

Root System Interactions of Common Vetch (*Vicia sativa* L.) and Triticale (*Xtriticosecale* Wittmack) Under Intercropping Conditions

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Abstract

Intercropping, mixed cropping, and rotation are commonly applied and some of the oldest crop production techniques to improve soil structure and nitrogen status. While rotation takes generally two-season with one triticeae and legume, inter-and mixed cropping takes place within the same season. Among many advantages of legume inter-and mixed cropping, the most important one is the sustainable use of soil resources and reduced input cost. Numerous studies evaluated the effect of inter -and mixed cropping on above-ground interactions, very few investigated below-ground interactions in these systems. This study aimed to investigate common vetch (*Vicia sativa*) x triticale (*Xtriticosecale* Wittmack) root system interactions at the seedling stage under intercropping conditions. The study was conducted with a semi-hydroponic plexiglass system. Five different ratios were used to test species level intercommunication and competition. The results suggest that, while triticale significantly affected the development of common vetch roots, common vetch did not affect triticale. Triticale with a fibrous root system was more aggressive on root growth compared to common vetch. It was seen that the most vigorous root system development (for both species) was at doses with higher legume ratios. Our results highlight the importance of below-ground interactions for the selection of best ratios to gain maximum outcome from the intercropping systems.

Key words: Intercropping, root architecture, roots length, lateral root, root depth

Karışım Ekim Koşullarında Yaygın Fiğ (*Vicia sativa* L.) ve Tritikale (*Xtriticosecale* Wittmack) Bitkisinin Kök Sistemi Etkileşimleri

Öz

Karışım ekim, sıraya ekim ve rotasyon, toprak yapısının iyileştirilmesi ve topraktaki azot durumunu artırmak için yaygın olarak uygulanan en eski üretim tekniklerindedir. Rotasyon, genellikle bir buğdaygil ve baklagil ile iki sezon sürerken, karışım ekim ve sıraya ekim ise bir sezonda yapılabilir. Baklagil bitkilerinin karışım ekimde başlıca avantajları, toprak kaynaklarının sürdürülebilir kullanımı ve azaltılmış girdi maliyeti olarak sıralanabilir. Karışım ve sıraya ekim ile yapılan çalışmalarda, karışım ekimin toprak üstü bitki gelişime olan etkisi bir çok kez araştırılmış, sınırlı sayıda çalışmada ise, kök sistemi etkileşimlerinin incelendiği görülmüştür. Bu çalışmada, fide döneminde adi fiğ (*Vicia sativa*) x tritikale (*Xtriticosecale* Wittmack) sıraya ekimlerinin detaylı kök sistemi etkileşimlerinin incelenmesi amaçlanmıştır. Çalışmada yarı hidroponik pleksiglas sistemi kullanılmıştır. Tür düzeyinde etkileşim ve rekabeti test etmek için beş farklı karışım oranı belirlenmiştir. Elde edilen sonuçlar, tritikalenin adi fiğ köklerinin gelişimini önemli ölçüde etkilediğini, adi fiğin ise tritikale'yi etkilemediğini göstermektedir. Saçak kök sistemine sahip olan tritikale, adi fiğ ile karşılaştırıldığında kök büyümesinde daha baskın bir gelişim göstermiştir. En iyi kök sistemi gelişiminin (her iki tür için) daha yüksek baklagil oranlarına sahip dozlarda olduğu görülmüştür. Elde edilen sonuçlar, karışım ekimden maksimum kazanç elde etmek için en iyi karışım ve sıraya ekim oranlarının belirlenmesi ve toprak altı etkileşimlerinin önemini vurgulamaktadır.

Anahtar kelimeler: Karışım ekim, kök mimarisi, kök uzunlukları, sekonder kök, kök derinliği

Introduction

The leading source of balanced feeding in animal production is forage crops. Forage crops have a significant effect on erosion and soil improvement, especially for the soil with low organic matter content. Rotation, inter-and mixed cropping contributes to the nitrogen cycle and enhances soil quality (Kavut and Geren, 2017). These systemic practices also help to loosen the soil, improve soil structure, nitrogen, and organic matter content (Yıldırım and Özasan Parlak, 2016; Iqbal et al., 2019).

