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Root trait variation within a wheat/faba bean intercrop system during the early growing stage

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Abstract

Intercropping with legumes can have various ecological benefits, such as contributing nitrogen to the system by symbiotically fixing atmospheric N_2. Faba bean (*Vicia faba* L.) has a large genetic diversity, and traits of agronomic interest have been identified and explored to improve crop performance. However, mapping root traits of different varieties of faba beans, and their response to changing environmental conditions, such as neighbouring plants, or water and nutrient stress, is not well established. Characterising root traits of different faba bean cultivars and their response to neighbouring plants, can contribute to identifying cultivars most suitable for intercropping.

This study examined root trait variation in multiple faba bean genotypes, identified differences in root traits between monocropped and intercropped roots in a wheat/faba bean intercrop system, and lastly, investigated the response of these root systems to water stress. Twelve faba bean genotypes were selected for comparison of root characteristics when grown individually in soil. Clear differences in root system size were found after two weeks of growth. However, these faba bean genotypes did not vary significantly in rhizosheath weight or root hair length.

Four faba bean cultivars varying in their root traits were then selected and intercropped with wheat. Overall, faba bean plants were negatively affected by being intercropped with wheat; intercropped plants had reduced root weight and total root length. Root characteristics of wheat were unaffected when grown with the different faba bean genotypes. Water stress reduced root growth of both faba bean and wheat, but intercropped faba bean plants were less affected by the drought treatment. The rhizosheath and root hair length of all plants were enhanced in response to the drought treatment.

Both faba bean and wheat appeared to lack plasticity in various root traits during the early stages of root growth when intercropped. Upon experiencing water stress, all plants enhanced their rhizosheath and lateral root hair length, but again these changes did not differ strongly with respect to the presence of neighbouring plants. To select genotypes most suitable for intercropping, further research should identify root traits that lead to improved outcomes when crop species are grown together. Genotypes showing extreme differences in root traits could be tested for their performance in intercrop systems, and their contribution to beneficial outcomes, such as increased yield, improved soil quality, and pest and disease suppression.

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I would also like to thank Lawrie Brown at the JHI for providing assistance with setting up my experiments and explaining how to analyse the roots; and Marta Maluk for providing the bean seeds and helping with seed germination.

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1. Introduction

Key to agricultural intensification has been the use of high-yielding crop varieties, often grown in monocultures, using chemical fertilizers and pesticides, non-renewable energy, and mechanisation (Therond et al., 2017). Intensive high-yielding agriculture has contributed considerably to the enormous increase in food production since ‘the Green Revolution’ in the 1950 and 60s (Evenson and Gollin, 2003). Concerns, however, have arisen over the long-term sustainability and environmental impacts of these intensive agricultural systems. Negative environmental effects include biodiversity loss, soil erosion, pollution of waterways and waterbodies, and large greenhouse gas emissions (Foley et al., 2011; Vermeulen et al., 2012). Concurrently, concerns about feeding a rapidly growing world population and reducing hunger remain ever-present. On top of that, food production and food security will be greatly influenced by increasing climatic variability. Shifting temperature and precipitation patterns are expected to lead to changes in nutrient cycling and soil moisture content, crop–weed interactions, and shifts in pest occurrences and plant diseases (Fuhrer, 2003). Thus, agriculture now has to face these intertwined challenges; meeting the growing demand for food, while reducing its environmental impact, and also developing adaptation and mitigation strategies towards climate change (Beddington et al., 2012; Raseduzzaman and Jensen, 2017).

Many suggestions for developing more resilient and sustainable agricultural systems have been proposed, including organic farming, conservation agriculture or precision agriculture (Alcon et al., 2020). Another approach is crop diversification, which can be achieved by increasing the number of cultivated species and varieties grown within a farm or region. Plant diversity is an essential agroecological principle and can potentially be used to promote resilient and sustainable production systems. Diversification of agricultural systems has been found to promote pest and disease control, pollination services, soil quality, and crop resilience (Kremen and Miles, 2012; Altieri et al., 2017; Hunt et al., 2019). Intercropping, also referred to as mixed cropping or polyculture, is the practice of cultivating two or more crops simultaneously on the same field. This agroecological practice enhances farm diversity and has been receiving renewed interest in recent years.

The component crops of an intercropping system are often from different species and different plant families, although they can be different varieties or cultivars of the same crop grown in variety mixtures (Lithourgidis et al., 2011). Intercropping has been widely practised by farmers for millennia and is still present in various cropping systems around the world. For example, in Mexico and Guatemala farmers often intercrop maize with beans, squash and other crops, according to ancient *milpa* traditions (Isakson, 2009). In rural sub-Saharan Africa, intercropping is a common practice aimed at minimizing risks associated with monocultures, with the predominant crop combinations being maize, bean/cowpea and pumpkin (Bedoussac et al., 2018).

Even though most crops in Western countries today are grown as sole crops, there is renewed interest in adopting intercropping practices, due to the various positive outcomes that can be associated with intercrop systems (Bedoussac et al., 2018). Intercropping systems have been shown not only to boost crop productivity (Qin et al., 2013) and improve land utilization efficiency (Agegnehu et al., 2008), but can also enhance soil quality (Cong et al., 2015), suppress pests, diseases and weeds (Jensen et al., 2015), increase yield stability (Raseduzzaman and Jensen, 2017), and reduce dependency on fertilisers and risks of nitrate leaching compared with sole cropping (Corre-Hellou et al., 2006; Hauggaard-Nielsen et al., 2003). These benefits, however, are not always achieved partly due to incomplete knowledge about the plant characteristics that optimise interactions between intercropped plants.

Features of an intercrop system differ around the world, depending on local climate, soil conditions, economic situation, and preferences of the local community (Lithourgidis et al., 2011). The different types of intercrop systems can be categorised based on the spatial and temporal variation of the crop mixture. These types of intercropping are usually divided into four main categories (Fig. 1):

1. Mixed intercropping or mixed cropping: the cultivation of crops that are randomly mixed in the available space with no distinct row arrangement.
2. Row intercropping: two or more crops are cultivated in separate alternate rows.
3. Strip cropping: several rows (= strip) of crops are alternated with several rows of another crop. Strips are wide enough to allow the use of modern equipment, but narrow enough for the crops to interact.
4. Relay intercropping: component crops are not sown and harvested at the same time, but the life cycle of one crop overlaps that of the other.

Next to these main four types, other types of intercropping are practiced. Undersowing is a type of intercropping in which a crop without direct economic importance is grown in the same field as the main crop (Theunissen and Schelling, 1996). This is the case for living mulches, which are sown either before or with a main crop and maintained as a living ground cover throughout the growing season (Hartwig and Ammon, 2002). Furthermore, intercrop systems can also include perennial plants such as trees. For example alley cropping, a kind of agroforestry system, involves the planting of timber, fruit, or nut trees in single or multiple rows, with other crops cultivated in the alleyways (Garrett et al., 2015).

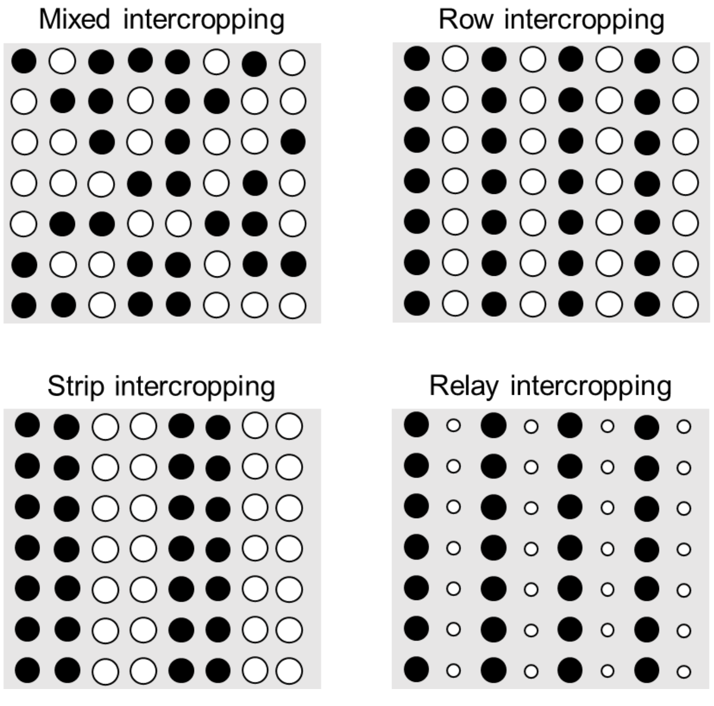

Fig. 1 Four main intercropping patterns, adapted from Ekanayake et al. (1997) and Geilfus (2019)

1.1. Intercropping of legumes and cereals

Various researchers have investigated the practice of intercropping cereals and legumes. Many authors consider legumes as key species in intercropping systems, due to their ability to fix nitrogen (N) from atmospheric N_2, through symbiosis with N-fixing bacteria. Legumes fix atmospheric N only when soil N is limited due to low availability or increased competition for N (Duchene et al., 2017; Raseduzzaman and Jensen, 2017). The latter is the case in intercropping systems, especially when legumes are intercropped with strongly N-competitive crops, such as cereals. Cereals, with high N demand and fast and deep root growth, can profit from intercropping with legumes. Within a cereal/legume intercropping system, legumes increase their reliance on symbiotic N-fixation (Li et al., 2009), and as a result, increased N becomes available for the cereal (Andersen et al., 2005; Corre-Hellou et al., 2006). Cereal/legume systems have been found to have additional benefits, such as an increase in grain protein content for the cereal (Jensen et al., 2006), an increase in phosphorus availability (Hinsinger et al., 2011; Latati et al., 2014), improved microbial activity and biomass (Tang et al., 2014), and an increase of nutrients released into the soil solution, due to legumes acidifying the soil rhizosphere (Li et al., 2008).

1.2. Faba bean (*Vicia faba* L.)

Faba bean, sometimes referred to as broad bean, horse bean or field bean, is a protein-rich legume, widely used for feed and food because of the high nutritional value of its seeds (Karkanis et al., 2018). Faba bean has the ability to grow in various climatic zones and is well adapted to most climatic areas of Europe (Crépon et al., 2010). The main producers of faba bean are China (2 Mt), Europe (1 Mt - principally U.K., France, Spain, and Italy) and Ethiopia (0.4 Mt) (Duc et al., 2010; Jensen et al., 2010). *V. faba* can be classified into three main botanical varieties according to seed size: 1) *V. faba* var. *major* with large seeds, 2) *V. faba* var. *minor* with small seeds, and 3) *V. faba* var. *equina* with medium seeds (Karkanis et al., 2018). Faba bean germplasm can also be categorised into spring and winter types, according to frost tolerance, sowing time, and time of flowering and maturity (Link et al., 2010).

