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Interaction Between Cereals and Pea Intercrops During Their Initial Development

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Abstract

The species that make up the intercrop interact with each other during their development, which can be beneficial, harmful, or indifferent to the participants. The aim of this study was to determine whether the developmental differences observed between intercropped and pure stands are already evident at early developmental stages. We conducted three experiments: (1) a laboratory germination test using filter paper rolls, (2) a laboratory growth test in plant pots, and (3) a small-plot field experiment comparing the development of mixtures and monocultures of winter cereals (winter wheat, winter durum wheat, winter einkorn, winter triticale, winter barley, and winter oats) and winter peas. Based on our results, only minor differences were observed (1–3%) in germination parameters (germination percentage, germination vigor, and seedling health). However, as plant development progressed, increasingly pronounced differences emerged (significance level of $p < 0.05$) between intercropped and pure stands based on which we assume allelopathic effects. Winter wheat and durum wheat had a positive effect on the initial development of winter pea, whereas barley, and oat had a clearly negative effect. Winter pea negatively affected the germination and early development of triticale and oat, while it had a positive effect on the initial development of durum wheat. Mutually beneficial interactions were identified in the winter wheat–pea and durum wheat–pea combinations during the early stages of plant association.

Keywords: germination; initial development; allelopathy; intercropping; cereals; pea

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1. Introduction

Currently, approximately two-thirds of Hungary's arable land is cultivated with cereals [1], primarily due to their economic competitiveness compared with oilseed and protein crops [2–4]. Since the cultivation of legumes is uneconomical, Hungary needs to import protein. Consequently, many farmers do not grow legumes, despite their well-documented benefits, including their capacity to provide a renewable source of nitrogen, reduce reliance on synthetic fertilizers [5–8], and enhance crop diversification. Furthermore, the inclusion of legumes can improve the environmental sustainability of cereal-based cropping systems [9–15]. If a competitive cultivation method were available to Hungarian farmers, the area sown with legumes would increase, allowing their beneficial effects to be more widely utilized. Since the second half of the last century, intensive crop production systems have dominated agricultural practices in developed countries [16]. The widespread

adoption of high-yielding cultivars has contributed to a marked simplification of cropping patterns and a substantial decline in agrobiodiversity [17,18]. The increasing prevalence of partial monoculture has been associated with greater tillage intensity and increased chemical inputs [19]. Simultaneously, agricultural intensification has exacerbated crop- and soil-related problems [20–22], while simplified cropping systems have increased farmers' exposure to market demands [2,23].

Owing to the disadvantages of intensive crop production and the need to increase arable land diversity and expanding the area cultivated with legumes, alternative cropping systems such as intercropping are receiving increasing attention in Hungary. Intercropping is defined as the simultaneous cultivation of two or more crop species in the same field during one growing season [24,25]. This practice is ancient and widespread in many tropical regions of the world [25,26], but remains much less common in European countries [27–30]. Interest in this valuable cropping system is particularly strong in low-input farming systems, especially in organic farming [31–33]. Intercropping is based on crop interactions among crop species that maximize productivity [34–37]. One of the most common advantages of intercropping is the increased quantity and stability of crop yields through more efficient use of available resources (light, water, and nutrients) compared with pure cropping [27,33,38]. Additional benefits include reduced weed competition and a lower incidence of insect pests and pathogens [9,34,39].

Despite its numerous advantages, intercropping also presents challenges for Hungarian farmers. These include sowing species with different seed sizes, nutrient management in mixed stands, crop protection, and harvesting [40,41], and the separation the harvested products by species. While many plant species are suitable for intercropping, others are difficult to integrate into such systems. One of the most important tasks is to select species that are compatible with one another and not only tolerate but also promote each other's development. Thus, plant species grown together influence one another, and these interactions are highly complex and continuously change throughout the plant development [42,43]. It can also be observed that species grown in intercrops compete for limited resources, resulting in one species suppressing the other [44].

However, differences can often be observed between plants grown in early-stage mixed stands and those grown in pure stands. Since resources (light, water, and nutrients) are generally available to young plants in sufficient quantities at such an early stage of growth, and competition for these resources is minimal, other factors are presumably responsible for the observed developmental differences. Based on the literature [45–52], we hypothesize that the early developmental differences between mixed and pure stands are primarily attributable to allelopathic interactions rather than competition for resources.

The aim of this study was to determine whether the species forming plant associations influence each other's initial development and to identify the earliest stage at which growth differences among the associated plant species become apparent.

2. Materials and Methods

This study comprises three experiments, conducted either under laboratory or field conditions. In all three experiments, we tested six different cereals, one pea, and their mixtures. The experiments were carried out using the GK Csillag winter wheat (*Triticum aestivum* L.) variety, the GK Julidur winter durum wheat (*Triticum durum* L.) variety, the MV Alkor winter einkorn wheat (*Triticum monococcum* L.) variety, the GK Maros winter triticale (×*Triticosecale* Wittm.) variety, the GK Aréna winter barley (*Hordeum vulgare* L.) variety, the GK Impala winter oat (*Avena sativa* L.) variety, and the Aviron winter field pea (*Pisum sativum* L.) variety.

2.1. Germination Test in Filter Paper Rolls

Germination tests were carried out under controlled laboratory conditions (20–23 °C) in February 2025. In the germination test, 50 seeds of each individual cereal or pea variety were germinated in rolls of germination filter paper, while 50 + 50 seeds were germinated in rolls of germination filter paper for the mixed treatments. The rolls were placed in perforated nylon bags to ensure constant humidity (relative humidity of approximately 80–85%). Each seed had the same area available, regardless of whether it was germinated in a pure or mixed treatment (6 cm^2 per seed). The experiments was replicated four times. During the experiment, germination percentage, germination vigor, seedling health, root and shoot length, and root and shoot dry weight were determined as follows:

- Germination percentage: The percentage of seeds that germinated by the 8th day of germination from a given germination batch (50 seeds). Germination percent (%) = number of seedlings × 2 on 8th day of germination.
- Germination vigor: Percentage of the total germinated seedlings (number of seedlings on 8th day of germination) germinated by the 4th day of germination. Germination vigor (%) = number of germinated seedlings on the 4th day of germination/number of germinated seedlings on the 8th day of germination × 100.
- Determination of seedling health: Intact, healthy seedlings (containing at least three strong roots and intact shoot buds) were separated from abnormal or diseased seedlings on the 8th day of germination. Seedling health (%) = number of healthy, intact seedlings on the 8th day of germination × 100/total number of germinated seedlings on the 8th day of germination.
- Root length: The root length of 20 healthy seedlings was measured on the 4th and 8th days of germination.
- Shoot length: The shoot length of 20 healthy seedlings was measured on 4th and 8th days of germination.
- Root dry weight: The roots of 20 healthy seedlings were cut and dried and their mass was measured on the 8th day of germination.
- Shoot dry weight: The shoots of 20 healthy seedlings were cut and dried and their mass was measured on the 8th day of germination.

Germination tests were performed according to the regulations issued by the International Seed Testing Association (International Rules for Seed Testing) and the harmonized Hungarian national standards (MSZ 6354-3:2008, MSZ 6354-9:2016, MSZ EN ISO 11269-1:2013) [53–55].

2.2. Growing Test in Plant Pots

The laboratory growing tests were carried out under controlled laboratory conditions (20–23 °C, relative humidity of approximately 60–65%) from March to April 2025. Plants were grown under a 16/8 h light/dark cycle. Illumination was provided by blue (400–450 nm) and red (620–680 nm) LED lamps. In the laboratory growing test, 25 seeds of each individual cereal or pea variety were sown in round plant pots with a diameter of 23 cm (monocrop), while 25 + 25 seeds were sown in round plant pots with a diameter of 32.5 cm for the mixed treatments (intercrop), ensuring that plants in both cultivation methods (pure stands and mixtures) had the same growing area available (16.6 cm^2 per plant). Seeds were sown at a depth of approximately 3–3.5 cm. The same soil was used in the plant pots as in the field experiment (Table 1). All monocrop and intercrop treatments were replicated four times. During the experiment, plant number, root and shoot length, and root and shoot dry weight were determined as follows:

- Plant number: Plants were selected during the 3rd week of growth from each growing batch (25 seeds).