The cultivation of legume forage crops combined with cereals is one of the oldest agricultural practices (Mariotti et al., 2009; Lizarazo et al., 2020). The main reasons for farmers to prefer rotation, inter-and mixed cropping systems is to increase the forage yield and keep soil healthy with a sustainable approach (Ghanbari-Banjar and Lee, 2003; Deak et al., 2007; Lithourgidis and Dordas, 2010; Chen et al., 2019; Vidal et al., 2019). Legumes can also increase, available phosphorus (Bargaz et al., 2012) and the nutritional status of the plant (Contreras et al., 2019). They also allow low input production (Dai et al., 2019) and provide pest control without chemical pesticides (Allen-Perkins and Estrada, 2019). Mixed cropping is reported to contribute to reduced weed infestation and increased water use efficiency (Iqbal et al., 2019).

Inter-and mixed cropping systems may cause competition among neighboring plants for water, nutrients, and light (Lithourgidis et al., 2011). Unequal competition may reduce overall productivity, and one or the other side may be negatively affected in various aspects such as biomass, growth, or chemical compositions (Zoric et al., 2015). In grain and legume mixed cropping systems, sowing ratios of each cultivar or species may affect nitrogen supply and demand balance (Fan et al., 2019). Therefore, selection of the species and mixture ratios are key factors to obtain maximum sustainable benefit from the inter-and mixed cropping systems (Önal Aşçı and Eğritaş, 2017).

Although ecologists and agronomists considered spatial root distribution (architecture) of plants in the rhizosphere important for inter-species interactions in natural and agricultural ecosystems, very few empirical studies examined the root distribution dynamics and their effects on inter-species interactions (Zhang et al., 2002; Zhang and Huang, 2003; Li et al., 2006). Maximum crop growth for biological or grain yield in inter-

and mixed cropping systems is significantly affected by the root distribution in the rhizosphere

which determines water and nutrient uptake efficiency (Adiku et al., 2001). Önal Aşçı and Eğritaş (2017) reports that competition between plants depends on the mixture ratio. However, competition on roots and other root-to root interactions is still an unexplored field. Therefore, the objectives of this study were; (i) to evaluate seedling root architectural traits under controlled conditions in a common vetch and triticale intercropping system, (ii) to test root competition phenomenon between species at the seedling stage, and (iii) to obtain optimum mixture ratios, for minimum competition and maximum growth at the seedling stage.

Materials and Methods

The study was aimed to analyze the root system competition between triticale (*Xtriticosecale* Wittmack) x common vetch (*Vicia sativa* L.) intercropping system. The experiments were conducted in the Agricultural Biotechnology Laboratory, Faculty of Agriculture, Siirt University in April 2019. Triticale cultivar Karma 2000 and common vetch cv. Alper was selected to evaluate species-level interactions. The temperature ranges were between 25-27 °C and relative humidity was 60-70%. The study was established according to the factorial design with 6 replications and 5 plants per replication.

Seeds were surface sterilized with 70% ethyl alcohol (C₂H₅OH) and 5% sodium hypochlorite (NaClO) for 5 minutes in each. The sterilized seeds were rinsed under running water for 1 minute. The seeds were then imbibed in water for 24 hours to initiate homogeneous seed germination. Germinated seeds were placed in a plexiglass system consisting of two germination papers (40x40 cm) as 5 seeds/germination paper. The entire system was covered with plexiglass plates and kept upright with a 45° angle in a tub with a 15 cm water level. The experiment was completed at the end of the 15th day.

Root images were obtained by separating plexiglass plates and scanning each plant with a handheld scanner (Iscan Color Mini Portable Scanner) at 600 DPI resolution. Root images were analyzed using ImageJ (imagej.nih.gov; Rueden et al., 2017) image analysis software. Legume and grains were tested in five different ratios; 100% common vetch (100V), 100% triticale (100T), 50% common vetch and 50% triticale (50V-50T), 80% common vetch, and 20% triticale (80V-20T), and 20% common vetch and 80% triticale (80T-20V). The names, abbreviations, and references for the traits are given in Table 1.

Statistical analysis was performed using Statistix software v10 (Analytical Software;

Tallahassee, FL, USA) to measure the variation between and within the species. The Least Significant Difference (LSD) multi comparison test was applied for species, and mixture ratios (Steel et al., 1997). A comparative analysis was made between 100% common vetch and intercropping

for the number of roots and root lengths at depth zones of 0-5, 5-10, 10-15, and 15+ cm. The ratio of change was calculated as the percent value of intercropping (50V-50T, 80V-20T, and 80T-20V) in 100% common vetch (100V).