Ecological benefits of introducing faba bean to cropping systems, include (i) the ability to contribute N to the system by symbiotically fixing N_2, (ii) diversification of the system, leading to a reduction in pests, diseases and weeds, and (iii) increase of soil phosphorus availability to subsequent crops (Köpke and Nemecek, 2010; Fouad et al., 2013). Faba bean is highly efficient in establishing symbiosis with specific *Rhizobium* bacteria, and in turn very effective in performing biological N-fixation (Karkanis et al., 2018). The amount of N that can be fixed is dependent on several factors, such as cultivar, local farming practices, soil properties, and the presence of symbiotically effective rhizobia in the soil (Argaw and Mnalku, 2017). Faba bean, along with soybean, has relatively high levels of N_2-fixation of up to 200 kg N ha^{-1}, compared with pea and lentil (85 kg N ha^{-1}) and chickpea and common bean (50 kg N ha^{-1}) (Hardarson and Atkins, 2003; Neugschwandtner et al., 2015; Argaw and Mnalku, 2017). The introduction of legumes into N-fertilizer-based cropping systems will lead to lower demand of mineral N-fertilizers and thus to a reduction of fossil energy used for N-fertilizer manufacture, transport and spreading, and a reduction in accompanying CO_2 emissions (Nemecek et al., 2008), making them an important component of future low-carbon agriculture.

A large genetic diversity in faba bean has been developed over several centuries and includes local landraces, open-pollinated populations, inbred lines, and cultivars. To preserve these genetic resources, while reducing and slowing down its erosion, *ex-situ* gene banks have been developed. At present, more than 38,000 accessions of faba bean germplasm are conserved globally in these gene banks (Duc et al., 2010). Due to this wide genetic variability, efforts have been made to evaluate germplasm for the utilization for crop improvement. Traits of agronomic interest have been discovered for faba bean, including biotic and abiotic stress tolerance, the efficiency of symbiotic nitrogen fixation, yield potential and seed composition (Duc et al., 2010). Despite this, hardly any focus is put on characterising the root system of *V. faba*, even though it is known that root traits of European accessions vary profoundly (Zhao et al., 2018).

1.3. Root traits for intercropping

Despite the potential benefits associated with intercropping, significant gaps remain in our understanding of belowground interactions that govern the outcomes when crop species are grown together. Many plant interactions take place belowground and are mediated by the roots. The relative contribution of belowground interactions to yield advantages in intercropping, through increased water and nutrient uptake, can be substantial (Mu et al., 2013; Zhang et al., 2001). However, crop varieties currently used in intercropping systems are often modern varieties bred for monocultures, since crop breeding programs for intercrops are rare. Modern crop varieties are not necessarily the most suitable for intercrop systems, as they have been designed and bred for monoculture cropping, typically with high input levels, and plant traits that are considered beneficial in these conditions may not be optimal for intercropping (Lithourgidis et al., 2011). Finding ways to reduce competition and increase complementarity and facilitation between intercropped plants is an essential quest to maximise intercrop performance. Belowground competition for soil resources may be even more intense than aboveground competition for light. Therefore, identifying specific root traits that can promote beneficial plant-plant interactions and minimise intercrop competition, and thereby enhance intercrop productivity, would be a step forward to design crops suitable for intercropping.

Quantifying root trait variation of different faba bean genotypes, and their response to intercropping, can lead to a better understanding of which cultivars might be suitable for an intercrop system. Not only do root traits vary between different genotypes of *V. faba*, root systems are also known to show plasticity in relation to changing environmental conditions, such as neighbouring plants (Bardgett et al., 2014). Root plasticity is the ability to change and adapt in response to variations in the belowground environment. It is thus likely that root traits of different varieties of faba bean, observed under sole cropping will change when intercropped. Furthermore, the impact of climate change, with shifting temperature and precipitation patterns, will lead to changes in nutrient cycling and soil moisture content (Fuhrer, 2003). Under water or nutrient stress, plants will further modify their root behaviour, and the response of intercropped plants to these stresses might be different than the response of monocropped plants. For example, intercropping faba bean with wheat under low water availability increased overall nodulation in deeper soil layers (Bargaz et al., 2015); intercropping soybean and wheat under phosphorus deficient conditions increased microbial diversity and increased root allocation to deeper soil layers for intercropped wheat (Bargaz et al., 2017).

The objective of this study was to (i) examine root trait variation in multiple faba bean genotypes, (ii) identify how roots in a wheat/faba bean intercrop system respond to intercropping compared with monocropping; in order to find which root traits show plasticity, and (iii) investigate the response of these root systems to water stress. Various root traits were studied, including root weight, total root length, rhizosheath weight, root hair length and average root diameter. **Root weight** and **total root length** give an indication of the size of the root system and the consequent soil volume explored, which can indicate the amounts of soil nutrients and water potentially available to the plant. The **rhizosheath**, defined as the weight of soil that adheres strongly to roots on excavation, plays an important role in protecting the root from various abiotic stresses, including drought and heat stress, and nutrient deficiencies (Brown et al., 2017). **Root hairs**, which are extensions of root epidermal cells specialised for nutrient uptake (Jungk, 2001), are considered important for the acquisition of relatively immobile nutrients and the interaction of roots with soil microbes (Zhu et al., 2010). Root hairs enmesh soil particles around the root and are essential for the formation of rhizosheaths (Brown et al., 2017). Lastly, root systems are composed of a heterogeneous assembly of roots with a variety of diameters. Roots with a relatively large diameter are involved in plant storage of resources and the transport of water and nutrients, whereas fine roots are responsible for the absorption of soil resources (Zadworny et al., 2016).

A shift in the **average diameter** of the root system might indicate a change in the root system’s overall function with respect to nutrient uptake capacity; a smaller average root diameter might signify a larger fraction of absorptive roots.

2. Materials and methods

This study consisted of three separate experiments, one for each objective of the study. All experiments were conducted in a glasshouse at the James Hutton Institute, Scotland (56° 46’ N and 3° 6’ W) between February and July 2020. For each experiment, plants were grown in special containers allowing easy access to plant roots. These Rootrainers have a volume of 175 cm^3 (12 cm deep × 4 cm × 4 cm tapering) (Deep Rootrainers™, Ronaash Ltd., Kersquarter, Kelso, UK) (Fig 2A and B). For all three experiments, Rootrainers were filled with with approximately 140g of soil collected from James Hutton Institute land which was typical of arable soil of the region and defined as a Cambisol (FAO, 1994).

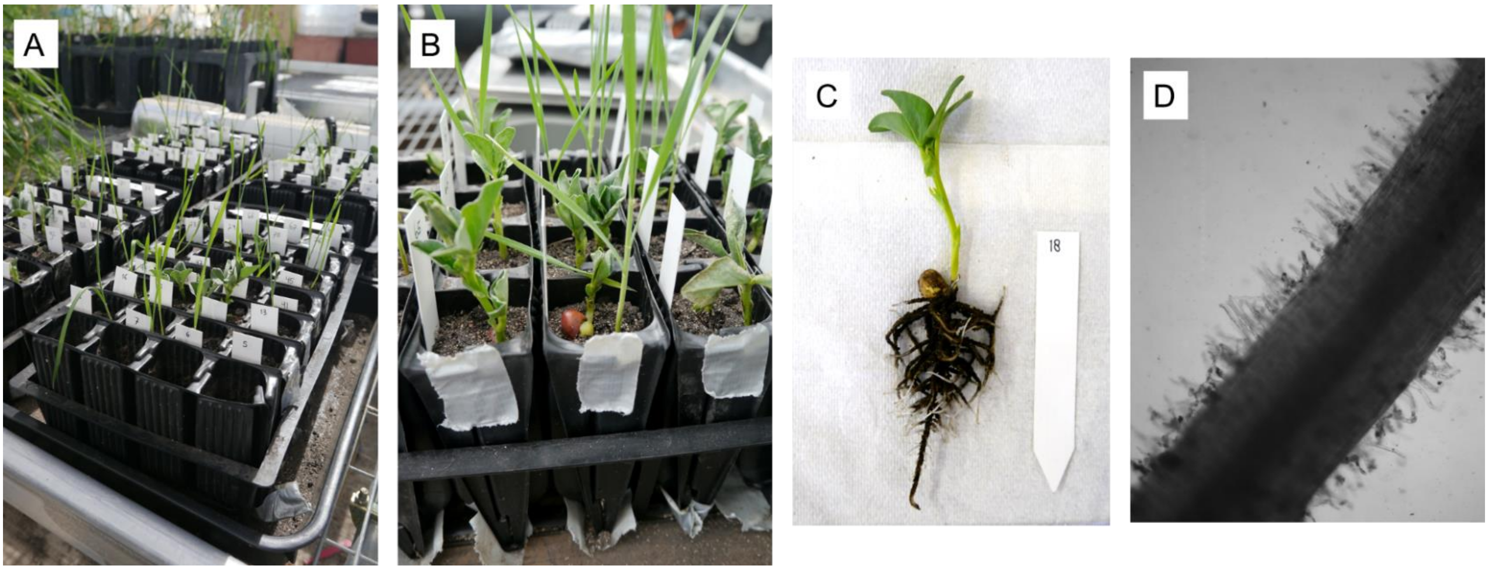

Fig 2. Rootrainers used for the three experiments; Rootrainer set up used for the third experiment, showing both faba bean and wheat plants (a and b). Harvested faba bean plant (cv. Fanfare) with its rhizosheath intact (c). Root hairs on the lateral root of a faba bean plant (cv. Fanfare) used for determining root hair length

2.1. Experiment 1: root trait variation in multiple faba bean genotypes

The first experiment aimed to investigate root trait variation in multiple faba bean genotypes. Twelve Faba bean genotypes were selected for comparison of their root characteristics. This selection included various commercial cultivars (Arthur, Boxer, Clipper, Fanfare, Fuego, Honey, Maris Bead, V134, Vertigo), a mutant with absence of nodules (Ascott Nod- mutant) (Duc, 1995), parent 1 of a Recombinant Inbred Line (RIL) population (*V.faba* var. *paucijuga* accession 172) a small seeded landrace from Afghanistan, and parent 2 of the RIL population (*V. faba* cultivar Optica) (Ramsay, 1997).

