UNIVERSITÀ DEGLI STUDI DI TRIESTE
UNIVERSITÀ DEGLI STUDI DI UDINE

XXXVI CICLO DEL DOTTORATO DI RICERCA IN AMBIENTE E VITA

Validation of Practices to Optimize the Production of Protein Crops in Organic Systems

Settore scientifico-disciplinare: **AGR/02**

Dottorando – Ph.D. Student
Daniel Marusig

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ANNO ACCADEMICO 2022-2023

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Summary

Abstract

The increase in global population coupled with reduced crop yield due to climate change have arisen a serious concern in terms of food security. In order to cope with the increasing pressure on the current system of food production and supply, innovative and sustainable approaches are needed. We need to shift our diet towards plant-based substitutes of proteins. We call this shift the protein transition.

Switching to plant-based protein requires increasing the area and productivity of protein-rich seeds. The economic competitiveness of feed crops (cereals and oil rich crops) is very strong and largely explains farmers' lack of interest in protein-rich crops.

From an agricultural perspective, however, the adaptability of these minor crops in different pedo-climatic zones must be verified.

In this thesis we report about the experiments carried out with the aim to test optimization practices for protein crops production in organic agriculture in North of Italy (Friuli-Venezia Giulia region). We investigated the performance of crops under organic agriculture practices, which are recognized as a key strategy in the Common Agricultural Policy framework of the European Union (EU). This policy aims to ensure a fair standard of living for farmers, stabilize agricultural markets, provide a secure and affordable food supply for consumers, and promote sustainable agriculture. Within this framework, organic farming is acknowledged as a main factor in providing environmental sustainability, biodiversity conservation, and meeting consumer demand for organic products.

The studied species were: chickpea, faba bean and lentil, which have been poorly cultivated in this region.

Since the lack of knowledge about these crops in this region, they were studied to assess the varieties' adaptability to water stress, heat and cold stress, low soil fertility, also evaluating seed

and protein yield. This was conducted through multiple experiments which are collected in the 5 chapters of this thesis.

In the first chapter we present a study aimed to evaluate performance of various *cvs* of chickpea, faba bean and lentil, grown in Udine during summer 2021. By the results of this experiment, we decided to focus on the two more promising species, chickpea and lentil.

In the second chapter, we present a study conducted during the growing season 20222, where we investigated the adaptability to water and heat stress of chickpea and lentil. Tolerance of these crops was assessed by treating these crops with an increasing gradient of irrigation performed during the critical phase of grain filling. During both the growing seasons 2021 and 2022 we paid particular interest in assessing the performance of crops with multispectral data acquired with remote sensing techniques by unmanned aerial system (UAS).

In the third chapter, have deepened the investigation of remote sensing of these crops, which has been poorly studied in chickpea and lentil Hence, we present an investigation aimed to assess the most used agronomical parameters and yield by remote, with remotely sensed vegetation indices, calculated from data collected by UAS. In particular, we tested an ensemble of indices as predictors of crop biomass, leaf area index, crop status and yield.

One of the main constraints in organic agriculture consists in managing weed competition. The Sustainable Use of Pesticides Directive of the European Union promotes the adoption of Integrated Pest Management approaches as a key strategy to achieve sustainable pesticide use. For this reason, in the fourth chapter we present a study where we tested chickpea and lentil intercropped with buckwheat to verify the allelopathy and competition functions with the main objective of managing weed competition.

As a side project, in the fifth chapter, we present a methodological investigation aimed to perform an unsupervised classification model to test the possibility of automatically classifying chickpea, lentil, faba bean and quinoa, by using a combination of spectral and photogrammetric data acquired by remote with UAS.

Faba bean resulted being the species with low and unstable yield, probably due to biotic stress. Chickpea seems to be a suitable crop for this region with high grain and protein yields also under drought stress.

Lentil performance was variable as in the first trials it did not produce grains, while on the second experimental year it got high production.

Irrigation influenced crop phenology of chickpea by extending the duration of the flowering and grain setting phases, with some negative effects in terms of seed quality.

The use of remote sensing data was effective in monitoring crops development. Significant correlations were assessed between vegetation indices and field-measured parameters. The use of time-integrated vegetation indices was particularly effective in correlating biomass and yield. Intercropping with buckwheat did not provide significant evidence about being an effective strategy to mitigate weed competition; however, the use of buckwheat water extracts had a significant effect on seeds germination. Random Forest modelling with remote data acquired by UAS resulted being a useful tool, able to classify with high accuracies the investigated protein crops at the species level. This result is of particular interest in automated classification of crops using commonly used precision agriculture data.

Riassunto

L'aumento della popolazione mondiale associato alla riduzione del rendimento delle colture a causa dei cambiamenti climatici ha suscitato una forte preoccupazione in termini di sicurezza alimentare. Per affrontare la crescente pressione sul sistema attuale di alimentazione, sono necessari approcci innovativi e sostenibili. È necessario un cambiamento nelle abitudini alimentari attuali, integrando più fonti vegetali nell'apporto proteico della nostra dieta Questo cambiamento è definito "transizione proteica".

La transizione proteica richiede un incremento nella quantità di superfici coltivate e nella produzione di colture proteiche. Data la maggiore resa economica delle attuali colture (cerealicole e oleaginose) rispetto alle colture proteiche minori, gli agricoltori non sono propensi ad adottare sistemi produttivi alternativi.

L'adattabilità di queste colture minori nelle diverse zone pedoclimatiche deve essere ancora investigata. In questa tesi illustriamo delle prove sperimentali volte a validare le pratiche di ottimizzazione della produzione di colture proteiche in agricoltura biologica, nella regione Friuli-Venezia Giulia. Abbiamo valutato la prestazione di una serie di colture coltivate in regime di agricoltura biologica, una delle strategie di maggior rilievo nel quadro della Politica Agricola Comune dell'Unione Europea (UE). Questa politica mira a garantire uno standard imprenditoriale equo per gli agricoltori, stabilizzare i mercati agricoli, garantire un mercato alimentare sicuro e accessibile per i consumatori e promuovere l'agricoltura sostenibile. All'interno di questa politica, l'agricoltura biologica è riconosciuta come un elemento fondamentale al fine di promuovere la sostenibilità ambientale, la conservazione della biodiversità e nel soddisfare la domanda dei consumatori per i prodotti biologici.

Le specie studiate sono cece, favino e lenticchia, colture poco diffuse nella regione FVG.

Data la poca conoscenza delle prestazioni di queste colture nella regione d'interesse, sono state predisposte una serie di prove sperimentali finalizzate a valutarne l'adattabilità in termini di

stress idrico e termico, bassa fertilità dei suoli e resa in granella e proteina. Le sperimentazioni sono state raggruppate ed esposte nei cinque capitoli che compongono questa tesi.

Nel primo capitolo viene presentato uno studio finalizzato a valutare la prestazione di varie cultivar di cece, favino e lenticchia, coltivate a Udine durante la stagione estiva 2021. Dai risultati di questo esperimento si è deciso di focalizzare l’attenzione sulle due colture reputate più promettenti, cece e lenticchia.

Nel secondo capitolo viene presentato uno studio atto a investigare la risposta di cece e lenticchia allo stress idrico. La tolleranza allo stress è stata valutata trattando le colture con trattamenti di irrigazione crescente, effettuati durante la fase critica di riempimento dei baccelli.

Durante entrambe queste sperimentazioni è stato posto particolare interesse all’utilizzo di tecniche di telerilevamento con dati multispettrali acquisiti da drone (*unmanned aerial system* – UAS) col fine di valutare la prestazione delle colture da remoto.

Nel terzo capitolo viene presentata una indagine finalizzata a valutare con maggiore dettaglio l’utilizzo di tecniche ti telerilevamento, che sono state scarsamente studiate in cece e lenticchia. Lo studio presentato ha come obiettivo di correlare i principali indici di vegetazione con i principali parametri agronomici e la resa in granella delle colture. Nello specifico, sono stati valutati molteplici indici come potenziali *proxy* per la biomassa delle colture, l’indice di area fogliare, lo status idrico e la resa.