- Root length: The root length of 10 plants was measured during the 3rd and 6th weeks of growth.
- Shoot length: The shoot length of 10 plants was measured during the 3rd and 6th weeks of growth.
- Root dry weight: The roots of 10 plants were cut, dried, and weighed during the 3rd and 6th weeks of growth.
- Shoot dry weight: The shoots of 10 plants were cut, dried, and weighed during the 3rd and 6th weeks of growth.

Table 1. Soil characteristics of the growing area.

Parameters	Unit	Tested Soil Depth (cm)	
		0–20	20–40
Slightly alkaline reaction	pH	7.07	7.14
Organic matter (SOM)	%	2.83	2.67
$CaCO_3$	%	0.78	0.98
$NO_3^- + NO_2^-$	mg kg^{-1}	19.2	12.3
P_2O_5	mg kg^{-1}	367	396
K_2O	mg kg^{-1}	276	385
SO_4^{2-}	mg kg^{-1}	22.3	21.6
Na	mg kg^{-1}	112.3	98.5
Mg	mg kg^{-1}	267	286
Cu	mg kg^{-1}	4.67	3.52
Zn	mg kg^{-1}	4.58	6.98
Mn	mg kg^{-1}	26.3	15.7

2.3. Field Investigation

The field investigation was carried out in Szeged, Hungary, on chernozem soil (Table 1), which is well suited for the cultivation of peas and cereals. The experimental area was fertilized with a multi-nutrient autumn fertilizer before sowing at a rate of 200 kg ha^{-1} ($N:P_2O_5:K_2O$ = 8:15:15). Seedbed preparation consisted of plowing (20–25 cm), disk harrowing (10–12 cm), and cultivation (5–8 cm). Sowing was carried out in 10 m^2 plots arranged in a randomized design on 15 October 2024 at a sowing depth of 3–4 cm, with a seed density of 300 seeds m^{-2} for cereals and 100 seeds m^{-2} for pea. The same seed densities (300 and 100 seeds m^{-2}, respectively) were maintained in the mixtures. All monocrop and intercrop treatments were replicated four times. The experimental plots were managed using identical plant protection and agronomic practices. The meteorological conditions during the experiment and the corresponding 25-year averages are presented in Figure 1. During the experiment, plant number per square meter, shoot length, and shoot dry weight were determined as follows:

- Plant number per square meter: The number of plants per square meter was counted in autumn (27 November 2024—leafy development of cereals) and early spring (19 March 2025—tillering of cereals).
- Shoot length: The shoot length of 30 plants was measured in autumn (27 November 2024) and early spring (19 March 2025).
- Shoot dry weight: The shoots of 30 plants were cut, dried, and weighed in autumn (27 November 2024) and early spring (19 March 2025).

2.4. Statistical Analysis

Data were analyzed using Microsoft Excel 2010 and IBM SPSS Statistics version 27 (IBM Corp., Armonk, NY, USA). The experiment was conducted using a completely randomized design. The effects of cereal species and cropping system on initial plant development were evaluated using a two-way multivariate analysis of variance (MANOVA), with cereal

species and cropping system (pure cereal stand versus cereal–pea mixture) included as fixed factors. The cereal species × cropping system interaction was also tested. Statistical significance was set at $p < 0.05$. When the MANOVA indicated a significant multivariate main or interaction effect, follow-up two-way univariate ANOVAs were performed separately for each response variable. To control the family-wise Type I error rate across these multiple univariate tests, Bonferroni correction was applied, with the adjusted significance threshold calculated as α adj = $0.05/m$, where m was the number of dependent variables included in the corresponding MANOVA. The effect of cereal species on the initial development of pea was evaluated separately using a one-way ANOVA, with cereal species treated as a fixed factor. Effect sizes were expressed as partial eta squared (ηp^2) for the effects of cereal species, cropping system, and their interaction. Univariate normality and homogeneity of variances were assessed using the Kolmogorov–Smirnov and Levene's tests, respectively. Following significant univariate effects, pairwise comparisons were conducted using Tukey's HSD test when the assumption of homogeneity of variances was met and the Games–Howell test when variances were unequal, whereas Tukey's post hoc test was used when equal variances were assumed.

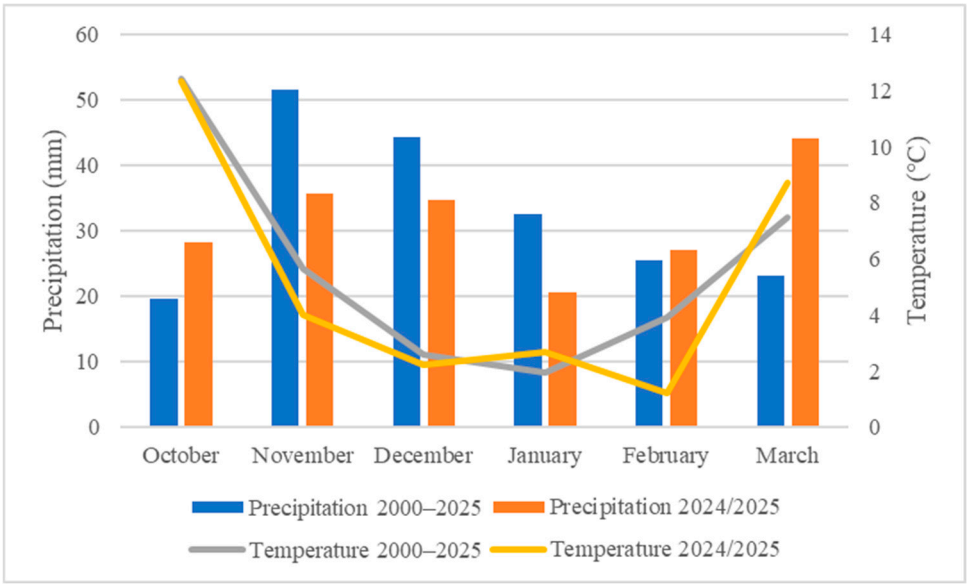


Figure 1. Monthly precipitation (mm) and mean temperature (°C) in the studied seasons of 2024/2025, and over the last 25 years (2000–2025).

3. Results

3.1. Germination Test in Filter Paper Rolls

Applying MANOVA (Table 2), the effect of cereals species was significant (cereals Wilks' $\lambda = 0.154$, $F_{[45;128.354]} = 1.483$, $p < 0.05$, $\eta p^2 = 0.312$). No significant effect of intercropping was detected (intercropping Wilks' $\lambda = 0.704$, $F_{[9;28]} = 1.308$, $p = 0.277$, $\eta p^2 = 0.296$). However, the interaction between cereal species and intercropping was significant (cereals * intercropping Wilks' $\lambda = 0.143$, $F_{[45;128.352]} = 1.552$, $p < 0.05$, $\eta p^2 = 0.322$).

Table 2. MANOVA table for germination test of pure cereals and cereals mixed with winter pea.

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Cereals	Wilks' Lambda	0.154	1.483	45.000	128.354	0.045	0.312
Intercropping	Wilks' Lambda	0.704	1.308	9.000	28.000	0.277	0.296
Cereals * Intercropping	Wilks' Lambda	0.143	1.552	45.000	128.354	0.030	0.322

Figure 2 shows the results of germination tests of pure cereal stands and cereal–winter pea mixtures conducted in filter paper rolls.