Table 1. The names, abbreviations, and related references for the investigated root traits

Abbreviation	Trait Name	References
NOR	number of roots	Mia et al., 1996; Ceritoglu et al., 2020; Acikbas et al., 2021
NLR	number of lateral roots	Lynch and van Beem, 1993; Orman-Ligeza et al., 2014;
LRL	longest root length	Kashiwagi et al., 2015; Bektas, 2021.
TRL	total roots length	Lynch and van Beem, 1993; Orman-Ligeza et al., 2014
TLRL	total lateral roots length	Mia et al., 1996; Ye et al., 2018
NOR 0-5	total number of roots at 0-5 cm	
RL 0-5	lengths of roots at 0-5 cm	
NOR 5-10	total number of roots at 5-10 cm	Orman-Ligeza et al., 2014; Chen et al., 2017
RL 5-10	lengths of roots at 5-10 cm	
NOR 10-15	total number of roots at 10-15 cm	
RL 10-15	lengths of roots at 10-15 cm	
NOR 15+	total number of roots at 15+ cm	
RL 15+	lengths of roots at 15+ cm	

RL: Root length, NOR: Number of roots, SE: Standard error, Dif: Difference

Results and Discussion

The effect of intercropping system in root system development and interactions on common vetch and triticale were evaluated at the seedling stage. In this study, the importance of the seeding rate in the seedling growth was discussed. There were no previous reports evaluating root system interactions in a multi-species root system analysis platform. According to the results, the roots of triticale significantly affect common vetch roots. Understanding the underlying physiological processes and mechanism of action is considered to be the main challenge for inter-and mixed cropping systems and rhizosphere dynamics (Brooker et al., 2015; Novoplansky, 2019). In the study, root developments of two species under five different ratios (100V, 100T, 80T-20V, 80V-20T, and 50T-50V) were evaluated, and the root competition phenomenon was investigated.

Root growth parameters of NOR, NLR, LRL, TRL, TLRL, NOR0-5, NOR5-10, RL5-10, NOR10-15, RL10-15, NOR15 +, and RL15 + were analyzed. Intercropping (50V-50T, 80T-20V, 80V-20T,) showed significant ($p < 0.01$) differences in the root number, distribution, and length parameters compared to 100T or 100V. It has been determined that common vetch and triticale roots are affected by the presence of another species in the same environment. Previous reports suggest that legume

and grain inter-and mixed cropping affect root biomass, architecture, and development (Corre-Hellou and Crozat, 2005; Li et al., 2006; Zuo and Zhang, 2008; Xia et al., 2013; Streit et al., 2019).

The Effect of Intercropping on Common Vetch Root Architecture

Root to root interactions was evaluated from the common vetch perspective. While the highest value (17.95 per plant) for NOR in triticale-common vetch intercropping were in the 100V, common vetch in the 50V-50T and 80V-20T ratios had similar values according to LSD analysis (Table 2). When common vetch growth in 100V is compared with the growth in other ratios, it was seen that the NOR value of the common vetch in the intercropping was lower than 100V. The presence of triticale is thought to have a negative effect on the root development of common vetch. In cases where the common vetch ratio was equal or more than triticale (80V-20T and 50V-50T), common vetch development was sufficient despite triticale, on the other hand when the common vetch ratio was lower than the triticale ratio (80T-20V), root development of the common vetch was more suppressed (Table 2).

The number of lateral roots (NLR) was calculated for common vetch, while only the

number of roots (NOR) was calculated for triticale. The highest value (16.93 per plant) for NLR was determined in the 100V. The difference between the common vetch values planted in 100V, 50V-50T (15.14 per plant), and 80V-20T (14.04 per plant) was not significant.

Common vetch TRL values varied between 37.45 and 49.98 cm. While the highest TRL value was observed in the 80V-20T ratio, the lowest value was found in the common vetch in the 80T-20V ratio (Table 2). While triticale forms few seminal roots, vetch is a species with advanced lateral root development. Therefore, lateral root development in common vetch is more likely to be affected by the presence of another crop. The effect of triticale was seen in the lateral root

lengths. Higher ratios of triticale seem to limit lateral root lengths compared to 100V. This might be due to strong competition of triticale compared to legumes due to their relatively quick root initiation, large root size, and deeper root distribution (Gregory et al., 1995; Gregory and Eastham, 1996; Hauggaard-Nielsen et al., 2001).