Una delle principali criticità legate all’utilizzo di pratiche di agricoltura biologica consiste nella gestione della competizione attuata dalle malerbe infestanti. La Direttiva sull'Uso Sostenibile dei Pesticidi dell'Unione Europea promuove l'adozione di approcci di Gestione Integrata degli Infestanti come strategia chiave per raggiungere un uso sostenibile dei pesticidi. Per questo motivo, nel quarto capitolo presentiamo uno studio in cui abbiamo valutato la tecnica della consociazione per la gestione delle infestanti in cece e lenticchia, coltivate con una coltura allelopatia, il grano saraceno.

Nel quinto capitolo viene presentato un progetto secondario con finalità metodologiche, finalizzato all’implementazione un modello di classificazione *unsupervised* per testare la possibilità di classificare automaticamente cece, lenticchia, favino e quinoa, utilizzando una combinazione di dati multispettrali e di fotogrammetria acquisiti con tecniche di telerilevamento da UAS.

Il favino è risultato essere la specie con il minore rendimento, probabilmente a causa di stress biotici.

Il cece è risultato essere una coltura adatta per questa regione, dato l’alto rendimento e la produzione di proteina, anche in condizioni di siccità.

La lenticchia ha avuto prestazioni variabili. Il primo anno non ha prodotto seme, mentre quello successivo ha avuto una produzione elevata.

L'irrigazione ha influenzato la fenologia del cece, prolungando la durata della fase di fioritura e riempimento del baccello, con alcuni effetti negativi sulla qualità del seme.

L'uso dei dati di telerilevamento è stato efficace nel monitorare lo sviluppo delle colture. Sono state individuate correlazioni significative tra gli indici di vegetazione e i parametri misurati in campo e l'elaborazione di indici cumulativi di vegetazione è stato efficace nel correlare biomassa e resa. La consociazione con il grano saraceno non è risultata essere una strategia efficace per mitigare la competizione da infestanti, tuttavia, l'uso degli estratti acquosi di grano saraceno ha avuto effetti di inibizione sulla germinazione dei semi. Il modello *Random Forest* si è rivelato uno strumento utile in grado di classificare con alta precisione le colture proteiche a livello di specie.

General introduction

Climate change effects have arisen serious concern in terms of food security, impacting various aspects of food production, distribution and access (Wheeler and Von Braun 2013). As the global population is projected to increase to over 9 billion by 2050, there will be an increase in demands for nutrient-dense foods, including high-quality protein sources (Grafton et al. 2015). In response to this challenge, there is a need for the food production and supply systems to proactively develop innovative and sustainable approaches, as they are currently not suited to address the foreseen increase in pressure (Miraglia et al. 2009). The 2030 Agenda for Sustainable Development, adopted by all United Nations Member States and the signing of the Paris agreement on climate change (COP21) in 2015 created a juridical obligation for Member States and pushed for a re-think of our food system. Alternative protein sources are urgently needed to respond to the increasing protein demand from a growing world population and the need for a more resource-efficient production. Global food production is the largest pressure caused by humans on Earth, threatening local ecosystems and the stability of the Earth system (COP21 2016).

Nowadays, a significant portion of the protein in diet is based on animal products. This is mostly due to the food consumption habits of people, then to an agricultural constraint (Gotor and Marraccini 2022). In fact, the consumption of plant-based proteins has significantly decreased after 1960s, especially in Europe, along with a shift towards the consumption of animal-based proteins. Hence, the decreasing demand for plant-based proteins in food, mostly derived from pulses, has induced a progressive decrease in their production area in the last five decades (Watson et al 2017). The EU27 livestock sector (based on pig and poultry accounting for 75% of 45Mt of meat production) requires energy-rich cereals and to import 80% of protein-rich feed from third countries like Brazil, Argentina and the United States. The EU currently devotes only 3% of its arable land to protein crops used for feed or for food. This consolidated production system entails major issues in terms of environmental sustainability. Livestock-based farming contribute significantly to greenhouse gas emissions (Wood and Tavan 2022), necessitate extensive land use (Poore and Nemececk 2018) consume and pollute vast amounts of water (Aschemann-Witzel et al 2020). Hence, the need to develop promote and adopt new, sustainable food system shifting the diet including a greater than 50% reduction in global consumption of unhealthy foods such as red meat, fat and sugar, and a greater than 100% increase in consumption of healthy foods, such as nuts, fruits, vegetables, and legume (Willett et al. 2019).

Despite their importance, grain legume and in particular pulses production is declining globally, especially in Europe, where in 2018 they accounted for only 1.3% of the EU27 Utilized Agricultural Area (UAA) (Gotor and Marraccini 2022). This decline can be attributed to a multitude of factors, however, a major contribution consists in the increase in importing affordable soybean for feed from abroad, especially North and South America, which led to deflation in meat price. As a consequence, pulse cultivation reduced also in the number of cultivated crops (Watson et al. 2017). Recently, Gotor and Marraccini (2022) conducted a systematic review of 269 papers from 1974 to 2019 to identify the most suitable pulse species for the Western European Temperate Regions. They investigated a list of 41 species and, based on their agronomical characteristics (*e.g.* temperature and water requirements, pest risk, yield) and nutritional profile (i.e. energetic value and content in protein, oil, carbohydrate and fiber). According to crops thermal requirements and climatic suitability, they selected 21 minor crops that are currently underutilized but have the potential to thrive in such regions.

Organic agriculture is a farming approach aimed to apply sustainable cropping practises while avoiding the use of synthetic compounds (herbicides, pesticides and fertilisers), also avoiding other practises of conventional agriculture (e.g., use of genetically modified organisms, and growth regulators), being reputed harmful for the environment and potentially for human health (Lotter 2003). This approach aims to promote environmental sustainability, soil health, agroecosystem diversity, climate change mitigation, sustainable resource use and consumer health (Willer and Lernoud 2017). Organic agriculture is recognized as a key strategy in the Common Agricultural Policy framework (CAP) of the European Union (EU). This policy aims to ensure a fair standard of living for farmers, stabilize agricultural markets, provide a secure and affordable food supply for consumers, and promote sustainable agriculture. Within this framework, organic farming is acknowledged as a main tool in providing environmental sustainability, biodiversity conservation, and meeting consumer demand for organic products (Pânzaru et al. 2023).

Major constrains in organic agriculture compared with conventional, are represented by lower yield (estimated to be in average equal to 20%) due to biotic and abiotic factors like pest, and diseases, weeds and mineral nutrients (e.g. NPK) availability (Lotter 2003). Acknowledging for these issues, cropping of legumes, particularly grain legumes, represent a key method in organic farming. Grain legumes can support the development of sustainable and climate-resilient cropping systems. Legumes have the capability to biologically fix Nitrogen (BNF), reducing the use of mineral N fertilizers. Furthermore, the BNF has a legacy for the following crop promoting soil fertility and reducing the synthetic N fertilizers requirements (Watson et al. 2017).

The BNF function of legumes is also used in cropping system diversification as intercropping of legumes with cereals. This method is well known from the past but now is part of specific research in order to better value: i) yield of mixes legumes-cereals under varying climatic conditions; ii) resistance to drought, water and heat/cold stress; iii) capacity to control weeds emergence and competition; iv) susceptibility to pests and diseases. Experiments are required also to validate at farm level the introduction of legumes and intercropped legumes in combination with other agronomic practices.

The Smart Protein HO2020 project has been established with the aim of harness sustainable protein (plant and microbial) knowledge to significantly enhance the sustainability and resilience of a new European protein supply chain by developing alternative protein ingredients and products for humans which have a positive impact on economy, environment, biodiversity and food security. In this framework, the aim of this thesis was to focus on food security by promoting grain yield and quality in organic production system. In the Smart Protein project, this aim was pursued by the working package 1 (WP1), composed by a group of universities and companies that has investigated the selected crops: chickpea (*Cicer arietinum*, CH), lentil (*Lens culinaris*, LN), faba bean (*Vicia faba*, FB) and quinoa (*Chenopodium quinoa*, QN). These species have been investigated by various experimental trials in a European North to South transect in order to account for diverse pedo-climatic and cropping systems conditions.