Ten seeds of each genotype were pre-germinated on 0.5% distilled water agar plates for four days and five germinated seeds of each genotype were then transferred to the Rootrainers. Genotypes were sown in a completely randomized design with five replicates. Plants were grown during February 2020 in a glasshouse at 18/14 °C (day/night) with an approximate 16 h day-length at minimum light intensity of 200 µE ensured by supplementary lighting. Containers were watered daily to a predetermined weight to maintain a moisture content close to 80% field capacity.

Harvest and measurements

Methods used for plant harvest and roots analysis were adapted from Brown et al. (2017) and Haling et al. (2014). Plants were harvested 10 days after sowing by opening the Rootrainers, removing the plants and shaking each plant gently by hand until no more bulk soil became detached. The rooting depth of each plant was measured with a ruler. Those roots that had reached the bottom of the container were given a rooting depth of 11 cm. Roots were separated from the shoots and weighed with soil attached. The remaining soil attached to the roots was considered to be the rhizosheath (Fig 2C). This soil was then carefully washed from the roots with water over a sieve. The roots were dried with tissue paper and reweighed to establish the rhizosheath mass by subtraction. Shoots and remaining seeds were oven-dried for three days at 70 °C and shoot dry weight and remaining seed dry weight were recorded.

The roots were stored at 4 °C in 50% ethanol after which they were scanned (400 dpi; Epson Expression 1640xL flatbed scanner) and analysed for total root length and average root diameter using the program WinRHIZO (Regent Instruments, Quebec, Canada). Root hair length was determined using a compound light microscope (Leica MZF111, Leica Microsystems, Bannockburn, IL, USA) with camera attachment (Leica DC480). Root hairs on both the taproot as well as a lateral root were analysed. An image of the taproot at approximately 3 to 4 cm from the root tip was taken. A lateral root close to the root tip was selected for measurement (Fig 2D). Measurements of the root hair length was aided by ImageJ software (US National Institutes of Health, Bethesda, Maryland, USA). A total of six individual root hair measurements were taken per root image. The mean root hair length was then calculated for each taproot and lateral root. After analysing all the roots, they were oven-dried for three days at 70 °C and root dry weight was recorded.

2.2. Experiment 2: effect of intercropping on root traits of faba bean and wheat

A second experiment was conducted to investigate how intercropping faba bean and wheat (*Triticum aestivum* L. cultivar Talisker) would affect root traits. For this experiment a smaller selection of four faba bean genotypes was made, namely Fuego, Vertigo, Fanfare and Boxer. Plants were grown either as a single plant, two of the same cultivar together (simulating monoculture), or as an intercrop (one faba bean cultivar with one wheat plant).

Plant combinations were divided over three blocks, with a delay in sowing of one week between each block. Within each block, plants were sown in a completely randomized design, with two replicates per block, resulting in a total of six replicates for each plant combination. Before the seeds were germinated, all seeds were rinsed with tap water and left to soak in water for about an hour. Seeds were then germinated in similar fashion to experiment 1 and transferred to the Rootrainers. During May to July 2020, plants were grown under similar glasshouse conditions as experiment 1, and watered daily to maintain a moisture content close to 80% field capacity.

Harvest and measurements

The plants were harvested 27 days after sowing as described for experiment one; rhizosheath mass was determined, roots were carefully washed and root fresh weight was measured, and shoot and seed dry weights were recorded. For the plant combinations where two plants were growing in the same container (monocropped and intercropped), roots were carefully separated from each other by gently teasing the two root systems apart. Roots were stored at 4 °C in 30% ethanol during the period of analysis. The roots were analysed in a similar way as experiment 1; roots were scanned, scanned images were analysed, and the root hair length of the lateral roots was measured. After all roots were analysed, the roots were oven dried and root dry weight was recorded.

2.3. Experiment 3: effect of water stress on root traits in a wheat/faba bean intercrop system

The third experiment was conducted to investigate the response of faba bean and wheat roots to water stress, and to see if these root systems would respond differently to water stress when intercropped. For this experiment, the faba bean cultivars Fuego and Vertigo were used. These cultivars were chosen because they are popular commercial cultivars of faba bean with a large seed stock available at the James Hutton Institute.

Plants were grown in a similar way as experiment 2; either as a single plant, two of the same cultivar together (simulating monoculture), or as an intercrop (one faba bean cultivar with one wheat plant). Again, seeds were pre-germinated and then transferred to the Rootrainers and grown under same conditions as in previous experiments during July/August 2020.

Plant combinations were divided over four trays. Two trays, containing six replicates of each plant combination, were assigned as the control group, and received normal daily watering throughout their growing period. The plants in the other two trays, also containing six replicates of each plant combination, were assigned to experience water stress. For the first 13 days, all plants in these trays were watered daily, after which they did not receive any water for 7 days, thereby simulating water stress.

Root traits were analysed as described for experiments 1 and 2.

2.4. Data analysis

For all experiments, the rhizosheath was determined on a per unit root length basis by dividing the rhizosheath weight by the total root length of each plant.

Before the data analysis was carried out, the normality test and variance homogeneity test of the data were carried out by the Shapiro-Wilk and Levene tests, respectively. For experiment 1, the data for root fresh weight and total root length did not fit the assumptions of the normality criteria, and a non-parametric alternative to a one-way ANOVA (Kruskal-Wallis rank sum test) was applied to these two root characteristics. All other root traits of experiment 1 adhered to normality assumptions and were thus analysed using an analysis of variance (ANOVA) using the software R version 3.6.3.

The Spearman correlation method (non-parametric) was used to identify how the measured root characteristics influence plant growth. All measured root traits were plotted against the total plant dry weight, which was calculated as the sum of root and shoot dry weight.

For experiment 2, the data for the faba bean rhizosheath weight and root diameter did not fit the assumptions of the normality criterion, and were therefore log transformed. All faba bean root traits of experiment 2 were analysed using a two-way ANOVA, with factors genotype and plant combination. The root traits of wheat were analysed with a one-way ANOVA, with the factor plant combinations categorized as single, monocrop, intercrop with Boxer, intercrop with Fanfare, intercrop with Fuego, and intercrop with Vertigo.

For experiment 3, none of the measured root traits for the faba bean plants, except for rhizosheath weight, fitted the assumptions of the normality criteria and were therefore log transformed. The faba bean root traits of experiment 3 were analysed using a three-way ANOVA, with factors genotype (Fuego and Vertigo), plant combination and water treatment. For the wheat plants, root fresh weight and rhizosheath weight did not meet the normality criteria, and were log transformed. Wheat root traits were analysed with a two-way ANOVA, with factors plant combination and water treatment.

The significance threshold for all analyses was set at 0.05.

Land equivalent ratio calculations

The land equivalent ratio (LER) reflects the yield of two species in intercropping compared with monoculture. The LER is calculated as below, whereby the yield of intercropped and monocropped faba bean are represented as Y_{ib} and Y_{mb}, respectively, and the yields of intercropped and monocropped wheat are represented as Y_{iw} and Y_{mw}, respectively. LER is usually used for crops grown in the field, with yield expressed as kg ha^{-1}.

$$\text{LER} = \frac{Yib}{Ymb} + \frac{Yiw}{Ymw}$$

Since the yield of the plants used in this experiment cannot be expressed on a kg ha^{-1} basis, the shoot dry weight of each plant was used as its yield, as this represents harvestable biomass. When LER is greater than one, this indicates intercropping to be more efficient than sole cropping in terms of land use.

3. Results

3.1. Experiment 1: root trait variation in multiple Faba bean genotypes

The selection of faba bean genotypes showed clear differences in their root characteristics. Unfortunately, not all beans germinated or grew; none of the Maris Bead beans germinated, and 2 out of the 5 var. *paucijuga* beans and 2 out of the 5 V134 beans also did not grow.

Root size
Root fresh weight was significantly (H(10) = 36.25, $p < 0.001$) different between genotypes and ranged from 0.09 g (var. *paucijuga*) to 1.5 g (Optica) per plant (Fig. 3). The total root length was also significantly (H(10) = 36.72, $p < 0.001$) different between genotypes and spanned a similar magnitude of range, from 10 cm (var. *paucijuga*) to 202 cm (Fanfare) per plant (Fig. 4). Out of all the studied genotypes, *V.faba* var. *paucijuga*, and the cultivar Honey had the smallest root systems, as can be seen from their root fresh weight and total root length (Fig. 3 and 4). Fanfare and Optica were on the other end of the scale, with the largest root systems.