The effect of intercropping on the TLRL was not significant for common vetch. The highest value was obtained as 29.24 cm in 100V and the lowest was found in 80T-20V mixture as 16.90 cm (Table 2). When the common vetch intercropping ratios were compared for NLR, it can be said that common vetch planted in 100V showed better root development compared to those planted in the mixture.

Table 2. Mean values of root traits for common vetch and triticale under monocropping and intercropping conditions in a semi-hydroponics plexiglass system

	Mixture ratio	NOR	NLR	LRL (cm)	TRL (cm)	TLRL (cm)
Common vetch	100V	17.95 a	16.93 a	20.12	49.36 bc	29.24
	80V+20T	15.03 ab	14.04 ab	21.95	49.98 bc	28.01
	50V+50T	16.25 ab	15.14 ab	20.71	48.87 bc	28.16
	80T+20V	11.42 b	10.23 b	20.55	37.45 c	16.90
Triticale	100T	3.95 c	NA	27.93	76.34 a	NA
	80V+20T	3.27 c	NA	25.72	52.94 bc	NA
	50V+50T	3.63 c	NA	23.05	54.16 bc	NA
	80T+20V	4.13 c	NA	24.80	60.86 b	NA
F value		11.78**	17.58**	2.66 ^{ns}	3.83**	3.44 ^{ns}

Means followed by different letters within columns are different according to least significant difference test, **: p<0.01, NA: Not applicable, ns: not significant, NOR: number of roots, NLR: number of lateral roots, LRL: longest root length, TRL: total root length, TLRL: total lateral root length

To evaluate the effect of intercropping on rooting ability in depth zones of 0-5, 5-10, 10-15, and 15+ cm (Fig. 1-2.), the number and lengths of roots in each zone was calculated. Common vetch roots had a decrease in all depth zones due to the increasing triticale ratio and common vetch in 80T-20V had the lowest number and lengths of lateral roots (Fig. 1). There was a 45 to 100% reduction in lateral root lengths in the 80T-20V ratio in depth zones compared to 100V. Similarly, the number of lateral roots in common vetch decreased between 18.45 to 100% in depth zones (Table 3). Total root lengths of 50V-50T at RL5-10 depth zone and 80V-20T at RL10-15 depth zone were 68.60 and 33.03% longer than root lengths at 100V, respectively (Fig. 2. and Table 3). According to results obtained in triticale x common vetch intercropping trials, there was a negative

correlation between the ratio of triticale and root development of common vetch at various rooting depth zones. Triticale seems to suppress or compete with the roots of common vetch (Acar et al., 2006). The reasons may be various and may need in-depth physiological and molecular analysis to uncover the mechanism.

The Effect of Intercropping on Triticale Root Architecture

The highest value for the NOR in triticale was obtained in the 80T-20V ratio with 4.13 roots per plant and the lowest value was in 80V-20T with 3.27 per plant (Table 2). Triticale did not show any significant difference between control (100T) and intercropping ratios. It constituted the lowest group in terms of NOR, including all mixtures and 100T plantings, which is expected due to the fibrous rooting nature of triticale compared to tap

and lateral roots in common vetch. When the effect of intercropping with legumes evaluated for the NOR in triticale, it was seen that legumes did not limit triticale root growth (Table 2). The NLR and TLRL parameters were not taken into consideration for triticale since these traits are only related to taproot systems. It was determined that the TRL values of the triticale varied between 52.94 (80V-20T) and 76.34 cm (100T) per plant. Triticale in the 80T-20V, 50V-50T, and 80V-20T intercropping were statistically in the same group and formed the second group after 100T (Table 2). Intercropping seems to affect TRL in triticale, but it was not a strong effect to consider or the results of this study were biased due to genotype or other factors.