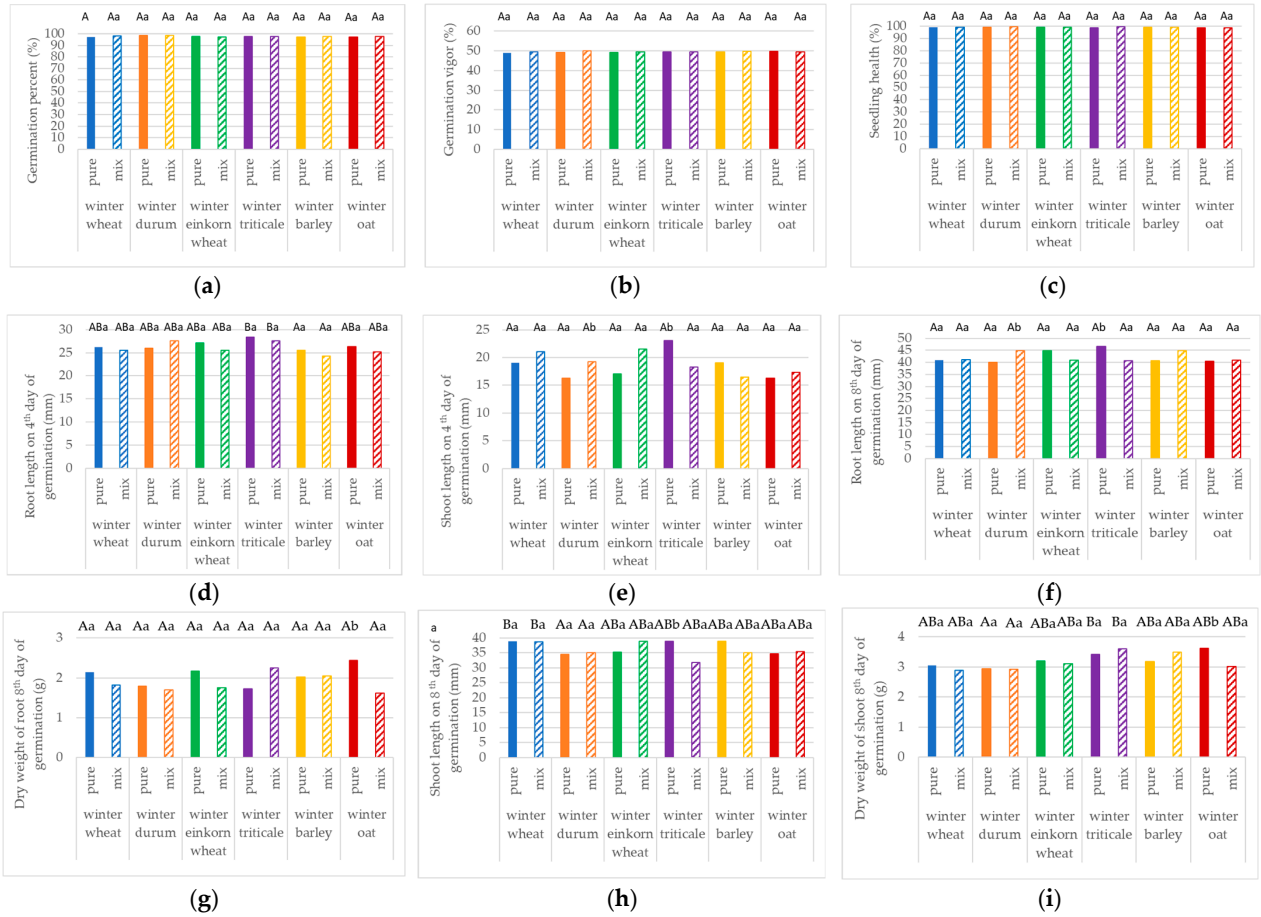


Figure 2. Germination test of pure cereals and cereals mixed with winter pea in filter paper rolls: (**a**) germination percentage; (**b**) germination vigor; (**c**) seedling health; (**d**) average root length (mm) of 20 healthy seedlings on 4th day of germination; (**e**) average shoot length (mm) of 20 healthy seedlings on 4th day of germination; (**f**) average root length (mm) of 20 healthy seedlings on 8th day of germination; (**g**) average dry weight (g) of root of 20 healthy seedlings 8th day of germination; (**h**) average shoot length (mm) of 20 healthy seedlings on 8th day of germination; (**i**) average dry weight (g) of shoot of 20 healthy seedlings on 8th day of germination (Within a cereal species, the values of the pure and mixed sowing methods are significantly different at a $p < 0.05$ significance level if they are marked with different lower letters. Values for individual cereal species are significantly different at a significance level of $p < 0.05$ if they are marked with a different capital letter).

Based on the two-way analysis of variance, significant differences among the examined cereal species were observed only for root length on the 4th day of germination (mm), shoot length on the 8th day of germination (mm), and shoot dry weight on the 8th day of germination (g). In contrast, most germination parameters did not differ significantly ($p > 0.05$) among the cereal species.

When comparing pure and mixed sowing within each cereal species, no significant differences were found between pure cereals and their pea mixtures in terms of germination percentage, germination vigor, or seedling health, and only slight variation was observed. Root length measured on the 4th day of germination decreased in einkorn, triticale, barley, and oat when grown in mixtures with pea compared with pure cereal stands, whereas it increased in durum wheat. However, none of these differences were statistically significant. Shoot length measured on the 4th day of germination decreased in triticale and barley grown in pea mixtures compared with pure stands but increased in wheat, durum wheat,

einkorn, and oat. No significant differences were detected among the treatments. Root length measured on the 8th day of germination decreased in einkorn and triticale when grown in mixtures with pea, whereas it increased in wheat, durum wheat, barley, and oat compared with pure stands. The pea mixture significantly reduced root length in triticale but significantly increased root length in durum wheat on the 8th day of germination. Root dry weight measured on the 8th day of germination decreased in wheat, durum wheat, and einkorn grown in mixtures with pea. In the oat–pea mixture, pea significantly reduced the root dry weight of oat. In contrast, root dry weight increased in triticale and barley grown in mixtures with pea, although these differences were not significant. Shoot length measured on the 8th day of germination decreased in wheat, triticale, and barley grown in mixtures with pea compared with pure cereal stands, whereas it increased in durum wheat, einkorn, and oat. The reduction in shoot length caused by pea was already statistically significant in triticale by the 8th day of germination. By the 8th day of germination, shoot dry weight decreased in wheat, durum wheat, einkorn, and oat grown in mixtures with pea compared with pure stands but increased in triticale and barley. However, no significant differences were detected among the treatments.

The one-way analysis of variance (Table 3) showed that, during the germination test conducted in filter paper rolls, significant effects were observed for pea root length on the 4th day of germination, pea shoot length on the 4th day of germination, and pea shoot length on the 8th day of germination.

Table 3. ANOVA table for germination test of pure winter peas and peas mixed with different cereals in filter paper rolls.