In this thesis we reported the results of three years of experimental trials supported by Smart Protein in Udine (Italy). These crops are rpresent in the Friuli-Venezia Giulia region, in only 4 farms. According to the local farmers, these crops have generally low yields and high costs of processing, resulting in low incomes.

The aim of the research was to select the most performing cultivars of selected protein crops for this region and validate the practices to optimize their production under organic farming. This aim was pursued in five different steps which form the main structure of this thesis.

In a first step we conducted a crops and cultivar screening to validate the species and cultivars adaptability and assess yield and seeds quality. We then evaluated the adaptability of chickpea and lentil to drought conditions. Furthermore, we investigated irrigation and intercropping as optimization strategies to improve yield and protein production. and the last experiment tested the precision agriculture technology to monitor crops performance using a Random Forest classification model.

These research activities are designed to meet the needs of organic production, but of course the knowledge generated is also of primary interest to conventional production.

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Chapter 1. Comparison of High Protein Crops Cultivars Under Organic Agriculture

Keywords: Chickpea, Faba bean, Lentil, LAI, Yield, UAV, NDVI

1 Introduction

Agriculture plays a fundamental role whithin the framework of policies of the European Green Deal. In particular, the Farm to Fork Strategy stands out as a major component, comprising a set of policies and strategies aimed to promote more sustainable agricultural practices and food system (Boix-Fayos and de Vente 2023). One of the key goals of the Farm to Fork strategy is to enhance the sustainability of the entire food supply chain. In this optic, it is recognised the environmental impact of protein production and consumption, and it aims to encourage more sustainable practices (Billen et al. 2024).

To move towards an innovative and sustainable food system supported by alternative protein production, a robust supply of high-quality raw materials is essential. For this purpose, the Smart Protein project has targeted three grain legumes (faba bean, lentil and chickpea) and a protein-rich grain crop (quinoa), to be promoted in different pedo-climatic regions of Europe.

Use of legumes represent a key strategy as they capitalize on their biological ability to fix Nitrogen (N) from the atmosphere. This capability reduces the input requirements in terms of nutrients for these crops, moreover, part of the Nitrogen is stocked in the soil into a plant-usable form promoting soil fertility and reducing the synthetic fertilizers requirements of the following crops (Watson et al. 2017). The Nitrogen amount that can be fixed varies according to the species and cultivar and it depends as well on the environmental factors like temperature and water availability (Watson et al. 2017). It has been estimated that in Europe, the average Nitrogen fixed by grain legumes amounts to 133kg ha^{-1} (Baddeley et al. 2013) and can reach up to 250kg ha^{-1} in some species like faba bean (Duc et al. 1988).

However, the production of large quantities of raw material addressed to the production of ingredients for plant-based food requires productive cultivars with high yields and a quality that are suitable for industry demand. Plant-based food production at larger scales is developing and there are different challenges to face.

As a main issue, yield stability of protein crops is generally less stable than spring cereals, especially in the Northern countries of Europe (Peltonen-Sainio and Niemi 2012). Hence, the challenges in scaling up their production include ensuring yield stability through adapted varieties, enhancing tolerance to abiotic and biotic stress conditions, and developing optimized production systems for these new protein crop species (Cernay et al. 2015, Watson et al. 2017, Röös et al. 2020, Olesen et al. 2021).

In 2018, grain legumes accounted for only 1.8% of the Utilized Agricultural Area (UAA) of Europe (Gotor and Marraccini 2022) and the more widely cultivated species were soybean, pea and faba bean, resulting a 70% deficit of high protein materials for feed (Watson et al. 2017). In general, the cultivation of dry pulses occurs within intensive production systems, while only 2.2% is grown in organic farms, primarily in Italy, Germany, Austria, and France (Eurostat 2017).

Among pulses, faba bean has a broad adaptability across different pedoclimatic regions of Europe. However, its production is primarily oriented toward animal feed and it is highly susceptible to drought, diseases, and pests (Alandia et al. 2020, Sellami et al. 2021). On the contrary, lentil is mainly produced for human consumption due to high cost of production, mostly processing. It is mainly grown in the Mediterranean region as it requires minimal inputs in terms of water and nutrients and its seeds have a high nutritional profile, with protein content that can reach up to 30% (Romano et al., 2021). Chickpea is also cultivated in the Mediterranean region, mostly due to its capability of drought tolerance. It is mostly produced in Spain and the main challenges for its cultivation are related to biotic stress (Stoddard 2017). Quinoa has been recently introduced in Europe and its adoption has been increasing in various countries, mainly Spain, France and Italy, while tt is less cultivated in the Northern region. Quinoa yield can reach up to 2ton ha^{-1} and its seeds have a high nutritional value, also containing all essential ammino acids (Vilacundo et al. 2017, Alandia et al. 2020). Quinoa is particularly adapted to drought conditions, however it is quite susceptible to diseases (Jacobsen 2003).

The aim of this research is to validate the species and cultivars adaptability and assess yield and seeds quality of chickpea, faba bean and lentil, in the Friuli-Venezia Giulia region (NE Italy). For this purpose, we aim to combine the most used agronomical parameters, coupled with vegetation assessment by remote with multispectral data acquired by unmanned aerial system (UAS).

2 Materials and Methods

2.1 Study Area

The experiment was conducted during the growing season 2021 in Udine (NE Italy) at the experimental farm “A. Servadei” of the University of Udine (46.03°N, 13.22°E), in a field that had previously been cultivated with maize.

Soil characteristics were obtained from a combined sample of 20 sub-samples taken at sowing at 25cm depth. The soil textural class is Loam (36%sand. 35% silt 22% clay, 7% gravel), having 1.9% of Soil Organic Carbon, 6.8 pH and 9 C/N. The soil has a low fungi/bacteria ratio of 0.5 and microbial activity is low. The SOC balance indicates a steady state supply of C equal to 1.6 tC ha^{-1} $year^{-1}$ assuming an annual mineralization rate of 2.9% of the actual SOC. Deficiencies of Mn are foreseen for legumes; Fe, Zn, P and K plant available are evaluated rather low. The soil Water Holding capacity (at 0.25m depth) is 52mm.

2.2 Climate and weather conditions

Climate in Udine is temperate, with cold winters (minimum temperature usually below 0°C) and warm, relatively dry summers (average maximum temperature higher than 27°C). Precipitation is relatively evenly distributed throughout the year, with some variation in intensity and frequency between the seasons, being maximum in autumn. Climate diagram of Udine is represented in Figure 1.1.