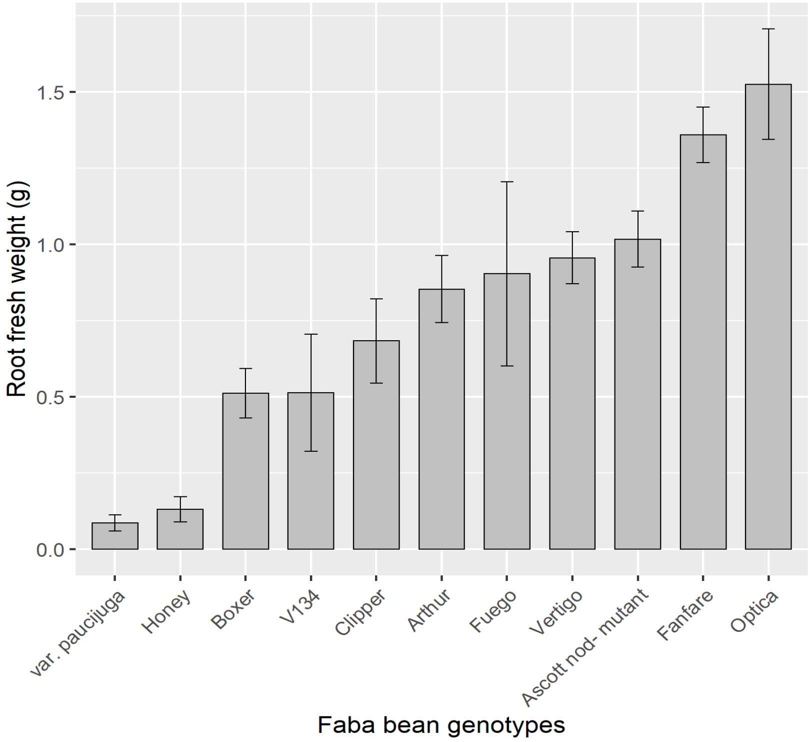

Fig 3. Mean root fresh weight for several faba bean genotypes. Error bars show ± Standard Error (SE). (n=5, except for var. paucijuga and V134 where n=3)

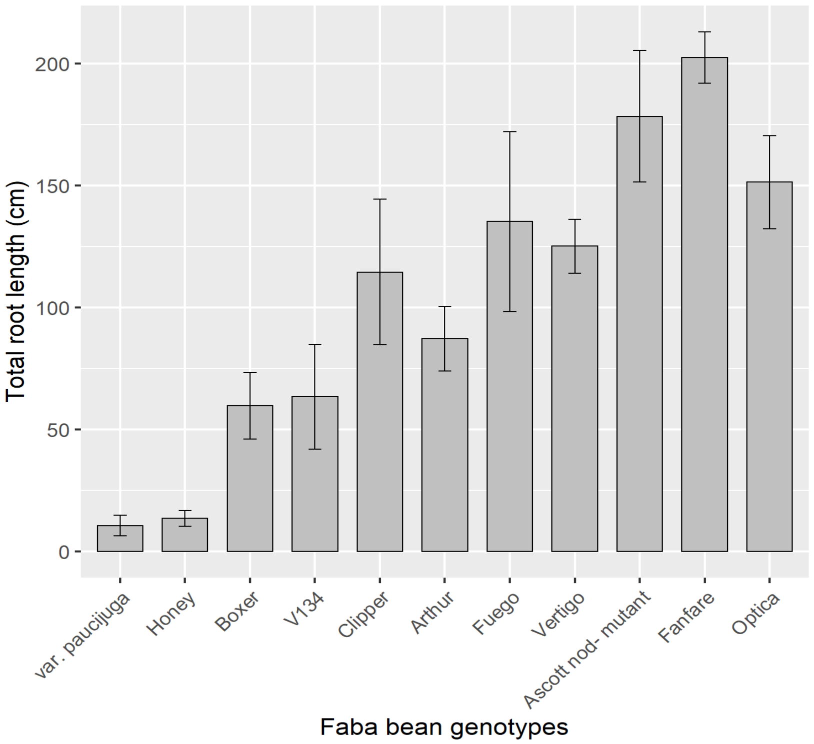

Fig 4. Mean root length for several faba bean genotypes. Error bars show ± SE. (n=5, except for var. paucijuga and V134, where n=3)

Rooting depth

Most of the faba bean genotypes grew their taproot all the way to the bottom of the Rootrainer. Consequently, this constraint prevented detection of potential differences in rooting depth. Only the bean plants with the smallest root systems (var. *paucijuga* and Honey) did not reach the bottom of the container. Var. *paucijuga* grew on average 3.5 cm deep, and Honey grew on avergae 3.0 cm deep.

Rhizosheath weight and root hair length

Although the root system size of the faba bean plants varied significantly, this variation was not observed in the rhizosheath weight per unit root length (Fig. 5). On average, the faba bean plants had a rhizosheath weight of 0.009 g of soil per cm root length. No significant differences ($F_{10,39} = 1.01$, $p = 0.45$) in rhizosheath weight were found between the faba bean genotypes.

No significant differences ($F_{9,33} = 1.57$, $p = 0.16$) in root hair length of the lateral roots were found between the genotypes. A trend towards significance was found for the root hairs on the taproot ($F_{10,39} = 1.99$, $p = 0.06$) (Fig. 6), indicating that there is variation in the root hair length of the taproot for the various faba bean genotypes. Root hairs on the lateral roots of the faba bean plants had an average length of 0.34 mm, and the root hairs on the taproot had an average length of 0.41 mm.

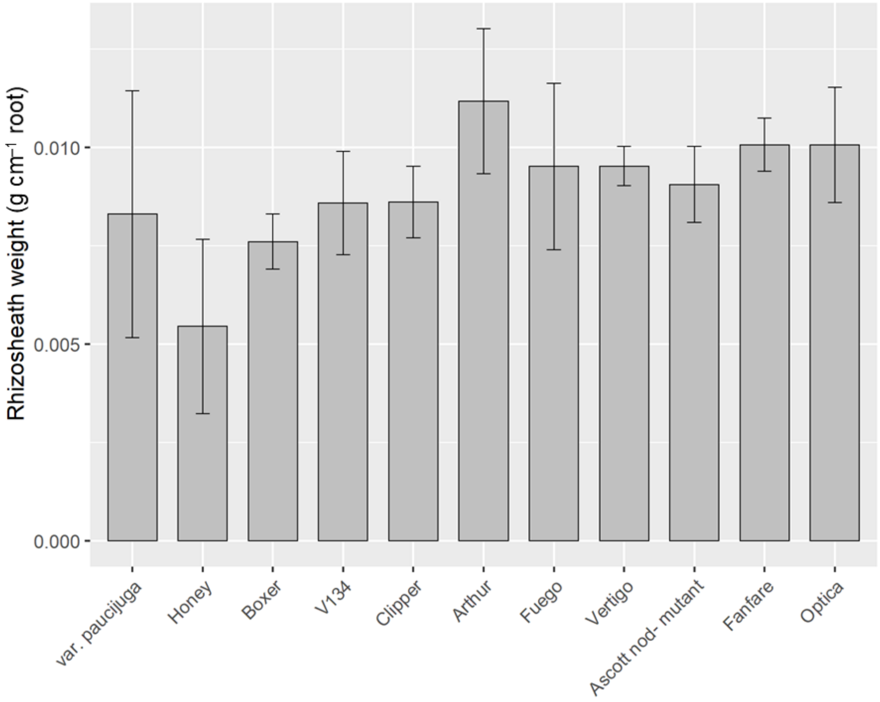

Fig. 5 Soil attached to the root system after shaking (rhizosheath weight) for several faba bean genotypes, presented on a per unit root length basis. Faba bean genotypes are ordered based on root weight, ascending from small to large. Error bars show ± SE. (n=5, except for var. paucijuga and V134 where n=3)

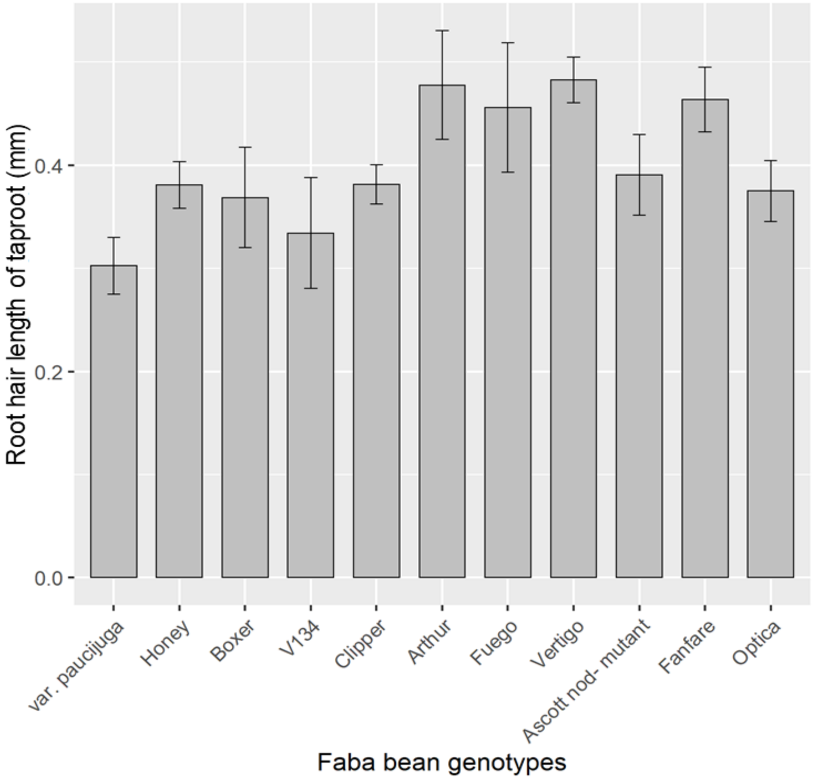

Fig 6. Average root hair length of the taproot for several faba bean genotypes. Faba bean genotypes are ordered based on root weight, ascending from small to large. Error bars show ± SE. (n=5, except for var. paucijuga and V134 where n=3)

Average root diameter

Average root diameter was significantly ($F_{10,40}$ = 3.08, p = 0.005) different between the faba bean genotypes. However, out of all the genotypes, only Honey was found to have a significantly smaller root diameter than Arthur ($p < 0.01$) and Optica ($p < 0.01$) (Fig. 7).

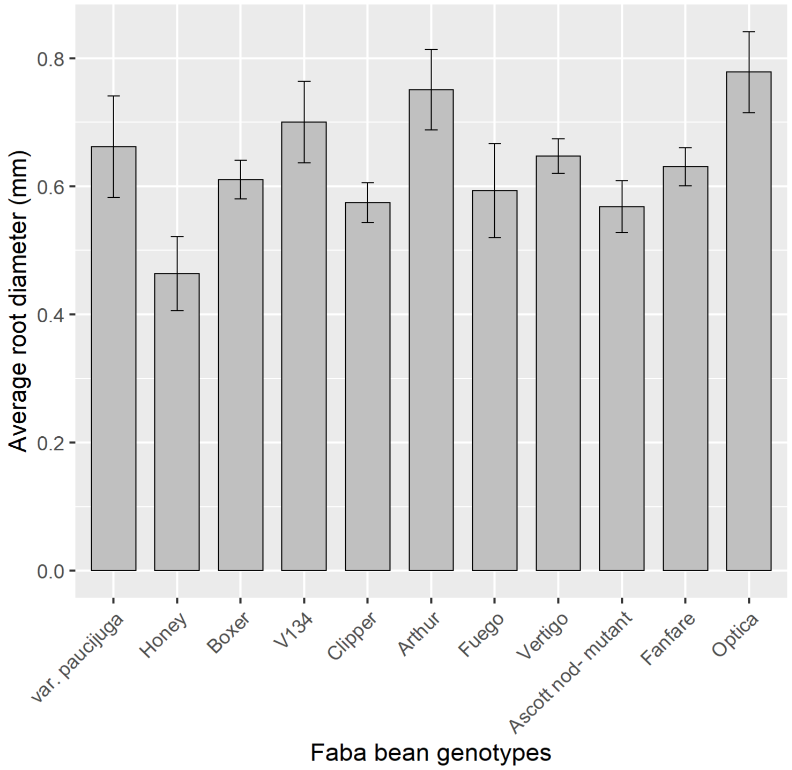

Fig. 7 Average root diameter for several faba bean genotypes. Faba bean genotypes are ordered based on root weight, ascending from small to large. Error bars show ± SE. (n=5, except for var. paucijuga and V134 where n=3)

Correlation between root traits and plant growth

Most of the beans had a seed length ranging between 1.4 and 2.0 cm (Fig. 8a), and within this range, no clear relation between seed length and total plant dry weight can be observed. However the smallest and the biggest seeds grew into the smallest and biggest plants, respectively, indicating that seed size has an effect on plant growth only for the exceptionally small or large seeds.