		Sum of Squares	df	Mean Square	F	Sig.
Germination percent	Between Groups	1.466	6	0.244	0.082	0.997
	Within Groups	62.748	21	2.988		
	Total	64.214	27			
Germination vigor	Between Groups	2.561	6	0.427	0.817	0.569
	Within Groups	10.967	21	0.522		
	Total	13.528	27			
Seedling health	Between Groups	3.839	6	0.640	0.584	0.739
	Within Groups	23.003	21	1.095		
	Total	26.842	27			
Root length on 4th day of germination	Between Groups	62.775	6	10.463	9.421	<0.001
	Within Groups	23.322	21	1.111		
	Total	86.097	27			
Shoot length on 4th day of germination	Between Groups	133.922	6	22.320	10.241	<0.001
	Within Groups	45.770	21	2.180		
	Total	179.692	27			
Root length on 8th day of germination	Between Groups	41.420	6	6.903	1.141	0.374
	Within Groups	127.052	21	6.050		
	Total	168.472	27			
Shoot length on 8th day of germination	Between Groups	78.580	6	13.097	4.195	0.006
	Within Groups	65.564	21	3.122		
	Total	144.144	27			
Dry weight of root 8th day of germination	Between Groups	1.504	6	0.251	1.495	0.228
	Within Groups	3.520	21	0.168		
	Total	5.024	27			
Dry weight of shoot on 8th day of germination	Between Groups	2.867	6	0.478	2.049	0.104
	Within Groups	4.898	21	0.233		
	Total	7.765	27			

Compared with pure winter peas, the germination percentage of winter pea decreased when mixed with einkorn and barley, but increased when mixed with wheat (Table 4). The germination vigor of winter pea decreased in mixtures with all cereal species (wheat, durum wheat, einkorn, triticale, barley, and oat) compared with pure pea. Seedling health also declined when pea was mixed with einkorn, barley, and oat. In contrast, pea seedlings grown with wheat, durum wheat, and triticale showed better seedling health than those grown in pure stands. Root length measured on the 4th day of germination decreased in pea mixed with einkorn and oat but increased in mixtures with wheat, durum and triticale compared with pure peas. Barley significantly reduced pea root length on the 4th day of germination. Shoot length measured on the 4th day of germination was significantly greater in pea plants grown in mixtures with durum and triticale than in pure pea. No significant treatment effects were detected in the remaining mixtures: shoot length decreased in mixtures with wheat, einkorn, and oat but increased in the barley mixture. By the 8th day of germination, pea root length increased, although not significantly, in mixtures with durum wheat and triticale, whereas it decreased in mixtures with wheat, einkorn, barley, and oat compared with pure pea. Compared with pure pea seedlings, shoot length on the 8th day of germination was shorter in mixtures with wheat, einkorn, and oat but longer in mixtures with durum wheat, triticale, and barley. Regarding root and shoot dry weight measured on the 8th day of germination, both parameters were lower in all pea mixtures than in pure pea. On average, root dry weight decreased by 18%, while shoot dry weight decreased by 7%. However, no statistically significant treatment effects were detected.

3.2. Growing Test in Plant Pots

In applying MANOVA (Table 5), a significant effect of the cereal species was observed (cereals Wilks' $\lambda = 0.099$, $F_{[45;128.354]} = 1.93$, $p < 0.05$ $\eta p^2 = 0.370$). No significant effect of intercropping was detected (intercropping Wilks' $\lambda = 0.799$, $F_{[9;28]} = 0.781$, $p = 0.635$, $\eta p^2 = 0.201$). The interaction between cereal species and intercropping was also not significant (cereals * intercropping Wilks' $\lambda = 0.752$, $F_{[45;128.354]} = 0.188$, $p = 1.000$, $\eta p^2 = 0.055$).

Based on the two-way analysis of variance, significant differences among the examined cereal species were observed only for root length in the 3rd week (mm), root dry weight in the 3rd week (g), shoot length in the 3rd week (mm), and root length in the 6th week (mm). In contrast, most growth parameters did not differ significantly ($p > 0.05$) among the cereal species.

Three weeks after sowing, the number of cereal plants in the wheat–pea and triticale–pea mixtures was lower than that in the corresponding pure cereal stands. In contrast, the number of oat plants in the oat–pea mixture was higher than in the pure oat stand, and this difference was statistically significant. The root length of 3-week-old durum wheat plants was shorter in the pea mixture than in the pure stand. In contrast, wheat, einkorn, triticale, barley, and oat developed longer roots when grown in mixtures with pea than when grown alone. However, these differences were not statistically significant. For the remaining parameters of the pot experiment, no significant treatment effects were detected; however, similar trends were observed. On average, the shoot length (4%), root dry weight (7%) and shoot dry weight (4%) of 3-week-old plants, as well as the root length (5%), shoot length (6%), shoot dry weight (4%), and root dry weight (4%) of 6-week-old plants, were higher in the pea mixtures than in the pure stands for all examined cereal species (wheat, durum, einkorn, triticale, barley, oat).

Table 4. Germination test of pure winter pea and mixed pea with different cereals in filter paper rolls.

				Average for 20 Healthy Seedlings					
	Germination Percent (%)	**Germination Vigor (%)**	**Seedling Health (%)**	**Root Length on 4th Day of Germination (mm)**	**Shoot Length on 4th Day of Germination (mm)**	**Root Length on 8th Day of Germination (mm)**	**Shoot Length on 8th Day of Germination (mm)**	**Root Dry Weight 8th Day of Germination (g)**	**Shoot Dry Weight on 8th Day of Germination (g)**
pure winter field pea	96.50 ± 3.41 a	49.73 ± 0.99 a	98.96 ± 1.20 a	21.50 ± 1.44 bc	14.19 ± 3.13 a	33.54 ± 1.88 a	29.04 ± 1.82 a	2.39 ± 0.53 a	3.61 ± 0.74 a
winter field pea near winter wheat	96.54 ± 0.98 a	49.24 ± 0.50 a	99.00 ± 1.16 a	22.56 ± 1.37 bc	13.40 ± 0.62 a	32.47 ± 2.61 a	27.95 ± 1.92 a	2.04 ± 0.48 a	3.23 ± 0.48 a
winter field pea near winter durum wheat	96.50 ± 2.52 a	49.64 ± 0.51 a	98.98 ± 1.09 a	23.02 ± 0.97 bc	18.29 ± 0.27 b	35.47 ± 1.62 a	31.44 ± 1.51 a	1.88 ± 0.24 a	3.22 ± 0.31 a
winter field pea near winter einklorn wheat	96.00 ± 0.05 a	49.23 ± 0.51 a	98.57 ± 1.02 a	21.26 ± 0.32 bc	13.00 ± 0.94 a	32.13 ± 2.87 a	26.82 ± 1.52 a	2.01 ± 0.41 a	3.40 ± 0.17 a
winter field pea near winter triticale	96.50 ± 1.00 a	49.54 ± 0.98 a	99.08 ± 1.18 a	23.52 ± 1.43 c	18.50 ± 1.21 b	34.97 ± 3.53 a	31.69 ± 1.71 a	1.57 ± 0.21 a	2.89 ± 0.28 a
winter field pea near winter barley	96.00 ± 0.03 a	49.55 ± 0.98 a	98.42 ± 1.01 a	18.66 ± 0.78 a	15.32 ± 0.76 ab	32.87 ± 1.36 a	32.42 ± 1.31 a	2.08 ± 0.42 a	3.49 ± 0.71 a
winter field pea near winter oat	96.50 ± 0.98 a	48.98 ± 0.02 a	97.96 ± 0.02 a	21.06 ± 0.37 ab	13.20 ± 1.43 a	32.47 ± 2.61 a	27.95 ± 1.98 a	2.11 ± 0.47 a	3.48 ± 0.38 a

Within a column, the values of pure peas and mixed peas are significantly different at $p < 0.05$ if they are marked with a different letter.

Table 5. MANOVA table for growing test of pure cereals and cereals mixed with winter pea.

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Cereals	Wilks' Lambda	0.099	1.930	45.000	128.354	0.002	0.370
Intercropping	Wilks' Lambda	0.799	0.781	9.000	28.000	0.635	0.201
Cereals * Intercropping	Wilks' Lambda	0.752	0.188	45.000	128.354	1.000	0.055

The one-way analysis of variance (Table 6) showed significant treatment effects for pea root dry weight in the 6th week, shoot length in the 6th week, and shoot dry weight in the 6th week.

Table 6. ANOVA table for growing test of pure winter peas and peas mixed with different cereals in plant pots.