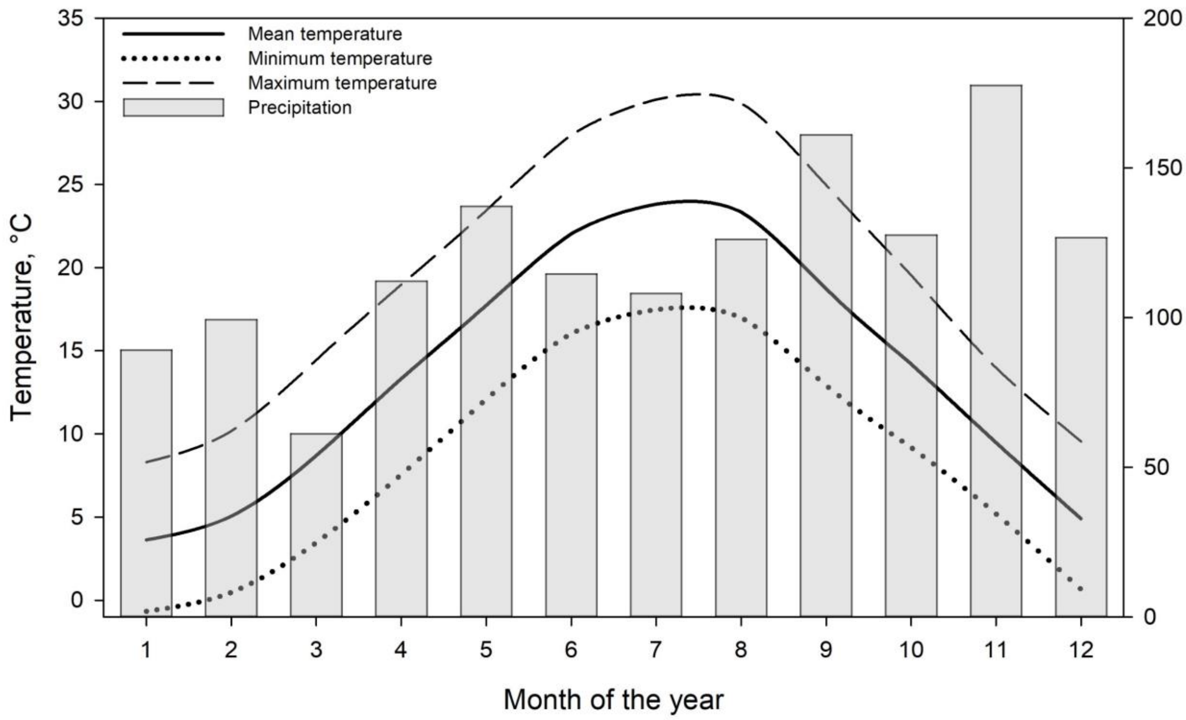

Figure 1.1. Climate diagram of Udine. Data by ARPA-OSMER weather station (Udine Sant'Osvaldo). Reference period 2001-2022.

Weather data have been acquired by the ARPA-OSMER weather station (Udine Sant'Osvaldo) located in the university's farm, close to the experimental area. Weather conditions of the 2021 growing season are represented in Figure 1.2. Compared to long term data (Figure 1.1), in 2021 spring temperature was cooler (~ -2°C). June was particularly dry (-83mm), however, the cumulative precipitation from sowing to harvest reached 545mm for faba bean and 568mm for chickpea.

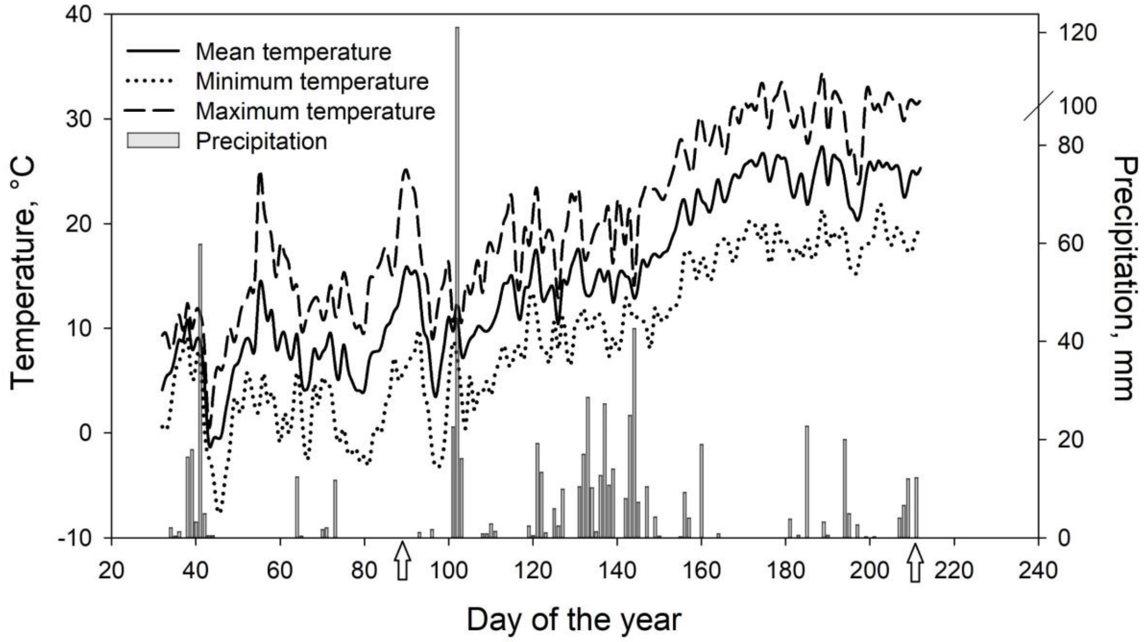

Figure 1.2. Weather conditions of the growing season in Udine during 2021. Sowing and harvest are marked by arrows. Data collected by ARPA-OSMER weather station (Udine Sant'Osvaldo).

2.3 Experimental design and crops management

Different varieties of four protein crops were tested:

- Chickpea (*Cicer arietinum*, CH) cvs: *Eq.3279, Eq.3282, Eq.3283, Eq.3284, Sultano*;
- Faba bean (*Vicia faba* var *minor*, FB) cvs: *Fuego, Tiffany, Fanfare, Lynxs, Taifun, Alexia, GL Emilia*;
- Lentil (*Lens culinaris*, LN) cvs: *Anicia, Flora, Itaca*;
- Quinoa (*Chenopodium quinoa*, QN) cvs: *Eq.1001, Eq.1002, Eq.1010, Eq.1014, Puno, Titicaca*.

Furthermore, for chickpea, two *cvs* mixes were tested as an optimization strategy:

- *Sultano* + *Eq.3282* (ratio 1:1),
- *Sultano* + *Eq.3284* (ratio 1:1).

The CVs of each crop were arranged according to a randomized block experimental design.

Plots dimensions were: 8m length and 1.52m width (4 rows with row-distance of 0.38m), resulting in a plot area of 12.16m^2. The CVs tested for each crop are listed in Tables 1.1-1.3. Treatments (cultivar/intercropped cultivars) were replicated in three blocks for chickpea, faba bean and quinoa, while lentil varieties were replicated four times.

Table 1.1. Tested cultivar of chickpea, faba bean, lentil and quinoa.

Chickpea	**Faba bean**	**Lentil**	**Quinoa**
C1.3279	F1.Fuego	L1.Anicia	Q1.Titicaca
C4.3282	F2.Tiffany	L2.Itaca	Q3.1001
C5.3283	F6.Fanfare	L7.Flora	Q4.1002
C6.3284	F7.Lynxs		Q5.1010
C25.Sultano	F9.Taifun		Q6.1014
C26.Sult+3282	F10.Alexia		Q8.Puno
C27.Sult+3284	F11.GL Emilia		

Sown seeds of chickpea and lentil were inoculated with specific Rhizobia; chickpea was inoculated with *Mesorhizobium cicero* and lentil with *Rhizobium leguminosarum*. Rhizobia, provided by Agrifutur S.r.l. (Alfianello-Brescia, IT).

All crops were sown on 30/03/2021. Sown seeds amount was defined according to values of thousand seeds weight (TSW, g), germination rate (G%) and targeted plant density (see Table 1.1). Target plant densities (plants m-^{2}) were chickpea: 45; faba bean: 35; lentil: 200; quinoa: 70. Sowing depth was 3 cm for chickpea and faba bean, and 2 cm for lentil and quinoa. Since quinoa varieties did not emerge, a second sowing was conducted on 05/05/2021 at 1 cm depth. However, since scarce emergence at second sowing as well, quinoa experiment was withdrawn.

Emergence took place approximately two weeks after sowing, while flowering occurred at beginning of June. Harvests of faba bean and chickpea were conducted respectively on 16/07/2021 and 29/07/2021. Due to heat stress during the flowering period, lentil varieties did not produce seeds and were not harvested.