Intercropping Competition

The fibrous root structure of triticale developed faster than common vetch in the seedling stage. As previously reported, the root growth of cereal grains normally exceeds that of legumes (Gregory et al., 1995; Turpin et al., 2002). Cereals also do not show a clear advantage over legumes in terms of relative shoot or root yield (Streit et al., 2019). Seedling development of perennial forage plants may be slower and weaker compared to annual cereals at the early growth stages. Therefore, the competitiveness of these

seedlings against weeds, especially monocotyledons, may be slow. The seedlings of perennial legumes generally form a few leaves and use resources to develop a root system. At this stage, weeds may compete for water and minerals,

leading to yield losses (Acar et al., 2006). In these and similar cases, an annual plant that can compete with weeds (biological control) as a friendly plant and that can bring income in the first year might be selected (Tan and Serin, 2004). Abdel et al. (1991) reported alfalfa (*Medicago sativa*) and wheat cropping to increase the yield and quality of wheat and protect it from the winter colds. They determined that the grain yield of wheat increased by 130-160 kg da⁻¹ in mixed cropping plots, while the crude protein ratio and biomass yield of alfalfa were also increased. It is widely known that legumes support neighboring non-legume plants through nitrogen fixation. However, sharing the nitrogen with other species may cause the risk of not meeting its own nitrogen needs. Therefore, it is important to determine the ratio of non-leguminous species in the mixture for the highest grain yield and biomass production.

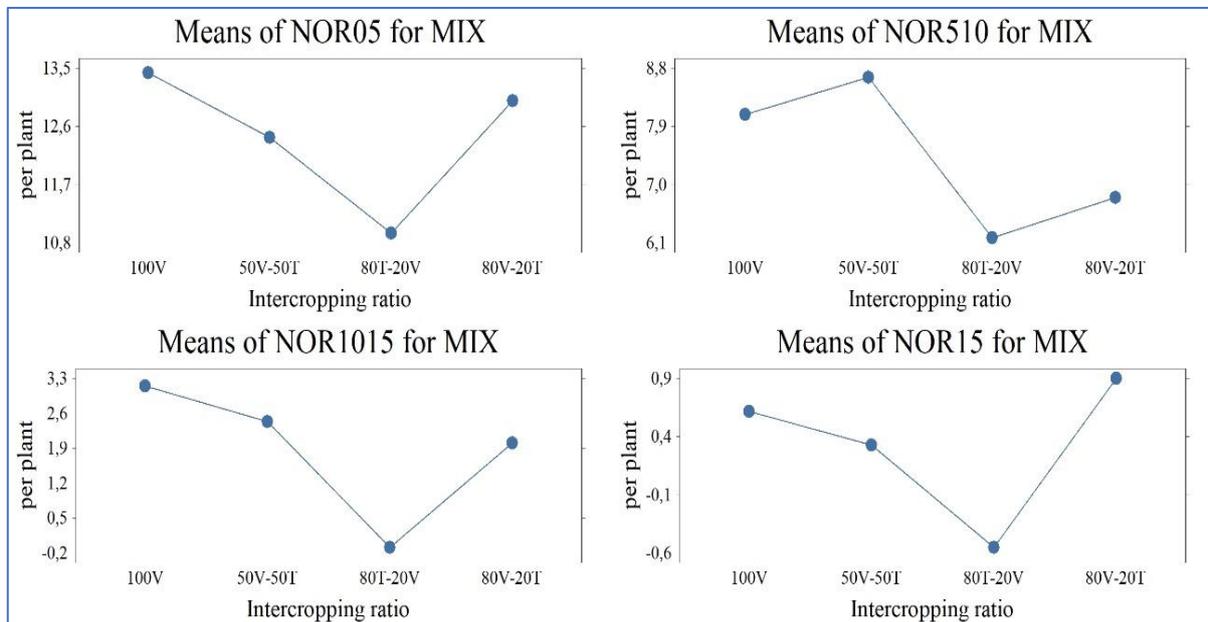


Figure 1. Mean values of number of roots (NOR) at depth zones for common vetch under monocropping and intercropping conditions in a semi-hydroponics plexiglass system