		Sum of Squares	df	Mean Square	F	Sig.
Plant number	Between Groups	6.429	6	1.071	0.776	0.598
	Within Groups	29.000	21	1.381		
	Total	35.429	27			
Root length on 3rd week	Between Groups	2.141	6	0.357	0.079	0.998
	Within Groups	94.440	21	4.497		
	Total	96.581	27			
Dry weight of root on 3rd week	Between Groups	0.244	6	0.041	0.472	0.821
	Within Groups	1.806	21	0.086		
	Total	2.050	27			
Shoot length on 3rd week	Between Groups	18.134	6	3.022	0.699	0.654
	Within Groups	90.832	21	4.325		
	Total	108.967	27			
Dry weight of shoot on 3rd week	Between Groups	0.138	6	0.023	0.473	0.821
	Within Groups	1.020	21	0.049		
	Total	1.158	27			
Root length on 6th week	Between Groups	343.091	6	57.182	1.768	0.155
	Within Groups	679.322	21	32.349		
	Total	1022.413	27			
Dry weight of root on 6th week	Between Groups	2.811	6	0.469	4.866	0.003
	Within Groups	2.022	21	0.096		
	Total	4.833	27			
Shoot length on 6th week	Between Groups	540.394	6	90.066	4.372	0.005
	Within Groups	432.634	21	20.602		
	Total	973.028	27			
Dry weight of shoot on 6th week	Between Groups	5.489	6	0.915	10.671	0.000
	Within Groups	1.800	21	0.086		
	Total	7.289	27			

The number of pea plants was higher than in the pure stand than in the cereal mixtures (wheat, durum wheat, einkorn wheat, triticale, barley, and oat) (Table 7). The root length of 3-week-old pea plants grown in pure stands was greater than that of peas mixed with durum wheat, einkorn wheat, triticale, and oat; however, it was shorter than that of peas mixed with wheat or barley. Root dry weight of 3-week-old plants was lower in mixtures with wheat, einkorn wheat, triticale, barley, and oat than in pure pea stands, whereas it was only in the durum wheat mixture. Shoot length of 3-week-old pea was shorter in mixtures with all cereal species than in pure pea stands, although differences were not statistically significant. Likewise, the shoot dry weight was lower in all cereal mixtures than in pure pea stands, but no significant treatment effects were detected.

Figure 3 presents the results of the growth test of pure cereal stands and cereal–winter pea mixtures conducted in plant pots.

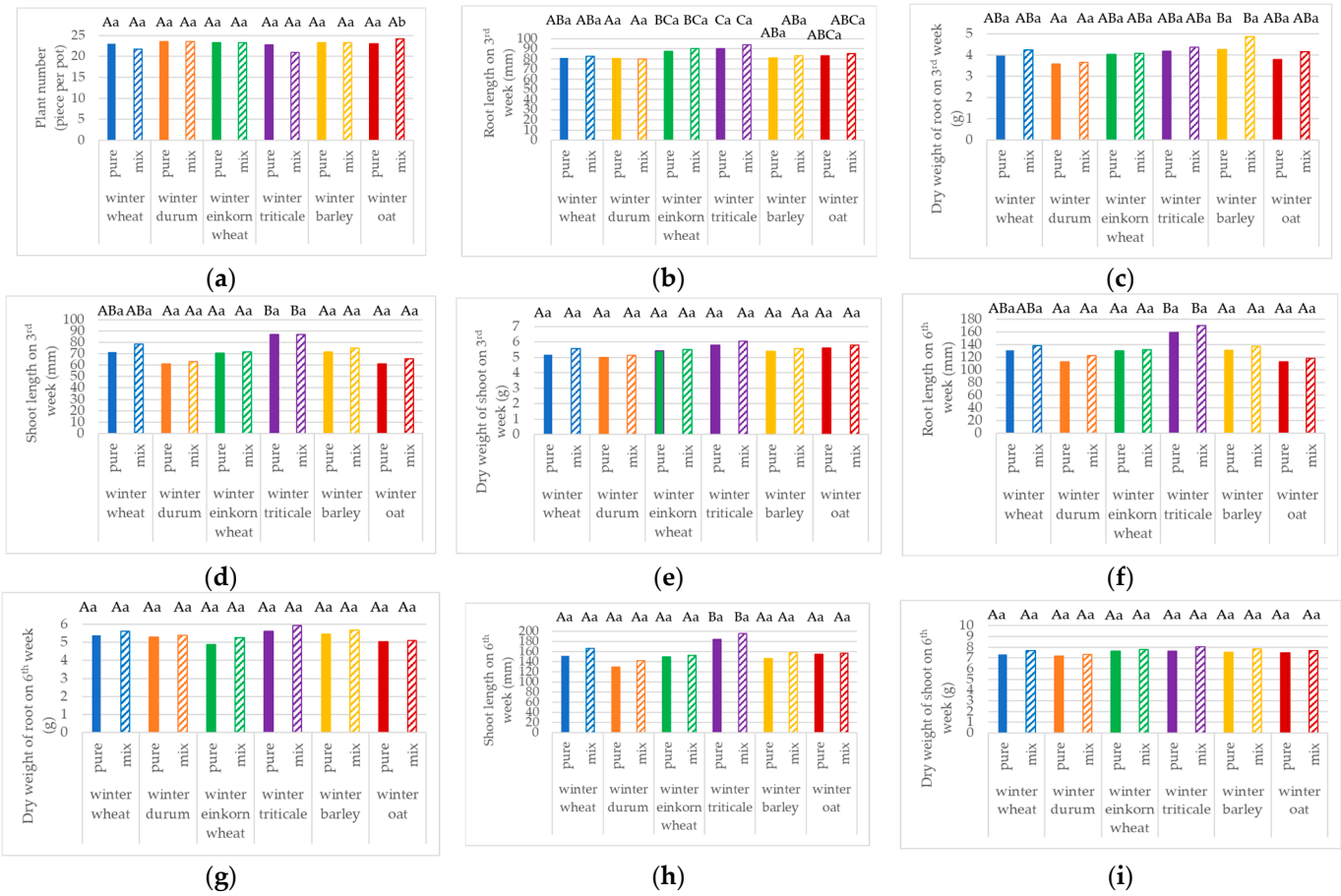


Figure 3. Growing test of pure cereals and cereals mixed with winter pea in plant pots: (**a**) plant number (plant per pot); (**b**) average root length (mm) of 20 plants on 3rd week; (**c**) average dry weight (g) of root of 20 plants on 3rd week; (**d**) average shoot length (mm) of 20 plants on 3rd week; (**e**) average dry weight (g) of shoot of 20 plants on 3rd week; (**f**) average root length (mm) of 20 plants on 6th week (mm); (**g**) average dry weight (g) of root of 20 plants on 6th week; (**h**) average shoot length (mm) of 20 plants on 6th week; (**i**) average dry weight (g) of shoot of 20 plants on 6th week (Within a cereal species, the values of the pure and mixed sowing methods are significantly different at a $p < 0.05$ significance level if they are marked with different lower letters. Values for individual cereal species are significantly different at a significance level of $p < 0.05$ if they are marked with a different capital letter).

At the 6th week, both root and shoot length were lower in peas grown in mixtures with einkorn wheat, barley, and oat than in pure pea stands, whereas both parameters were greater in mixtures with wheat, durum wheat, and triticale. However, these differences were not statistically significant. Similarly, root dry weight in the 6th week decreased in mixtures with einkorn wheat, barley, and oat compared with pure pea stands, although not significantly, while it increased in mixtures with wheat, durum wheat, and triticale. Shoot dry weight in the 6th week also decreased in mixtures with einkorn wheat, barley, and oat, whereas it increased in the triticale mixture compared with pure pea stands; however, these differences were not statistically significant. Statistical analysis showed that wheat and durum wheat significantly increased the root dry weight of pea plants compared with pure pea stands in the 6-week-old crop.