Total root length was positively correlated with plant dry weight (Fig. 8b); plants with a higher value of total root length also had a higher value of total plant dry weight. The correlation between rhizosheath weight and plant dry weight (Fig. 8c), and for average root diameter (Fig. 8d) were less clear. The length of the root hairs on the lateral roots of the bean plants appeared to correlate with total plant dry weight; plants with longer root hairs accumulated more dry matter (Fig 8e). For the root hair length on the taproots, this correlation was again less clear (Fig. 8f).

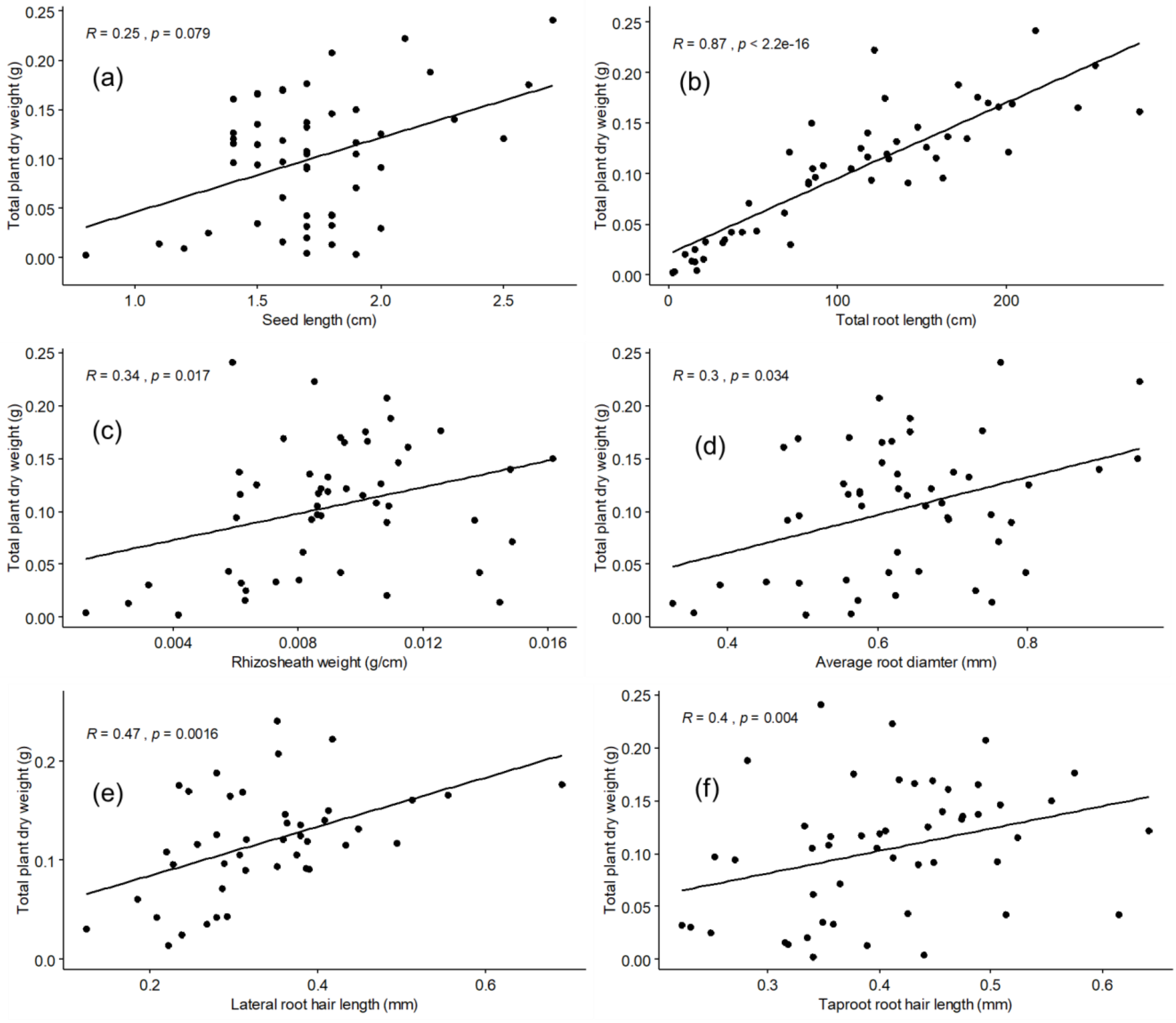

Fig. 8 Correlation graphs of all measured root characteristics against total plant dry weight; a) seed length, b) total root length, c) rhizosheath weight, d) average root diameter, e) lateral root hair length, and f) taproot root hair length. R values are Spearman correlation coefficients.

3.2. Experiment 2: effect of intercropping on root traits of faba bean and wheat

Intercropping with wheat had a mainly negative effect on the root growth of the faba bean plants; intercropped bean plants had a reduced root weight and a reduced total root length (Fig. 9). Root fresh weights of single plants were greatest (on average 0.70 g), whereas monocropping (on average 0.46 g) and intercropping (on average 0.29 g) reduced root weights in comparison. Similarly, total root length was largests for single plants (on average 77.5 cm), monocropping only slightly reduced the root length (on average 60.0 cm), and intercropped bean plants had the shortest root lengths (on average 27.9 cm).

For both root fresh weight and total root length, only plant combinations (single, mono- and intercropped) had a significant effect ($F_{2,70} = 11.4$, $p < 0.0005$; $F_{2,66} = 6.24$, $p = 0.003$, respectively). There was no significant difference between faba bean cultivars, and no interaction between cultivar and plant combinations for root weight and root length.

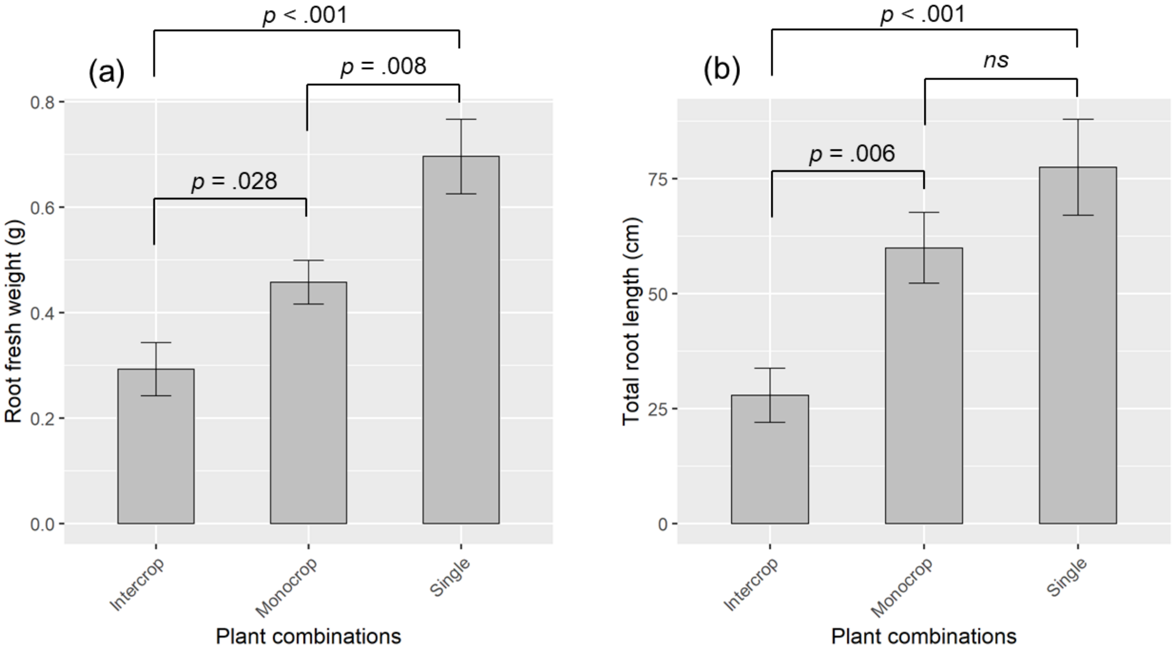

Fig. 9 Mean root fresh weight (a) and total root length (b) for faba bean when either grown alone (single) with the same cultivar (monocrop) or with wheat (intercrop). Error bars show ± SE.

Rhizosheath weight and root hair length

There was no significant effect of plant combination on either rhizosheath weight ($F_{2,66} = 0.8$, $p = 0.43$) or lateral root hair length ($F_{2,60} = 1.6$, $p = 0.21$). An interaction effect was found between genotype and plant combination for rhizosheath weight ($F_{6,66} = 2.6$, $p = 0.03$), indicating that the four faba bean genotypes change their rhizosheath weight differently from each other when grown with plant neighbours (Fig. 10).

For Boxer, the monocropped plants had a smaller rhizosheath weight than the single-grown plants ($p = 0.02$) and the intercropped plants ($p = 0.08$). Fuego and Vertigo displayed a similar pattern across the three plant combinations (Fig. 10). For Vertigo, single grown plants tended ($p = 0.10$) to have a higher rhizosheath weight than the intercropped plants. For Fanfare, no significant differences in rhizosheath weight between the different plant combinations were found.