Table 1.2. Information about tested CVs with corresponding target plant densities, varieties, thousand seeds weight (TSW) and seeds germination percentage (G%)

Species	Target plant density (#/m2)	cv / treatment	TSW (g)	G%
Chickpea	45	*Eq.3279*	434	82%
		Eq.3282	344	92%
		Eq.3283	454	81%
		Eq.3284	425	84%
		Sultano	268	89%
		Sultano + Eq.3282	*	*
		Sultano + Eq.3284	*	*
Faba bean	35	*Fuego*	546.2	87%
		Tiffany	573	86%
		Fanfare	530.9	92%
		Lynxs	625.8	89%
		Taifun	526.6	89%
		Alexia	434	89%
		GL Emilia	468.5	92%
Lentil	120	*Anicia*	25.1	100%
		Itaca	34.9	80%
		Flora	24	92%
Quinoa	70	*Titicaca*	2.92	97%
		Eq.1001	3.79	92%
		Eq.1002	4.28	96%
		Eq.1010	3.95	95%
		Eq.1014	3.70	94%
		Puno	2.18	89%

* Resulting values combining data from corresponding *cvs*.

Table 1.3. Characteristics of cultivars under investigation.

CV	Characteristics
Eq.3279	27% protein, Ascochyta Blight: medium tolerant, Fusarium wilt:tolerant, Kabuli, light color seeds Growth habit: semi-erect. TSW: 359gr
Eq.3282	21% protein, Ascochyta Blight: Light tolerant, Kabuli light color seeds Growth habit: semi-erect. TSW: 349gr
Eq.3283	23% protein, high yielding,, Ascochyta Blight: medium tolerant, Kabuli light color seeds Growth habit: semi-erect. TSW: 419gr
Eq.3284	22% protein, high yielding, Ascochyta Blight: medium tolerant, Fusarium wilt:tolerant, Kabuli light color seeds Growth habit: semi-erect. TSW: 423gr
Sultano	Medium-small size seeds, White flowers, erect plant, Plant height 75-80 cm, Medium Early Cycle, Good Cold Tolerance, Excellent Water stress resistance, Excellent Lodging Resistance, Excellent dehiscence resistance, Good Resistance to Ascochyta, protein 19-20%, Plant Density 30-50 pl/m2 (higher colder envir).
Sultano + Eq.3282	Combined characters of both varieties
Sultano + Eq.3284	Combined characters of both varieties
Fuego	Good leaf health, Pale hilum and stable yields; high 27.5 % protein
Tiffany	High stable yields, good leaf health, low in vicin and convicin, high 27.7 % protein
Fanfare	Resistance to dowry mildew, TSW: 545-556 g, 27.5% protein
Lynxs	Resistance to dowry mildew, Pale hilum, High Yielding 27.5 % protein
Taifun	Low tannin‡ cv, stable yields
Alexia	30% protein, high stable yield potential, Tolerance to root diseases, good performance towards Botrytis, virus and rust
GL Emilia	Tolerance to lodging, good tolerance to diseases, low vicine/convicine‡ content
Anicia	medium size plant, green seed color, French label reference, 30% protein
Itaca	Extern. seed colour Brown, Intern. seed colour orange, Medium-big seed size, erect plant, Plant height 37-40 cm, Medium Late, Seeds/pod 1/2, medium Cold Tolerance, Medium Water stress resistance, Excellent dehiscence resistance, protein 24-25%, Plant Density 220-300 pl/m2 (higher colder envir).
Flora	tall plant, blond seed color, tolerant to lodging, TSW=72 g
Titicaca	Danish bred cv high adaptation range, high and stable yields, Stress tolerance: drought, salinity, frost
Eq.1001	13% protein, Bitter cv (Saponin), TSW: 3.6 g
Eq.1002	14% protein, big seed size, BITTER CV saponin, compact growth, TSW: 3.9 g
Eq.1010	15% protein, bitter cv (saponin), compact growth, TSW:4 g
Eq.1014	14% protein, big seed size, BITTER CV saponin, compact growth, TSW: 4.37 g
Puno	Danish bred cv, tolerant to leaf diseases Stress tolerance: drought, salinity, frost Resistant to downy mildew, white grain TWS: 2.95 G, 17.6%9 PROTEIN

As a result of experienced drought stress, especially in June, all crops received irrigation four times throughout the growing season (total 109mm). Irrigation occurred on the following dates with the specified amounts: 15mm on 02/04/2021, 20mm on 17/06/2021, 37mm on 24/06/2021 and 30/06/2021.

Crops were grown under organic practices; no fertilization was applied, and weeding was periodically performed through hand hoeing.

2.4. Field measurements

Soil water content (SWC, m^3m^{-3}) was monitored throughout the season with CS-616 sensors connected to a CR-1000 datalogger (Campbell Scientific Ltd, Shepshed, England). A total of 8 sensors were randomly placed in the field, 4 in chickpea and 4 in faba bean plots. Crops water consumption (WC, mm d^{-1} was then estimated as:

$$WC = (SWC_T - SWC_{T-1}) * 0.3 * 1000 * -1 \ ,$$

Where SWC_T is the mean SWC of a specific day, SWC_{T-1} is the SWC of the day before, 0.3 is the depth of the layer of soil considered (*i.e.* 0.3m), 1000 is the rate of conversion from m^3m^{-2} to mm and -1 is used to convert from water loss to water consumption. Water consumption was calculated based solely on soil water content values without considering other external factors (e.g., rainfall, evaporation) that directly influence the soil water content. The depth of the soil layer considered in the calculation is determined by the placement of sensors at a depth of 30 cm, as specified by their technical specifications, also corresponding to the layer explored by the majority of the root system (>90%) of the investigated crops.

Plants height (H_p, cm) was assessed as the average of 10 plants per plot. H_p was measured three times during the growing season: on 21/05/2021, 03/06/2021 and 17/06/2021.

Measurements at flowering were conducted on 10/06/2021. For each crop we selected 2 *cvs*: chickpea: *Sultano* and *Eq.3282*; faba bean: *Taifun* and *Tiffany*; lentil: *Itaca* and *Ancia*. In each plot we sampled an area of 0.76m^2 (2 rows of 1m and row distance of 0.38m). For each sampling area we counted the total number of plants to estimate the plant density (PD, plants m^{-2}) and measured H_p of 5 plants. Plants were then cut at the ground level to measure the aboveground fresh biomass (FW_{abv}, kg m^{-2}) and oven-dried for 72h at 70°C to measure the aboveground dry biomass (DW_{abv}, kg m^{-2}). Dry matter content (DMC, g/g) was then calculated as the ratio between DW and FW. Moreover, considering the same CVs, 10 plants per plot were collected to record the number of leaves of each plant. After leaves and stems were separate. Leaves area (LA, m^2) was measured with LI-COR 3050C leaf area meter (LI-COR Biosciences, Lincoln, USA). Leaves and stems were then oven-dried for 72h at 70°C to measure stems dry weight (SDW_{sub}, kg), leaves dry weight (LDW_{sub}, kg) and estimate the leaves fraction of the total biomass (LF, kg kg^{-1}). Specific leaf area (SLA, m^2 kg^{-1}) was calculated as the ratio between LA and LDW_{sub}. Leaves total dry biomass (LDW_{tot}, kg m^{-2}) was calculated as the product of LF and DW. Leaf Area Index (LAI, $m^2 m^{-2}$) was then calculated as the product of LDW and SLA.

A further measurement of LAI was conducted on 23/06/2023 with the LI-COR LAI-2200C Plant Canopy Analizer. For each plot 2 sets of 5 measurements per row were conducted, following the diagonal transects' protocol for row crops specified by the producer.

At harvest, for all plots two sample areas were assessed. A first area of 0.76m^2 was sampled to measure PD, number of pods per plant, total DW and yield (g/m^2). Harvest Index (HI) was then calculated as:

$$HI = \frac{yield}{yield + DW}$$

A larger area of 3.04m^2 (2 rows of 4m length) was sampled on each plot to measure yield. Due to issues in data collection and lack of replicates for proper data correction, anomalous yield data from the larger area were replaced with yield measured in the smaller area of the same plot.

2.5 CHN analysis and protein content

Dry samples of crop residues and seeds were grounded into a fine powder with a ball mill to ensure uniformity in the sample. Nitrogen content (N_C, g g^{-1}) was then measured using Dumas' combustion using a CN Elemental Analyser (Vario Microcube, © Elementar) coupled to a stable isotope ratio mass spectrometer (IRMS; Isoprime 100, © Elementar).