Table 7. Growing test of pure winter pea and mixed pea with different cereals in plant pots.

		Average of 20 Plants							
	Plant Number (Plant per Pot)	**Root Length on 3rd Week (mm)**	**Dry Weight of Root on 3rd Week (g)**	**Shoot Length on 3rd Week (mm)**	**Dry Weight of Shoot on 3rd Week (g)**	**Root Length on 6th Week (mm)**	**Dry Weight of Root on 6th Week (g)**	**Shoot Length on 6th Week (mm)**	**Dry Weight of Shoot on 6th Week (g)**
pure winter field pea	22.75 ± 0.76 a	74.60± 1.78 a	3.56 ± 0.17 a	48.44 ± 1.33 a	3.82 ± 0.21 a	86.57 ± 4.57 ab	5.46 ± 0.40 ab	74.32 ± 4.54 ab	4.33 ± 0.29 a
winter field pea near winter wheat	21.75 ± 0.76 a	74.61 ± 3.29 a	3.53 ± 0.23 a	47.25 ± 1.47 a	3.81 ± 2.22 a	89.62 ± 5.97 b	6.12 ± 0.36 b	78.76 ± 4.98 b	5.24 ± 0.28 b
winter field pea near winter durum wheat	22.50 ± 0.5 a	74.50 ± 2.52 a	3.59 ± 0.30 a	47.63 ± 2.79 a	3.66 ± 0.30 a	87.24 ± 8.43 ab	5.87 ± 0.25 ab	78.42 ± 4.64 b	5.09 ± 0.27 b
winter field pea near winter einkorn wheat	22.00 ± 1.15 a	74.31 ± 1.34 a	3.31 ± 0.46 a	46.29 ± 1.44 a	3.72 ± 0.21 a	82.71 ± 5.85 ab	5.26 ± 0.40 a	71.66 ± 3.82 ab	4.25 ± 0.32 a
winter field pea near winter triticale	21.75 ± 1.7 a	74.43 ± 0.64 a	3.50 ± 0.27 a	46.03 ± 3.05 a	3.75 ± 0.21 a	89.90 ± 4.8 b	5.84 ± 0.19 ab	77.55 ± 4.96 b	4.37 ± 0.24 a
winter field pea near winter barley	21.50 ± 1.29 a	74.92 ± 2.66 a	3.37 ± 0.24 a	46.32 ± 2.21 a	3.63 ± 0.20 a	84.71 ± 4.85 ab	5.40 ± 0.18 a	71.98 ± 4.79 ab	4.15 ± 0.25 a
winter field pea near winter oat	22.50 ± 1.29 a	75.17 ± 1.34 a	3.45 ± 0.29 a	46.67 ± 1.5 a	3.67 ± 0.19 a	79.40 ± 4.25 a	5.25 ± 0.3 a	65.59 ± 3.98 a	4.01 ± 0.38 a

Within a column, the values of pure peas and mixed peas are significantly different at $p < 0.05$ if they are marked with a different letter.

3.3. Field Investigation

In applying MANOVA (Table 8), a significant effect of the cereals was observed (cereals Wilks' $\lambda = 0.063$, $F_{[30;126]} = 4.191$, $p < 0.05$ $\eta p^2 = 0.425$). A significant effect of intercropping was also detected (intercropping Wilks' $\lambda = 0.653$, $F_{[6;31]} = 2.74$, $p < 0.05$, $\eta p^2 = 0.347$). Furthermore, the interaction between cereal species and intercropping was significant (cereals * intercropping Wilks' $\lambda = 0.156$, $F_{[30;126]} = 2.482$, $p < 0.05$, $\eta p^2 = 0.31$).

Table 8. MANOVA table for field investigation of pure cereals and cereals mixed with winter pea.

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Cereals	Wilks' Lambda	0.063	4.191	30.000	126.000	<0.001	0.425
Intercropping	Wilks' Lambda	0.653	2.740	6.000	31.000	0.030	0.347
Cereals * Intercropping	Wilks' Lambda	0.156	2.482	30.000	126.000	<0.001	0.310

Figure 4 presents the results of the field investigation of pure cereal stands and cereal–winter pea mixtures.

Based on the two-way analysis of variance, significant differences among the examined cereal species were observed only for shoot length (mm) in autumn, plant number (plant m^{-2}) in spring, shoot length (mm) in spring and shoot dry weight (g) in spring. In contrast, the remaining field growth parameters did not differ significantly ($p > 0.05$) among the cereal species.

During the autumn field assessment, the number of plants decreased in durum wheat, einkorn, triticale, and oat when grown in mixtures with pea compared with pure cereal stands. plant number increased in wheat and barley grown in mixtures with pea. However, none of these differences were statistically significant. Autumn shoot length was lower in durum wheat, triticale, barley, and oat grown in pea mixtures than in pure cereal stands, whereas wheat and einkorn showed greater shoot length when grown with pea. Nevertheless, these differences were not statistically significant. Compared with pure cereal stands, autumn shoot dry weight was lower in wheat, durum wheat, triticale, barley, and oat grown in mixtures with pea, whereas einkorn showed higher values. However, these differences were also not statistically significant. During the spring field assessment, the number of pea plants per unit area for wheat, durum, einkorn, and oat in pea mixtures was not significantly lower than in pure cereal stands. In contrast, higher plant numbers were observed in triticale when grown in mixtures with peas compared to monocultures. In barley significantly higher plant numbers were observed when grown in mixtures with peas compared to pure. In the spring assessment, both shoot length (by 7% on average) and shoot weight (by 4% on average) of cereals increased in all pea mixtures compared to pure cereal crops, although these increases were not statistically significant.

By studying the ANOVA table (Table 9), it can be established that in the field studies, we can detect a significant effect the shoot length (mm) in autumn, the dry weight (g) of shoot in autumn, the shoot length (mm) in spring and the dry weight (g) of shoot in spring as well.

In the autumn field study, the number of pea plants per unit area in mixtures with einkorn, triticale, barley, and oat was not significantly lower than in pure pea stands (Table 10). Conversely, pea plant numbers were higher in mixtures with wheat and durum wheat than in pure pea crops. Regarding autumn shoot length, peas grown with einkorn, barley, and oat had shorter shoots, while those grown with wheat, durum wheat, and triticale exhibited non-significantly greater shoot length compared to pure pea stands. Peas grown in mixtures with einkorn, triticale, barley, and oat showed lower autumn shoot

mass, whereas those grown in mixtures with wheat and durum wheat had greater autumn shoot mass than peas grown alone. Although the results were not statistically significant, the trend observed in peas grown in wheat and durum wheat mixtures with respect to autumn plant number, shoot length, and shoot dry weight is noteworthy. In other words, under field conditions, winter wheat and winter durum wheat promoted the development of peas.