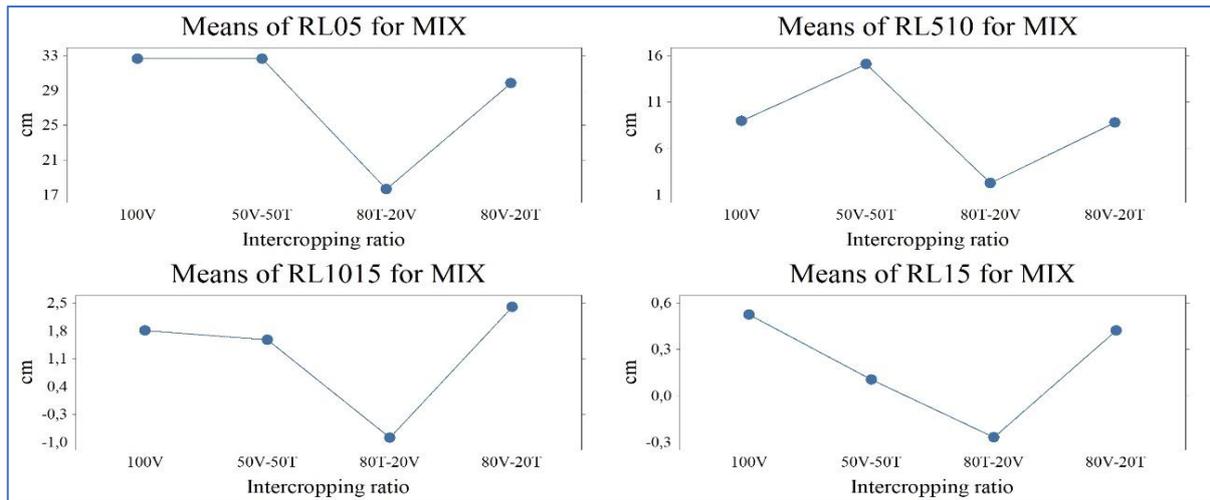


Figure 2. Mean values of total root length (RL) at depth zones for common vetch under monocropping and intercropping conditions in a semi-hydroponics plexiglass system

Table 3. Comparisons of root traits for common vetch under inter- and monocropping conditions

Means of RL 0-5 cm				Means of NOR 0-5 cm			
Mixture ratio	Mean	SE	Dif. (%)	Mixture ratio	Mean	SE	Dif. (%)
100V	32.62 ± 6.26		0.00	100V	13.43 ± 1.74		0.00
80V-20T	29.82 ± 4.60		8.59	80V-20T	13.00 ± 1.28		3.19
50V-50T	32.62 ± 6.26		0.02	50V-50T	12.43 ± 1.74		7.45
80T-20V	17.65 ± 6.78		45.91	80T-20V	10.95 ± 1.88		18.45
Means of RL 5-10 cm				Means of NOR 5-10 cm			
Mixture ratio	Mean	SE	Dif. (%)	Mixture ratio	Mean	SE	Dif. (%)
100V	8.94 ± 3.06		0.00	100V	8.09 ± 1.71		0.00
80V-20T	8.77 ± 2.24		1.91	80V-20T	6.80 ± 1.26		15.90
50V-50T	15.07 ± 3.06		-68.60	50V-50T	8.66 ± 1.71		-7.07
80T-20V	2.26 ± 3.31		74.74	80T-20V	6.18 ± 1.85		23.56
Means of RL 10-15 cm				Means of NOR 10-15 cm			
Mixture ratio	Mean	SE	Dif. (%)	Mixture ratio	Mean	SE	Dif. (%)
100V	1.81 ± 1.47		0.00	100V	3.14 ± 1.45		0.00
80V-20T	2.40 ± 1.08		-33.03	80V-20T	2.00 ± 1.07		36.36
50V-50T	1.57 ± 1.47		12.92	50V-50T	2.43 ± 1.45		22.73
80T-20V	0.21 ± 1.59		88.37	80T-20V	0.67 ± 1.57		78.68
Means of RL 15+ cm				Means of NOR 15+ cm			
Mixture ratio	Mean	SE	Dif. (%)	Mixture ratio	Mean	SE	Dif. (%)
100V	0.52 ± 0.51		0.00	100V	0.61 ± 0.85		0.00
80V-20T	0.42 ± 0.37		19.49	80V-20T	0.90 ± 0.63		-46.51
50V-50T	0.10 ± 0.51		80.39	50V-50T	0.33 ± 0.85		46.51
80T-20V	0.00 ± 0.00		100.00	80T-20V	0.00 ± 0.00		100.00

RL: Root length, NOR: Number of roots, SE: Standard error, Dif: Difference

Conclusion

This study evaluated the seedling root architectures of common vetch and triticale under monocropping and various intercropping ratios. The competition and inter-species interactions of root systems at the seedling stage are revealed in this preliminary study. According to our observations, triticale had a negative effect on the root development of common vetch, while triticale was not affected by the common vetch. The results

highlight the value of the topic and there is a need for in-depth evaluations with further species.

Conflict of Interest Statement: The manuscript's authors declare that they do not have any conflict of interest.

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