A subsample of grounded samples was oven-dried at 120°C for 48h to determine the residual water content and subsequently correct the CHN measurements.

Protein content (PC, g g^{-1}) of seeds was then estimated as:

$$PC = N_c * 6.25$$

where N_c is the Nitrogen content of seeds (g g^{-1}) and 6.25 is the conversion factor to estimate the protein content based on the assumption that, on average, proteins contain approximately 16% Nitrogen.

Protein yield (ton ha^{-1}) was calculated as the product of yield and Nc.

2.6 Spectral Vegetation Indexes

Remote sensing data were acquired with an unmanned aerial system (UAS) on 4 dates during the growing season at: i) crops development – 21/05/2021; ii) flowering – 04/06/2021; iii) early pods setting – 26/06/2021; iv) late pods maturation – 05/07/2021. All UAS flights were conducted between 10:00 to 11:00 a.m. (L.S.T.).

Altum and multispectral data were acquired with a MicaSense RedEdge MX camera (MicaSense Inc., Seattle, WA, USA) equipped on an unmanned aerial vehicle (UAV) piloted in manual mode. Spectral bands acquired were Blue (B, 475±32nm), Green (G, 560±27nm), Red (R, 668±14nm), Red Edge (RedEd, 717±12nm) and Near-Infrared (NIR, 842±57nm). Images were acquired with a multicamera system at 1 Hz frequency with minimum overlap of 80%. Pictures of the MicaSense calibrated reflectance panel were also acquired before and after each flight, in order to correct the acquired images on the day's lighting conditions and compare the results of different flights. Furthermore, to accurately georeferentiate the data, 17 ground control points were randomly placed in the field and their GPS positions and height accurately assessed.

Images processing has been conducted using the software Agisoft Metashape v.1.4.2 (Agisoft 2018) to obtain a digital elevation model (DEM), a digital terrain model (DTM) and reflectance raster maps for each spectral band. Single-plot data were extracted using the GRASS GIS v.6.4.5 software (GRASS Development Team 2010), by over-lapping a mask vector of 3.04m^2 (4m length, 0.76m width) on each plot and setting the geographic regions boundaries for the sampling areas with the "*g.region*" and "*r.mask*" functions. Camopy pixels were selected by subtracting the DTM to the DEM. Average plot values were then calculated with the R v2.0.1 software (R Core Team, 2021) and used to calculate the Normalized Difference Vegetation Index (NDVI, Rouse et al. 1974) as:

$$NDVI = \frac{(NIR - R)}{(NIR + R)}$$

2.6 Statistical analysis

All statistical analyses were conducted with the R software. All analyses were conducted separately for each species. After data normality a homoscedasticity assessment, differences between treatments were assessed by one way analysis of variance and Tukey's Honest Significance Difference as *post hoc*. Since sampling at flowering was conducted on two *cvs* per species, differences were assessed by Student's t-Test.

3 Results

3.1 Soil Water Content

Variation in soil water content of the upper layer (0-30cm) during the growing season is represented in Figure 1.3. We reported values for chickpea and faba bean up to harvest date, hence no datum was reported for lentil. SWC was lower in faba bean throughout the whole season, however, by looking to the estimated water consumption (Figures 1.4, 1.5), there was no difference between crops.

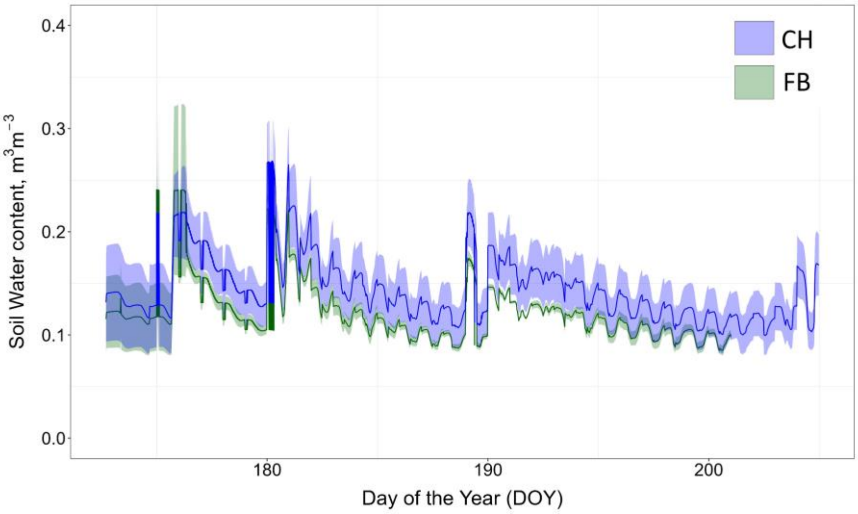

Figure 1.3. Soil water content (0-30cm) measured during the growing season 2021 in Udine, in fields cultivated with Chickpea (CH, blue line and ribbon) and Faba Bean (FB, green line and ribbon).

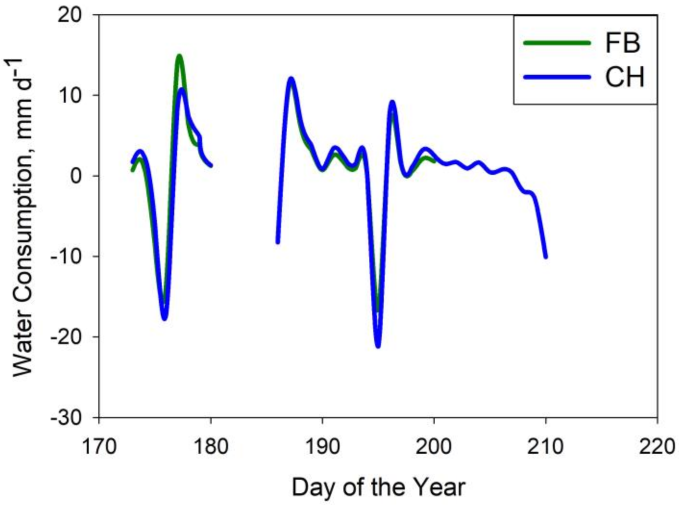

Figure 1.4. Daily water consumption (mm d^{-1}) calculated for chickpea (CH, blue line) and faba bean (FB, green line) from soil water content measured during the growing season 2021 in Udine.

