wheat 2009			Wheat 2010			Barley			Lentils		
Source	N%	CP†	TKM	N%	CP	TKM	N%	CP	TKM	N%	CP	TKM
Ratio (R, %)												
25	2.0 a	11.5 a	36.2 a	2.6 a	15.1 a	37.6 a	2.5 a	15.8 a	35.2 a	4.3	27.2	25.9 a
50	2.0 a	11.5 a	36.6 a	2.2 b	12.8 b	34.0 b	2.4 b	14.9 b	34.6 a	4.4	27.7	25.1 b
75	1.9 ab	10.9 ab	36.1 a	2.0 c	11.6 c	31.2 c	2.3 b	14.5 b	34.8 a	4.4	27.6	25.7 ab
100	1.8 b	10.3 b	35.2 a	1.9 c	11.0 c	31.2 c	2.2 c	13.7 c	33.3 b	4.4	27.4	25.5 ab
Year (Y)												
2009							2.2 b	13.5 b	33.8 b	4.2 b	26.4 b	25.6
2010							2.6 a	16.0 a	35.2 a	4.6 a	28.5 a	25.6

Significance (Pr > F)

	Wheat			Barley			Lentils		
Factor	N%	CP	TKM	N%	CP	TKM	N%	CP	TKM
R	< 0.001	< 0.001	0.007	0.002	0.002	0.04	0.12	0.11	0.03
Y	< 0.001	< 0.001	0.002	< 0.001	< 0.001	0.009	< 0.001	< 0.001	0.91
R×Y	0.001	0.001	0.02	0.09	0.09	0.34	0.08	0.08	0.71

† Crude protein (CP) = N content × 6.25 (barley and lentils), or N content × 5.7 (wheat). No significant differences for values followed by the same letters in the column within the same crop, P < 0.05

Tab. 3. Total crude protein yield (TCP, lentils plus one cereal crop) in lentil-wheat and lentil-barley mixed cropping systems with different mixing ratios (lentil:cereal) over two years (2009–2010) in organic farming
Gesamter Proteinertrag (TCP) von Linsen und Mischungspartner Sommerweizen oder Sommergerste beim Mischanbau in unterschiedlichen Anteilen 2009–2010, Ökologischer Landbau

Source	Value (TCP, g m^{-2})		
Interaction (ratio × cropping)			
Ratio	Cropping (lentil-wheat)	Ratio	Cropping (lentil-barley)
100:0	39.5 b†	100:0	39.5 b
75:25	55.9 a	75:25	46.0 a
50:50	51.4 a	50:50	39.8 b
25:75	49.7 a	25:75	34.5 c
0:100	35.0 c	0:100	21.6 d
Significance (Pr > F)			
Factor	DF	TCP	
Cropping (C)	1	< 0.001	
Ratio (R)	3	< 0.001	
Year (Y)	1	< 0.001	
R×C	3	0.004	

† No significant differences for values followed by the same letters in the column within the same cropping system, P < 0.05

spatial arrangements can affect crop yield components, such as ear numbers, kernels per ear, and the TKM (Marshall and Ohm, 1987). In the current study, both cereals and lentils produced grains with a higher TKM at low proportions in the mixture. As intra-specific competition among the cereal species may have decreased along with an increasing ratio of the companion crop, they might have compensated the lower density of heads to a certain extent by a higher TKM. However, the final grain yield of each crop in the study generally decreased as their proportion decreased (data not shown; Wang et al., 2012).

Unlike the cereal crops, the lentil grain crude protein remained constant, independent of the proportion in the mixture. Generally, legume protein content differs due to genetic factors, environmental and agronomic conditions and their interactions (Monti and Grillo, 1983; Burstin et al., 2011; Kesli and Adak, 2012). The protein content of lentils in the current study, with about 27–28% crude protein, is within the range of the varietal diversity from 18–30% protein described by Burstin et al. (2011). Seed protein in lentil is negatively correlated for instance with seed yield and harvest index (HI), and positively correlated with plant height, time to flower and to maturity (Hamdi et al., 1991). Though there were significant differences in HI and grain yield in the current study as a result of mixed cropping (Wang et al., 2012), this impact was not mirrored in protein content. As the protein content in lentil accessions is determined by a heritability of up to 80% (Burstin et al., 2011), genotypic effects might have had a stronger impact on protein content than the environment had in all mixtures.

The differences in the absolute crude protein content of crops between the years is probably a result of different water supply and temperature; high temperatures during grain filling are usually favorable to increase the grain crude protein of wheat (Rao et al., 1993), particularly in combination with drought (Altenbach et al., 2003).

The combination of the grain legume lentils with cereals (wheat or barley) resulted in a higher total crude protein yield of the whole system, compared with the monoculture of each crop. In countries with lack of protein supply in the food, mixed cropping can be therefore well used to increase the total protein supply for human nutrition. However, the effects of mixed cropping on protein quality and the composition of the essential amino acids are still not yet known. Malting barley does not seem a suitable companion crop for lentils from the point of a good malting and beer making process as there is the risk of too high crude protein levels. Wheat quality, on the other hand, may highly profit from mixed cropping with lentil, and maybe with other legumes because of increased protein contents. This system can be recommended particularly for organic farming where high protein contents are not easily achieved.

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