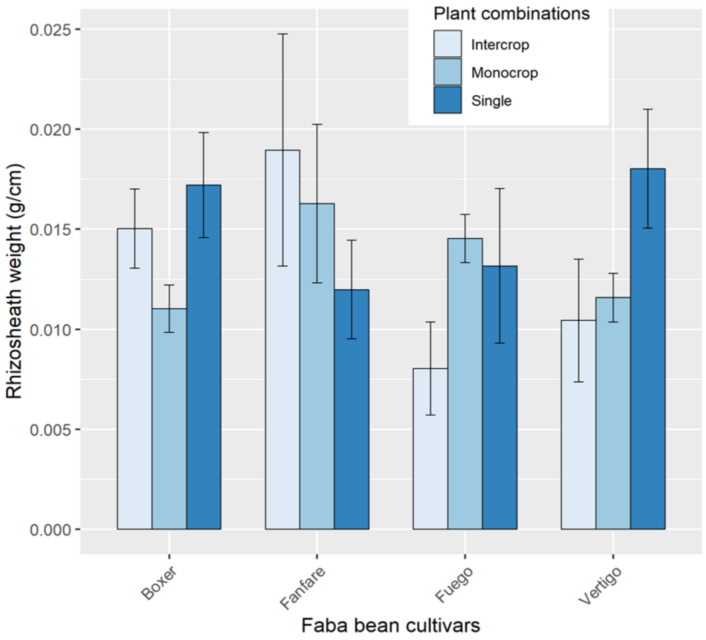

Fig. 10 Mean rhizosheath weight expressed on a per unit root length basis for four faba bean cultivars when either grown alone (single) with the same cultivar (monocrop) or with wheat (intercrop). Error bars show ± SE

Root diameter

There was no significant effect of plant combination on the average root diameter ($F_{2,66} = 1.68$, $p = 0.19$). Upon removal of outliers, differences in average root diameter remained non-significant.

Root characteristics of wheat

For all the measured root characteristics, wheat performed similarly among all plant combinations. No significant effects of plant treatment were seen on wheat root traits.

Land equivalent ratio

Despite a reduction in root size for all faba bean plants when intercropped, the LER was found to be on average 1.85 for the cultivars Boxer, Fanfare and Vertigo, indicating that intercropping with these three cultivars could be more efficient than monocropping in terms of land use. All the intercropped Fuego plants did not manage to grow aboveground biomass during the growing period, leading to a LER value of 0.8 for the Fuego/wheat intercrop pair.

3.3. Experiment 3: effect of water stress on root traits in a wheat/faba bean intercrop system

Water stress negatively affected the root growth of all plants. For the two faba bean cultivars (Fuego and Vertigo), both root fresh weight and total root length were reduced in most plants that received the drought treatment (Fig. 11). Root fresh weight was significantly ($F_{1,58} = 23.4$, $p < 0.001$) different between the control and drought treatment. A trend towards significance was found for plant combination ($F_{2,58} = 2.9$, $p = 0.067$), indicating that the root weight of intercropped plants tended to be less affected by the drought treatment than the monocropped and single plants, which was probably because faba bean plants grown with wheat had smaller roots to begin with (Fig. 11a). There was no significant ($F_{1,58} = 0.6$, $p > 0.4$) difference between the two bean cultivars and no interaction between plant combination and water treatment for root weight.

Root length was significantly ($F_{1,58} = 3.5$, $p = 0.04$) different between the three plant combinations, and there was a trend towards significance ($F_{1,58} = 3.5$, $p = 0.068$) between the control and drought treatment. Again, there was no significant difference between the two bean cultivars and no treatment interactions. Similar to root weight, root length was less affected by drought in the intercropped plants, but was greatly reduced in the monocrop and sole crop (Fig. 11b).

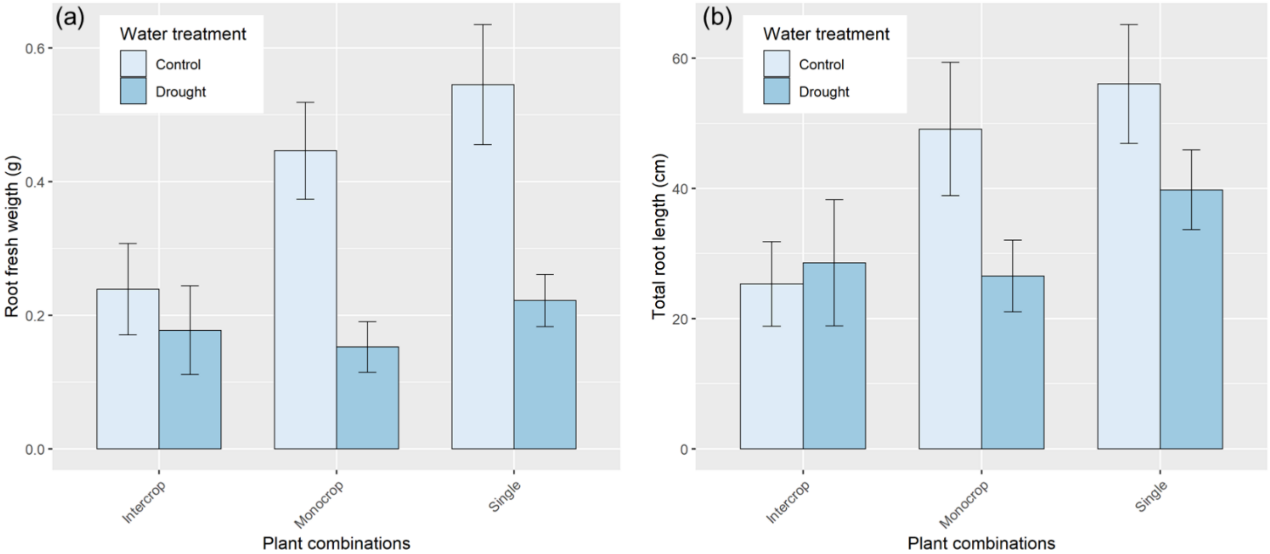

Fig 11. Mean root fresh weight (a) and total root length (b) for two faba bean cultivars combined (Fuego and Vertigo) when either grown alone (single) with the same cultivar (monocrop) or with wheat (intercrop), and receiving either normal watering (control group) or experiencing water stress (drought). Error bars show ± SE.

Rhizosheath weight and root hair length

Water stress had an effect on both rhizosheath weight and root hair length; for the faba bean plants, rhizosheath weight ($F_{1,58} = 3.8$, $p = 0.057$) tended to increas and root hair length ($F_{1,55} = 8.1$, $p = 0.006$) increased significantly under water stress (Fig. 12). For rhizosheath weight, the three plant combinations performed almost identically ($F_{2,58} = 0.002$, $p > 0.9$); and no significant differences ($F_{1,58} = 1.2$, $p = 0.3$) were found between the two faba bean cultivars.

There was no significant difference between the two bean cultivars for root hair length. ($F_{1,55} = 1.3$, $p = 0.3$), no significant difference between the three plant combinations ($F_{2,55} = 0.3$, $p = 0.7$), and no interaction between plant combination and water treatment ($F_{2,55} = 0.7$, $p = 0.5$).

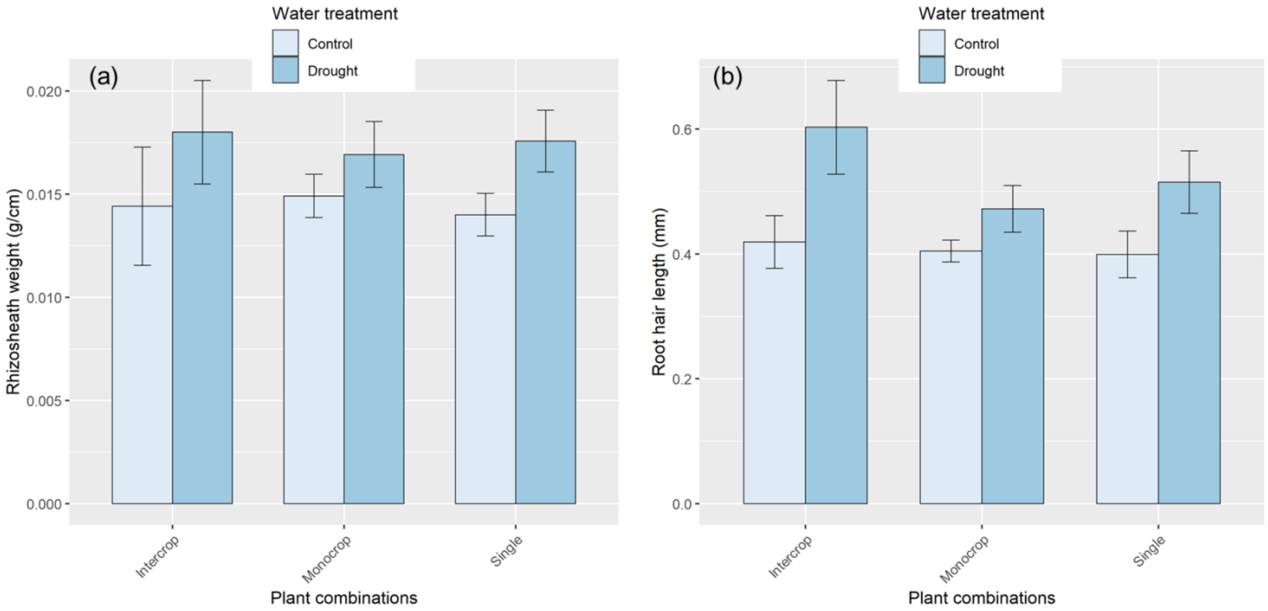

Fig. 12 Mean rhizosheath weight expressed on a per unit root length basis (a) and lateral root hair length (b) for two faba bean cultivars when either grown alone (single) with the same cultivar (monocrop) or with wheat (intercrop), and receiving either normal watering (control group) or experiencing water stress (drought). Error bars show ± SE.

Root diameter

Similar to the second experiment, there was no effect of plant combination ($F_{2,58} = 1.4$, $p = 0.3$) or water treatment ($F_{1,58} = 0.7$, $p = 0.4$) on the average root diameter of the faba bean roots.