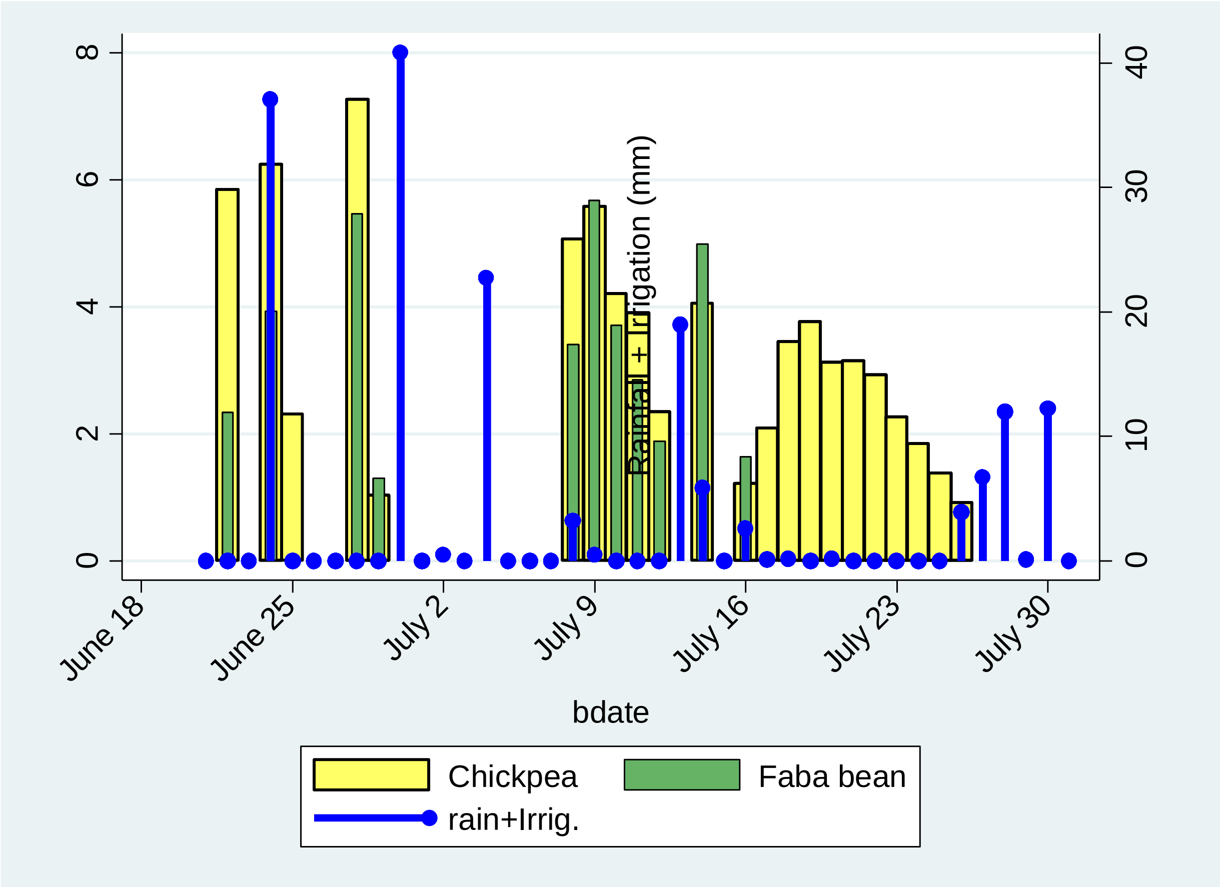

Figure 1.5. Daily water use (mm d^{-1}) for chickpea (CH, yellow bar) and faba bean (FB, green bar) from soil water content at 0.3m depth measured with soil moisture sensors from June 21st 2021 to harvest in Udine. Sum of Rainfalls and irrigations (mm·d^{-1}) are also indicated (blue line).

3.2 Crops phenology and development

Crops phenological development stages with corresponding thermal sums are reported in Table 1.4. Within each species, all cultivars exhibited a similar development. Emergence, flowering and harvest maturity occurred within a few days across all cultivars. Among species, chickpea had the highest thermal sum requirement, with 980°C d^{-1} at flowering and 2223°C d^{-1} at harvest.

Table 1.4. Overview of crops development during the growing season 2021 in Udine. For each phenological stage are reported the average date and corresponding Growing Degree Days (GDDs, base temperature = 0°C).

Species	Sowing	Emergence		Flowering		Harvest	
	Date	Date	GDDs (°C/d)	Date	GDDs (°C/d)	Date	GDDs (°C/d)
Chickpea	30/03/2021	14/04/2021	165	08/06/2021	980	29/07/2021	2223
Faba bean	30/03/2021	14/04/2021	165	02/06/2021	853	16/07/2021	1902
Lentil	30/03/2021	11/04/2021	138	08/06/2021	980	Not harvested	-

Values of H_p measured among the growing season are graphically represented in Figures 1.6-1.8 and reported in Table 1.5. Statistical differences among plants heights occurred differently according to the growth stage. At crops development (Figure 1.4) no differences were assessed among chickpea and faba bean cultivars. On the contrary, in lentil, cv *Flora* was significantly higher than *Itaca* and *Anicia* ($p < 0.01$). Similar results occurred at crops flowering (Figure 1.5) two weeks later. On the contrary, at flowering statistical differences were assessed in chickpea, where all treatments with *cv Sultano* were significantly higher than *Eq.3283* ($p < 0.01$).

Table 1.5. Mean values with corresponding standard deviation of plant height measured in various treatments of chickpea, faba bean and lentil during the growing season 2021 in Udine. Statistical differences are denoted by different letters, otherwise not significant.

Species	**Treatment**	**Plant height, cm**		
		21/05/2021	**03/06/2021**	**17/06/2021**
Chickpea	Sultano	20.5 ± 0.7	37.6 ± 2.7	55.3 ± 4.0 **b**
	Eq.3279	20.1 ± 2.2	34.3 ± 0.9	47.9 ± 3.3 **ab**
	Eq.3282	21.2 ± 0.5	33.2 ± 2.7	45.0 ± 2.4 **ab**
	Eq.3283	20.2 ± 1.5	35.3 ± 2.8	41.9 ± 2.9 **a**
	Eq.3284	21.4 ± 1.3	34.5 ± 5.7	47.2 ± 2.0 **ab**
	Sult+Eq.3282	24.2 ± 3.1	36.8 ± 2.2	55.2 ± 9.0 **b**
	Sult+Eq.3284	23.1 ± 2.3	42.8 ± 5.8	55.5 ± 3.9 **b**
Faba bean	Alexia	39.4 ± 0.5	63.7 ± 3.8	87.0 ± 7.8
	Fanfare	39.6 ± 5.8	61.4 ± 3.4	85.2 ± 5.6
	Fuego	39.3 ± 1.5	63.0 ± 2.7	83.0 ± 1.9
	GL Emilia	37.8 ± 1.3	60.6 ± 4.6	87.8 ± 2.6
	Lynxs	38.4 ± 0.5	60.9 ± 3.7	87.2 ± 2.0
	Taifun	37.9 ± 1.8	58.6 ± 2.8	83.1 ± 5.7
	Tiffany	41.0 ± 1.9	64.7 ± 0.6	89.0 ± 7.9
Lentil	Anicia	14.1 ± 0.2 **a**	21.1 ± 1.6 **ab**	31.4 ± 2.2
	Flora	18.9 ± 1.1 **b**	26.2 ± 2.4 **b**	36.0 ± 1.7
	Itaca	14.8 ± 0.8 **a**	18.6 ± 1.7 **a**	30.3 ± 6.0

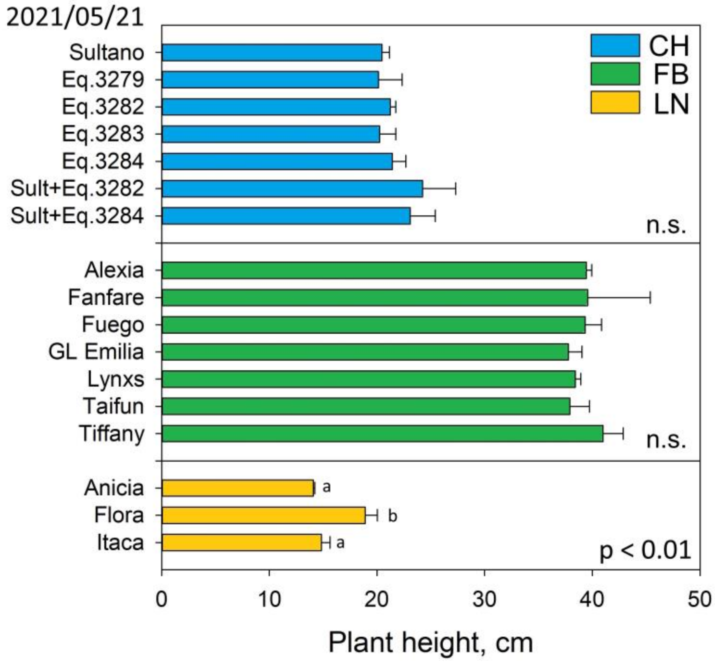

Figure 1.6. Plants height of Chickpea (CH, blue bars), Faba bean (FB, green bars) and Lentil (LN, yellow bars) varieties measured during crops development on 21/05/2021.Analysis of variance (ANOVA) probability values are specified whether statistical differences were assessed, otherwise not significant (n.s.)