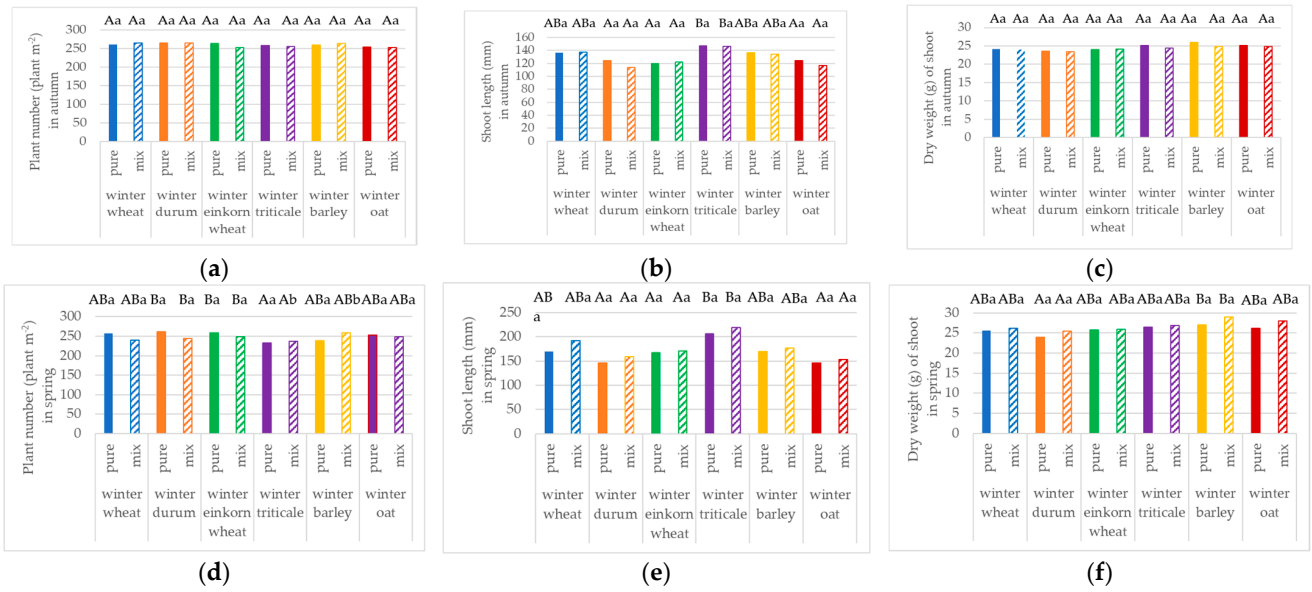


Figure 4. Field investigation of pure cereals and cereals mixed with winter pea: (**a**) plant number (plant m^{-2}) in autumn; (**b**) average shoot length (mm) of 30 plants in autumn; (**c**) average dry weight (g) of shoot of 30 plants in autumn; (**d**) plant number (plant m^{-2}) in autumn; (**e**) average shoot length (mm) of 30 plants in spring; (**f**) average dry weight of shoot of 30 plants in spring (Values for individual cereal species are significantly different at a significance level of $p < 0.05$ if they are marked with a different capital letter. Within a cereal species, the values of the pure and mixed sowing methods are significantly different at a $p < 0.05$ significance level if they are marked with different lower letters).

Table 9. ANOVA table for field investigation of pure winter peas and peas mixed with different cereals.

		Sum of Squares	df	Mean Square	F	Sig.
Plant number (plant m^{-2}) in autumn	Between Groups	181.357	6	30.226	2.111	0.095
	Within Groups	300.750	21	14.321		
	Total	482.107	27			
Shoot length (mm) in autumn	Between Groups	226.178	6	37.696	3.032	0.027
	Within Groups	261.109	21	12.434		
	Total	487.287	27			
Dry weight (g) of shoot in autumn	Between Groups	38.654	6	6.442	9.131	<0.001
	Within Groups	14.816	21	0.706		
	Total	53.470	27			
Plant number (plant m^{-2}) in spring	Between Groups	173.357	6	28.893	2.363	0.067
	Within Groups	256.750	21	12.226		
	Total	430.107	27			
Shoot length (mm) in spring	Between Groups	2379.662	6	396.610	13.323	<0.001
	Within Groups	625.140	21	29.769		
	Total	3004.802	27			
Dry weight (g) of shoot in spring	Between Groups	42.790	6	7.132	11.659	<0.001
	Within Groups	12.845	21	0.612		
	Total	55.635	27			

Table 10. Field investigation of pure winter pea and mixed pea with different cereals.

	Plant Number (Plant m^{-2}) in Autumn	Average of 30 Plants		Plant Number (Plant m^{-2}) in Spring	Average of 30 Plants	
		Shoot Length (mm) in Autumn	Dry Weight (g) of Shoot in Autumn		Shoot Length (mm) in Spring	Dry Weight (g) of Shoot in Spring
pure winter field pea	82.75 ± 2.36 a	59.46 ± 3.64 ab	14.34 ± 0.77 ab	82.25 ± 2.21 a	83.24 ± 5.08 ab	15.36± 0.86 bc
winter field pea near winter wheat	83.25 ± 4.03 a	63.01 ± 4.22 b	15.80 ± 0.77 b	82.00 ± 3.74 a	102.22 ± 5.65 d	16.43 ± 0.77 c
winter field pea near winter durum wheat	83.25 ± 2.98 a	62.49 ± 1.52 b	15.48 ± 0.62 b	82.50 ± 2.52 a	96.23 ± 7.32 cd	16.18 ± 0.60 c
winter field pea near winter einkorn wheat	78.00 ± 3.46 a	57.33 ± 3.06 a	13.21 ± 0.99 a	77.25 ± 3.2 a	80.26 ± 4.28 ab	14.10 ± 1.12 ab
winter field pea near winter triticale	82.50 ± 4.79 a	62.04 ± 3.97 b	14.12 ± 0.61 ab	82.25 ± 4.65 a	86.85 ± 5.56 bc	15.17 ± 0.22 bc
winter field pea near winter barley	79.25 ± 5.62 a	57.59 ± 3.83 a	12.92± 0.79 a	78.75 ± 5.12 a	80.62 ± 5.36 ab	13.77 ± 0.89 ab
winter field pea near winter oat	76.75 ± 1.71 a	54.97 ± 3.73 a	12.47 ± 1.16 a	76.25 ± 1.5 a	73.46 ± 4.36 a	12.77 ± 0.71 a

Within a column, the values of pure peas and mixed peas are significantly different at $p < 0.05$ if they are marked with a different letter.

In the spring field trial, the number of pea plants was lower in mixtures with wheat, einkorn, barley, and oat, while higher values were observed in mixtures with durum wheat compared to pure pea stands. In mixtures with einkorn, barley, and oat, pea shoot length was slightly lower (though not significantly), whereas triticale mixtures showed higher values compared to pure pea crops. In contrast, wheat and durum wheat caused a statistically significant increase in shoot length by spring. By spring, pea shoots were shorter in mixtures with einkorn, triticale, and barley, but longer in mixtures with wheat and durum wheat than in pure pea stands. In contrast, oat significantly reduced pea shoot dry weight compared with pure pea stands. Overall, the results indicate that the initial development (autumn and early spring) of winter pea under field conditions was promoted by winter wheat and winter durum wheat, which may also be beneficial for the subsequent development of pea grown in mixtures.

4. Discussion

The application of ecological principles, the enhancement of biodiversity, and the modeling of natural plant associations are gaining increasing importance in field crop production. Intercropping systems offer several potential advantages, including improved sustainability of crop production, enhanced soil biological activity, greater resilience to climate extremes, more efficient resource utilization, and reduced input requirements [25,56,57]. The nitrogen biologically fixed by legumes becomes available to cultivated plants, thereby reducing the need for synthetic nitrogen fertilizers and contributing to lower production costs, reduced greenhouse gas emissions, and decreased environmental pollution [58]. However, interactions between intercropped species are complex and continuously change throughout plant development [5,44,59,60]. Therefore, a more detailed understanding of intercrop interactions is essential for the development of effective intercropping systems. Numerous studies have focused on the agronomic aspects of intercropping systems, highlighting competition for limited resources such as land, light, water, and nutrients [31,32,61], as well as evaluating the after-effects of crop residues [62].

At the same time, interactions between mixture components begin as early as seed germination [45]. An initial advantage at this stage may influence subsequent development and play a crucial role throughout later growth stages. Likewise, difficulties encountered during the initial stages of development may also affect later plant growth and, consequently, crop yield. The developmental rate of a field crop species often differs when grown in monoculture compared with multispecies systems, even during the early stages of growth under non-limiting resource conditions. Such interactions between neighboring plants are frequently driven by biochemical processes involving secondary plant metabolites [46,47]. Allelopathy is defined as the direct or indirect, beneficial or harmful effect of one plant on another through the production of compounds released into the environment or affecting neighboring plants [63]. Although allelochemicals and plant extracts, were not measured directly in the present study, our results indicate the presence of significant allelopathic effects.