Root characteristics of wheat

Similarly to the bean roots, the wheat roots were negatively affected by the drought treatment, with reduced root fresh weight and total root length for all wheat roots that experienced water stress, regardless of plant combination (Fig. 13). Root fresh weight was significantly ($F_{1,45} = 12.1$, $p = 0.001$) smaller in the drought treatment. There was no significant ($F_{3,45} = 1.4$, $p = 0.3$) difference between the different plant combinations and no interaction between plant combination and water treatment. Root length was also significantly ($F_{1,45} = 4.9$, $p = 0.03$) smaller in the drought treatment. Again, no significant ($F_{3,45} = 0.9$, $p = 0.4$) difference was detected between the different plant combinations and no interactions.

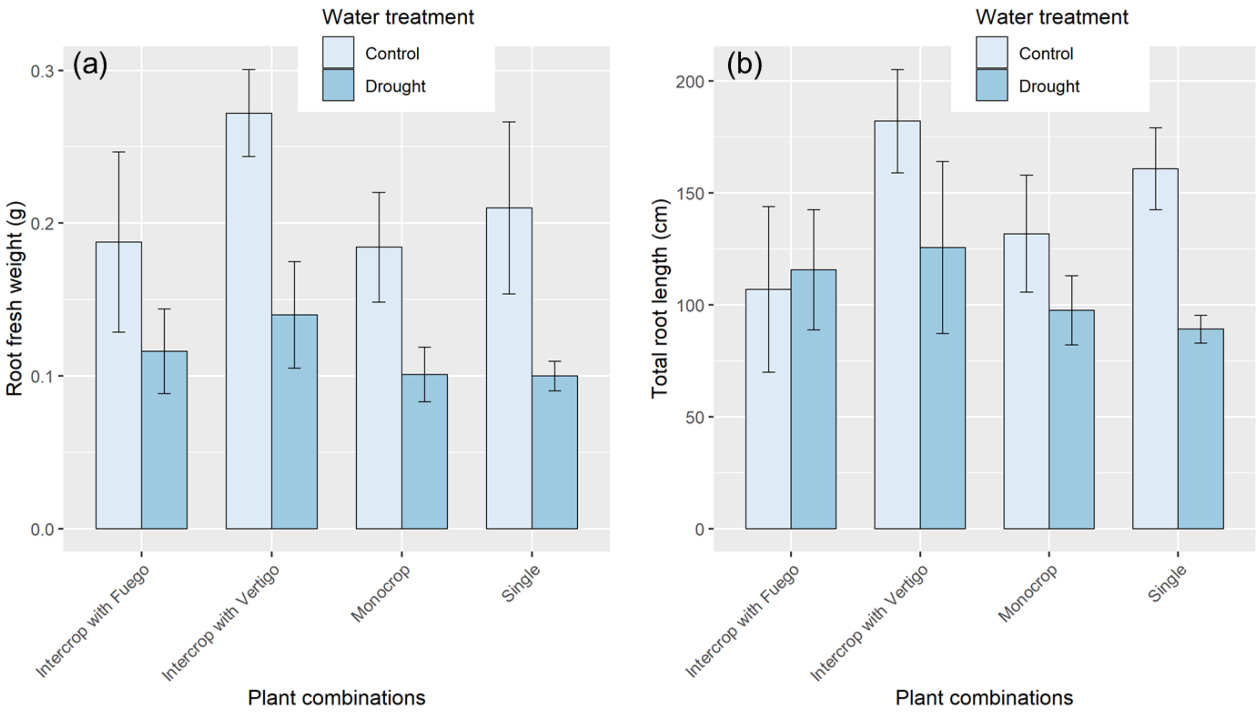

Fig 13. Mean root fresh weight (a) and total root length (b) for wheat (cv. Talisker) when either grown alone (single) with another wheat plant (monocrop) or with faba bean cultivar Fuego or Vertigo (intercrop), and receiving either normal watering (control group) or experiencing water stress (drought). Error bars show ± SE.

Rhizosheath weight and root hair length

Water stress had an effect on both rhizosheath weight and root hair length (Fig. 14). Rhizosheath weight was significantly ($F_{1,45} = 5.1$, $p = 0.03$) increased under drought treatment. There was no significant ($F_{3,45} = 1.4$, $p = 0.3$) difference between the different plant combinations and no interaction between plant combination and water treatment.

No significant effect ($F_{1,45} = 1.9$, $p = 0.18$) of water treatment on root hair length was found. However, a trend towards significance was found for plant combination ($F_{3,45} = 2.7$, $p = 0.059$) and for the interaction between plant combination and water treatment ($F_{3,45} = 2.7$, $p = 0.052$). Contrary to the other plant combination, it appears that monocropped wheat plants slightly reduced their lateral root hair length in response to water stress (Fig. 14b).

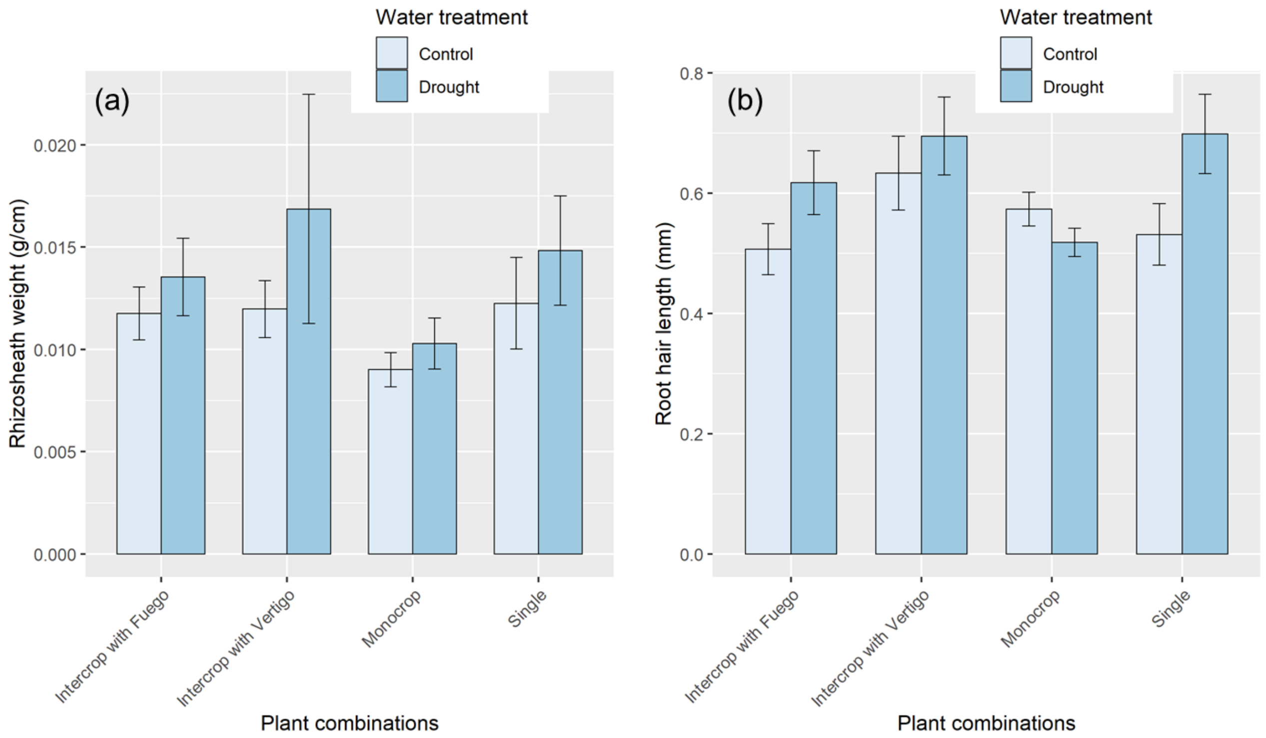

Fig 14. Mean rhizosheath weight expressed on a per unit root length basis (a) and lateral root hair length (b) for wheat (cv. Talisker) when either grown alone (single) with another wheat plant (monocrop) or with faba bean cultivar Fuego or Vertigo (intercrop), and receiving either normal watering (control group) or experiencing water stress (drought). Error bars show ± SE.

No significant effect ($F_{1,44} = 2.5$, $p = 0.12$) of water treatment on root diameter was found. However, an interaction ($F_{3,44} = 3.1$, $p = 0.04$) between plant combination and water treatment was found, indicating that the different plant treatments responded differently to the water treatment concerning root diameter (Fig. 15). Only for the wheat plants intercropped with Vertigo, the average root diameter reduced under water stress ($p = 0.03$). The other plants combinations were not significantly affected.

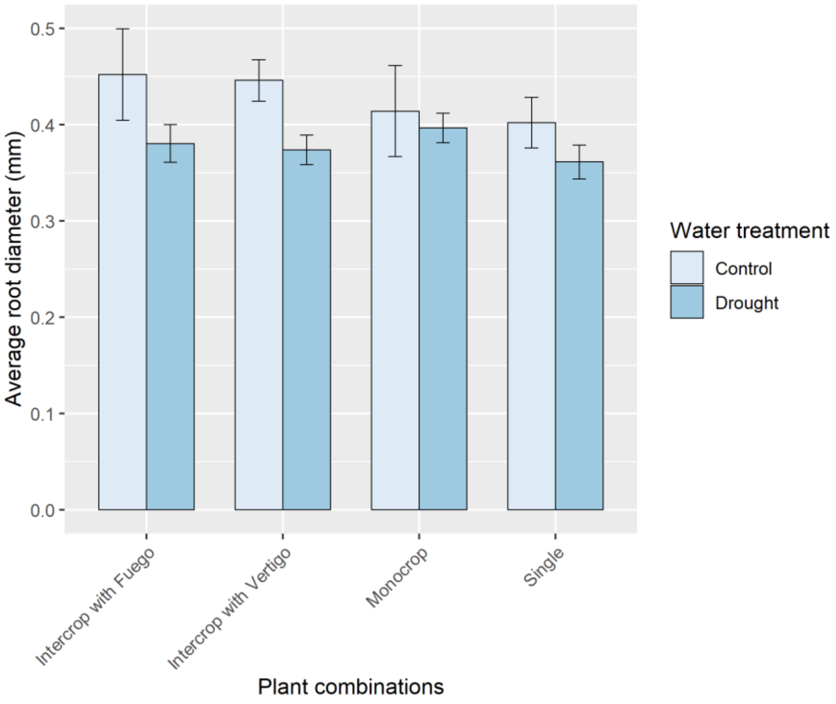

Fig 15. Plot of the average root diameter for wheat (cv. Talisker) when either grown alone (single) with another wheat plant (monocrop) or with faba bean cultivar Fuego or Vertigo (intercrop), and receiving either normal watering (control group) or experiencing water stress (drought). Error bars show ± SE.

Land equivalent ratio

The LER of the Fuego/wheat intercrop pair in the control group was found to be on average 1.4, and the Vertigo/wheat intercrop pair had on average a LER value of 1.8. Under drought conditions the LER of both intercrop pairs was found to be greater than those of the control group; the Fuego/wheat pair experiencing water stress had on average a LER of 2.3 and Vertigo/wheat had a LER of 2.6.

4. Discussion

This study has given insights into the behaviour of intercropped roots during the early growing stage, thereby providing novel information about the plasticity (or lack thereof) of certain root traits in response to neighbouring plants. The roots of the faba bean cultivars in this study showed a large variation in root system size (e.g. root weight and total root length) despite constituting a relatively small selection of genotypes compared with the large genetic diversity present among faba bean accessions (Duc et al., 2010). In a study on root characteristics of 16 European faba bean cultivars at maturity, Zhao et al. (2018) found similar results in the variation of overall root system size. Another leguminous crop species, lentil, has also exhibited variability in the root traits taproot length and lateral root number (Sarker et al., 2005). This variation in certain root traits implies that faba bean, and possibly other legumes, have a generally large diversity in root system size present among the different genotypes. This variation provides potential to explore which genotypes are most suited for growth with a specific plant partner. Faba bean plants with a large root system size might grow well together with specific crops compared with faba bean plants with a small root system size, or vice versa.

When intercropped with wheat, the four faba bean cultivars (Boxer, Fanfare, Fuego and Vertigo) were all negatively affected, and variations in root system size could no longer be observed, possibly because differences in root system size are harder to detect when root sizes are small. The reduction in root size of intercropped faba bean plants was expected; the total plant mass of an individual intercropped plant is likely to be smaller than that of a single grown plant, due more competition for soil resources when plants are grown together. However, the combined mass of the intercropped plants can be the same as or even larger than that of the monoculture. Although the root size of intercropped faba bean roots decreased on an individual level, the land equivalent ratio (LER) for the faba bean/wheat intercrop system was greater than one (except for Fuego), indicating that intercropping was more productive than monocropping, despite a reduction in root system size. Other studies on faba bean/wheat intercrop systems have also found LER values greater than one (Barker and Dennett, 2013; Xiao et al., 2018), but LER values of less than one have also been reported for this intercrop pair (Fan et al., 2006). This indicates that other factors beyond crop combination (e.g. location, soil type, activity of soil organisms) play a role in determining the outcomes of intercrop systems.

The other measured root traits, rhizosheath weight, root hair length, and root diameter, did not vary significantly between the faba bean genotypes grown in the present study, which might suggest that there is limited genotypic variation for these traits among faba bean genotypes. This lack of variation, displayed in these root traits when grown as single plants, can be misleading for selecting cultivars for intercropping, because plants can show trait plasticity in response to intercropping. In the case of rhizosheath weight, the four faba bean genotypes showed different responses to a growing partner (Fig. 10). For the cultivar Boxer, monocropped plants had the lowest rhizosheath weight, whereas the cultivar Vertigo had reduced rhizosheath weight when intercropped compared with mono- or sole cropped. This reduction could be the result of competition for soil resources between the intercropped plants. Plasticity in rhizosheath weight and root hair length have been reported in response to environmental stresses, such as drought stress (Basirat et al., 2019) and phosphorus deficiency (Zhu et al., 2010), but not yet, as in the current investigation, in response to neighbouring plants, even though these root traits could play a role in plant–plant interactions. The rhizosheath constituting part of the rhizosphere (Ndour et al., 2020), plays a role in interspecific root interactions between plants, such as facilitation in nutrient uptake (Zhang et al., 2004). The rhizosheath could thus be important for beneficial root interactions and those plants which maintain or enhance rhizosheath weight in response to neighbouring plants might be most suited for intercrop systems.

When a drought stress was imposed, both faba bean and wheat roots enhanced their rhizosheath weight and root hair length. Others have also concluded that the rhizosheath weight and root hair length are influenced by water stress (Liu et al., 2019; Hammac et al., 2011). However, the finding from this study that intercropped plants appeared to be less affected by the water limitation than single or monocropped plants is novel, as the response of these root traits to the combined effect of water stress and intercropping has not yet been addressed. Plasticity in root hair length when intercropped and exposed to water stress could lead to improved crop performance of intercropped plants experiencing drought. A greater LER value of intercropped plants experiencing drought, than plants growing under optimal soil moisture conditions, might further indicate that intercrop system could perform relatively well under environmental stress. This is in agreement with the conclusion of a global meta-analysis, consisting of 939 intercropping observations, that intercropping is beneficial under drought stress: it was found that irrigated and non-irrigated intercrop systems did not significantly differ in their land equivalent ratio (Martin-Guay et al., 2018). So, water stress did not lower their productivity of intercrop systems. The benefits of intercropping might thus not be visible under optimal growing conditions, but the resilience of intercrop systems to drought could be an essential feature for adapting agriculture to the uncertain future climatic conditions.

4.1. Reliability of the research setting

Rootrainers are practical growing containers for studying roots, since they can be easily opened. However, these containers are relatively small and plants have to be harvested in their early growing stages. In the first experiment, the faba bean roots were limited in their length by the depth of the Rootrainers. Growing two plants together in a small container is likely to enhance competition between the plants, due to the limited available soil volume. Although, this limitation in depth and volume is not representative of field growing conditions, forcing the two plants to interact with each other is needed to measure their response to a neighbouring plant. Moreover, expanding the research to bigger containers would allow intercropped plants to grow for a longer period of time. This would then allow researchers to study the response of roots to intercropping when plants have grown for more than just a few weeks. Keeping in mind that glasshouse conditions used for these studies are not identical to field conditions, and it should thus be demonstrated that the findings can be translated to field conditions.

In this study, six replicates of each plant combination were grown and analysed. However, in both the intercropping experiments, several germinated faba beans did grown after being transplanted from the agar plates to the rootrainers. Because of this, the total number of replicates did not actually reach six, making it more challenging to detect statistical differences between a smaller number of replicates. For instance, the root hair length of intercropped faba bean plants under drought conditions appeared to increase more than the monocropped and single grown faba bean plants (Fig. 12b). However, due to a loss of replicates during the growing period, this disproportional increase was not found to be significant. Differences between treatments that were not found to be significant in this study, might be detected with a larger number of plants.

Furthermore, in experiment 2 four faba bean cultivars were selected, and this selection was further narrowed down to two cultivars in experiment 3. These cultivars were chosen because of their current popularity among growers, however, as stated before, modern cultivars designed for high input monoculture growing conditions might not be the most suitable for intercropping. Including genotypes in the study which are not bred for monocropping, such as local land races, might have responded differently to a neighbouring plant than the modern cultivars. These locally adapted varieties might have shown higher levels of plasticity in response to intercropping.

4.2. Recommendation for future research

Although the findings of this study do not describe belowground interaction during the whole intercrop growing period, or the final yields of both crops when intercropped in the field, they give an insight into the response of faba bean and wheat roots in the early stages of their combined growth. However, root-root interactions take place during the complete growing period, and cannot be assumed to be static during this whole time. Measuring root characteristics at various times during the growing period could give an insight into temporal changes of intercropped roots. Although root characteristics of wheat were not affected by intercropping with faba bean during the first weeks of growth, this study did not quantify root traits at later growth stages. It is probable that the wheat roots will be affected by intercropping after a longer period of time. For example, in an investigation of a wheat/faba bean intercrop system, intercropping increased microbial biomass, and nitrogen and phosphorus availability at the end of the growing period, leading to increased yields of wheat (Song et al., 2007). In the present study, faba bean plants were negatively affected when intercropped with wheat during the first few weeks of co-growth. If grown together for longer, faba bean plants might be able to establish themselves better than they could during this experiment. It has been reported that intercropping increased nodulation of faba bean (Bargaz et al., 2015) and increased the percentage of nitrogen derived from air of faba bean in a wheat/faba bean system (Fan et al., 2006). These traits could contribute to an enhanced performance of intercropped faba bean plants, an effect that is not measurable during the first weeks of growth. Challenges, however, will arise with excavating, separating and studying bigger root systems, which will make studying intercropped roots at various time period more complicated than studying these younger and smaller roots.

Besides water availability, there is a myriad of other biotic and abiotic factors influencing intercrop systems. For instance, nutrient availability, soil type, the activity of belowground organisms and soil microbiome composition are all factors that can influence the behaviour of roots when intercropped. The management of intercropped plants, such as sowing patterns, planting densities, ratios of the intercropped species, timing of planting and harvesting, and preceding crops in the rotation, can further influence root behaviour in intercrops. Finding optimal crop partners is thus location specific, and a well performing crop combination in one location might not perform as well in another location. Considering all these factors that influence intercropped plants, there are still various research topics to further explore the optimisation of intercropped plant systems.

5. Conclusion

A large part of plant interactions take place belowground and mutualistic belowground interactions could contribute to a yield advantage in intercropping, through increased water and nutrient uptake. Although progress has been made to better understand belowground interactions between intercropped plants, significant gaps remain regarding root behaviour. This study confirmed that a large variation in root system size is present among faba bean genotypes, a feature which could be used to select cultivars for intercropping with specific crops. Furthermore, this study backs the hypothesis that variation in traits seen when plants are grown alone or in moncrops are not the same as when the plants are grown in intercrops. For instance, no significant differences in rhizosheath weight were found between the faba bean genotypes, but faba bean plants showed different responses to a growing partner. Choosing component crops based on traits measured in single grown plants might thus be misleading, because crops growing in mixtures can have different traits than they would have displayed when sole cropped. However, this was not the case for all root traits; for example average root diameter seemed to be nonresponsive to intercropping. Lastly, this study confirms that intercropping systems could be more resilient towards droughts than monocultures, and that an increase in root hair length and rhizosheath weight could play a role in this resilience. Knowledge about the response of roots to intercropping, and in particular the level of plasticity (or lack thereof) of various root traits, can be useful for breeding plants specifically designed for intercrop conditions, and thereby making intercropping more attractive for implementation as an agricultural practice.

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