In the present study, we investigated the effects of different cereal species (winter wheat, winter durum wheat, winter einkorn wheat, winter triticale, winter barley, and winter oat) and winter pea on each other's development during early phenological stages, when competition for resources is not yet a limiting factor (germination, emergence) or is so only to a minor extent (leaf development or tillering of cereals). Through this series of experiments, we aimed to demonstrate that the developmental interactions between cereals and winter pea in intercropping systems are likely influenced by allelochemicals with either positive or negative effects. Previous studies have shown that seed-level interactions influence not only early development but also later plant growth and yield [64]. Therefore,

the results of the present study provide new insights into our earlier findings on crop associations [3,7,33,37,54,65–67].

In some legumes, such as pea [48], secondary metabolites have allelopathic effects. In field experiments, residues and extracts of Pisum sativum plants inhibited the growth and population of several plant species [49,50]. In the present study, winter pea significantly reduced triticale root length, triticale shoot length, and oat root dry weight by the 8th day of the germination test in filter paper rolls. In contrast, winter pea significantly increased the root length of winter durum wheat by the same stage, the number of oat plants by the 3rd week of the pot experiment, and the number of barley plants during the autumn period of the field experiment. Although no statistically significant advantage was demonstrated for winter wheat, winter peas promoted its initial in almost all measured parameters.

During the experiment, we found that winter wheat promoted the initial development of winter pea. By the 6th week of the pot experiment, the shoot dry weight of pea had increased significantly. The association with winter wheat also proved beneficial during later developmental stages, as shoot length of pea significantly increased during the spring period of the field experiment. Other researchers likewise reported the positive effect of pea on wheat germination, attributing this response to phenolic compounds such as chlorogenic acid [51].

Durum wheat, owing to its strong stems, serves as an effective support crop for pea in intercropping systems. In our study, durum wheat increased pea shoot length by the 4th day of germination and enhanced shoot dry weight by the 6th week of the pot experiment. The results statistically confirmed that durum wheat promotes the initial development of winter pea, as evidenced by the greater shoot length of pea in the durum wheat–pea mixture compared with pure pea stands during the spring period of the field experiment. However, other studies have reported a negative allelopathic effect of durum wheat germ extract on the initial development of cucumber [64].

In the present experiments, einkorn wheat showed neither a significant positive nor a significant negative effect on the development of winter pea in the intercropping system.

Winter triticale significantly increased pea shoot length by the 4th day of the germination test conducted using filter paper rolls. However, its effect on pea development in the pot and field experiments remained unclear. Similarly, other researchers [45] have reported both positive and negative effects of triticale on pea development.

The effect of young barley on the development of young pea plants remains unclear in the literature. Some authors have reported that extracts of barley sprouts positively influence the initial development of pea [45], whereas others found no allelopathic effect but observed a reduction in the dry matter content of young pea plants caused by barley [68]. In our study, the negative effect of winter barley on pea root length was statistically confirmed on the 4th day of the germination test conducted in filter paper rolls. Later, in both the pot and field experiments, peas grown with barley generally showed weaker performance than pure pea stands in almost all measured parameters, although these differences were not statistically significant. In other words, throughout our experimental series, barley consistently exerted a detrimental effect on the initial development of pea.

Winter oat significantly reduced pea shoot dry weight during the spring period of the field experiment. Furthermore, in all three experiments (paper rolls, pots, and field trials), peas grown in association with oat consistently showed weaker performance than pure pea stands for all measured parameters, although the differences were generally not statistically significant. Similarly, previous studies [45] have shown that oat root exudates negatively affect pea germination. These exudates contain compounds with growth-inhibiting allelopathic potential [52].

Overall, it can be concluded that among the cereal species included in our experiments, winter wheat and durum wheat had a clearly positive effect on the initial development of winter pea, whereas winter barley, and winter oat had a clearly negative effect (Figure 5). Winter pea, in turn, negatively affected the germination and early development of triticale and oat, while exerting a positive effect on the initial development of durum wheat. Although of these interactions still require further investigation (such as metabolomic analysis of root exudates and substances leached from seeds), the present study, based on the observed phenotypic patterns—consistent with previous findings—indicates that allelopathic interactions can be detected as early as the initial stages of plant development in cereal–pea intercropping systems.

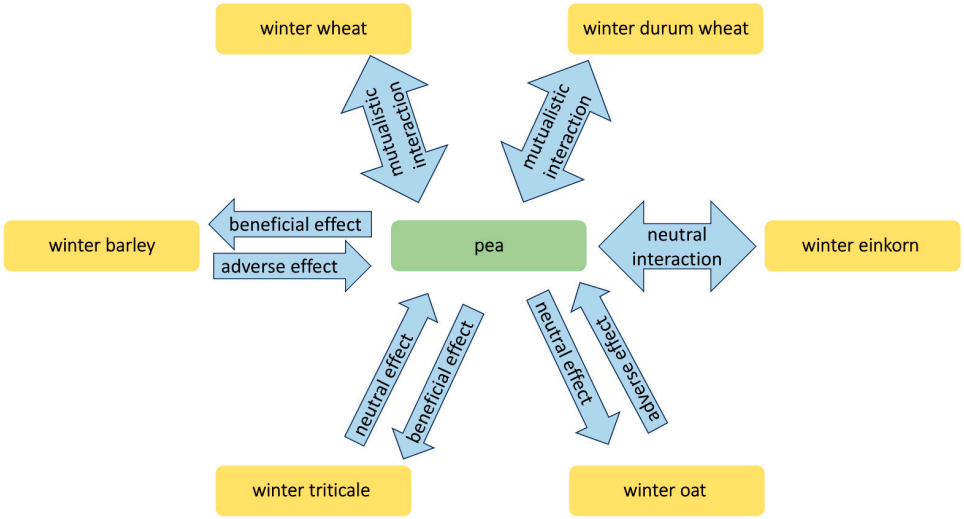


Figure 5. Summary of interactions between winter cereals and winter pea.

5. Conclusions

During the co-cultivation of cereals and pea, only minor differences were observed during the germination stage; however, these differences became increasingly pronounced as plant development progressed. Mutualistic interactions were observed in the wheat–pea and durum wheat–pea associations, whereas neutral interactions were found in the einkorn wheat–pea association. In other words, wheat–pea and durum wheat–pea associations, can be safely recommended for intercropping systems, as companion species should be selected based on their ability to support each other's development. In our experiments, winter pea had a beneficial effect on winter barley and winter triticale, whereas winter pea itself was negatively affected by winter barley and winter oat during its early development. Overall, however, winter oat had a neutral effect on winter pea. Although allelochemicals and plant extracts were not measured directly, phenotypic patterns strongly suggest allelopathic interactions as a primary driver during the early stages of plant development.

Future research may optimize the performance of pea–cereal mixtures to further improve the productivity of intercropping systems. At the same time, the results of this study highlight several important directions for future research. In particular, they emphasize the need to identify the allelochemicals produced by pea and different cereal species and to determine their specific effects in intercropping systems. A primary task for the future is to conduct metabolomic analysis of root exudates and seed leachates across the widest possible range of genotypes of cereals and peas, in order to precisely elucidate variety-specific allelopathic interactions. Therefore, the quantity of root exudates produced by the component species (and varieties) at different developmental stages, as well as the effects of these exudates on companion plants, should be determined through precise laboratory

analyses. Since interspecific competition is also influenced by agronomic factors, future research should investigate how early-stage interactions and developmental differences can be modified through agronomic practices, such as nutrient management, seeding rate, and variety selection, to achieve specific production goals.

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