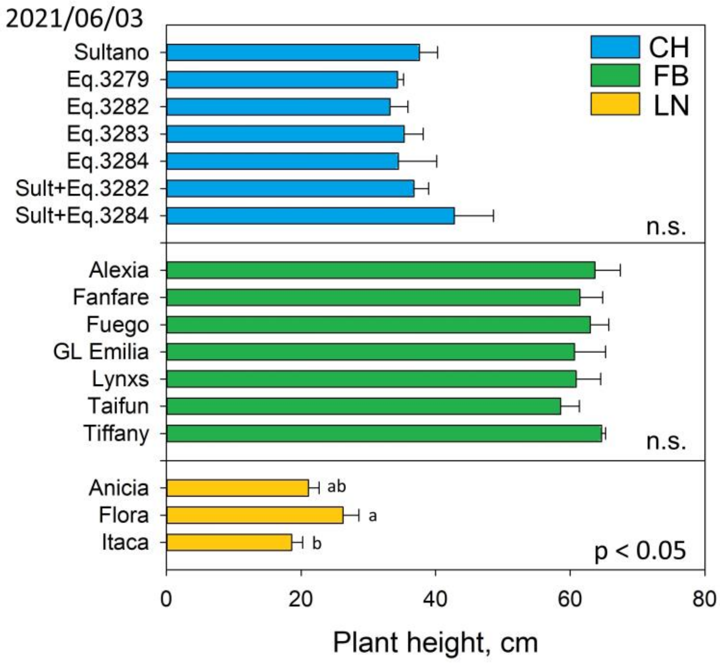

Figure 1.7. Plants height of Chickpea (CH, blue bars), Faba bean (FB, green bars) and Lentil (LN, yellow bars) varieties measured at crops flowering, on 03/06/2021.Analysis of variance (ANOVA) probability values are specified whether statistical differences were assessed, otherwise not significant (n.s.)

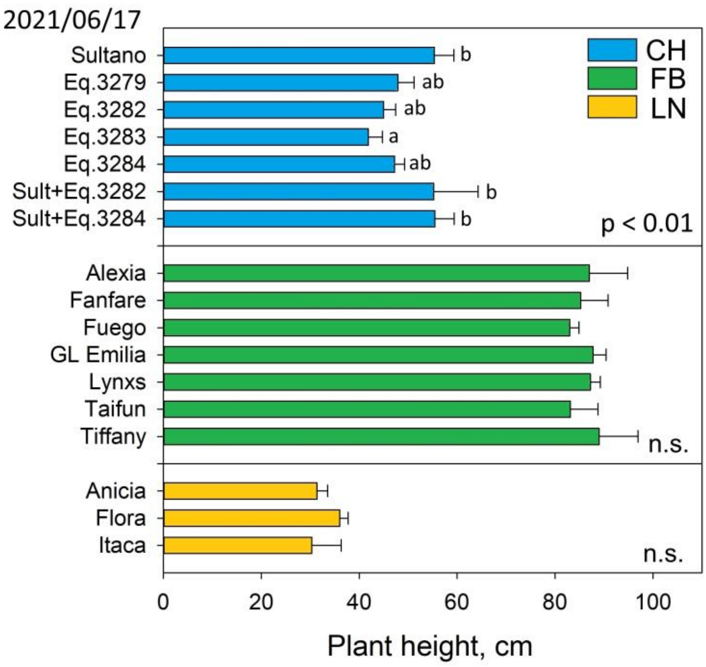

Figure 1.8. Plants height of Chickpea (CH, blue bars), Faba bean (FB, green bars) and Lentil (LN, yellow bars) varieties measured on 17/06/2021.Analysis of variance (ANOVA) probability values are specified whether statistical differences were assessed, otherwise not significant (n.s.)

Mean values with corresponding standard errors of LAI measured in chickpea and faba bean on June 23rd and July 2nd 2021 are reported in Table 1.6. On June 23rd, LAI in chickpea ranged from 1.72±0.32m^2m^{-2} to 2.57±0.12m^2m^{-2}, while in faba bean from 1.58±0.38m^2m^{-2} to 1.84±0.43m^2m^{-2}. On July 2nd, 2021, LAI in chickpea ranged from 1.66±0.18m^2m^{-2} to 2.52±0.22m^2m^{-2}, while in faba bean from 1.5±0.1m^2m^{-2} to 2.13±0.3m^2m^{-2}. From first to the second measurement, in chickpea, LAI increased in treatments: *Eq.3279, Eq.3283, Sultano, Sultano + Eq.3282* and *Sultano + Eq.3284*; and decreased in *Eq.3282* and *Eq.3284*. The increase in the intercropped treatments may be due to the increase in LAI of the *Sultano* variety. In faba bean, LAI increased in *Tiffany*, decreased in *Alexia*, *Fanfare* and *Taifun* and remained stable in *Fuego*, *GL Emilia* and *Lynxs*.

No statistical difference was assessed either considering CV and the interaction of date*CV in a two-way ANOVA (both $p > 0.05$).

Table 1.6. Mean values with corresponding standard error of leaf area index (LAI) measured in chickpea (CH) and lentil (LN) on June 23rd and July 2nd, 2021. No statistical difference was assessed.

Species	Treatment	LAI m^2m^{-2}	
		June 23rd 2021	**July 2nd 2021**
CH	Eq.3279	1.81 ± 0.14	2.23 ± 0.14
	Eq.3282	2.57 ± 0.12	2.29 ± 0.14
	Eq.3283	2.01 ± 0.16	2.3 ± 0.14
	Eq.3284	1.72 ± 0.32	1.66 ± 0.18
	Sultano	2.26 ± 0.21	2.52 ± 0.22
	Sultano + Eq.3282	2.1 ± 0.09	2.44 ± 0.25
	Sultano + Eq.3284	1.93 ± 0.27	2.32 ± 0.16
FB	Alezia	1.7 ± 0.23	1.6 ± 0.11
	Fuego	1.7 ± 0.4	1.72 ± 0.06
	GL Emilia	1.58 ± 0.38	1.6 ± 0.36
	Fanfare	1.75 ± 0.38	1.5 ± 0.1
	Tiffany	1.84 ± 0.43	2.13 ± 0.3
	Lynxs	1.74 ± 0.51	1.76 ± 0.3
	Taifun	1.73 ± 0.37	1.57 ± 0.18

3.3 Plant growth measurements at Flowering

Measurements were done just after flowering on 10/06/2021 considering two *cvs* per species: chickpea *Eq.3282* and *Sultano*; faba bean *Taifun* and *Tiffany*; lentil *Itaca* and *Ancia*. The cvs choice was addressed selecting the most productive ones as recorded in previous year experiments conducted in the project network.

Plant density (PD) of crops is represented in Figure 1.9. Measured values partially match the target plant density set at sowing (see Table 1.2). Statistical difference was assessed in chickpea, where *Sultano* reached the target PD of 45 plants m^{-2} while in *Eq.3282* germination was lower than expected.

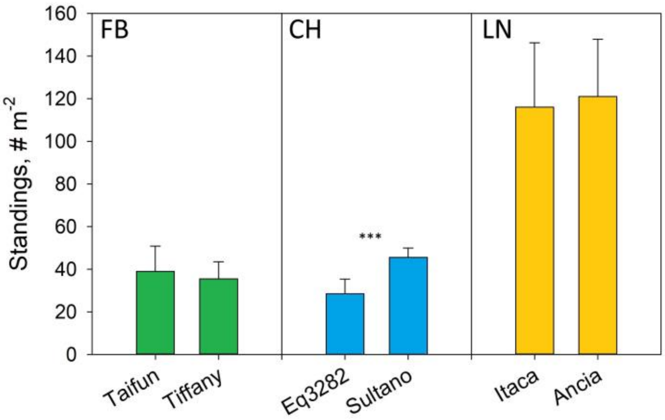

Figure 1.9. Plant density (Standings) measured in selected varieties of Chickpea (CH, blue bars), Faba bean (FB, blue bars) and lentil (LN, yellow bars) at flowering on 10/06/2021. Significance: * p<0.05; **p<0.01; ***p<0.001; otherwise not significant.

Number of leaves per plant are represented in Figure 1.11. Values were similar among varieties, ~28 leaves plant^{-1} in chickpea, 14 leaves plant^{-1} in faba bean and 24 leaves plant^{-1} in lentil. No statistical difference was assessed.

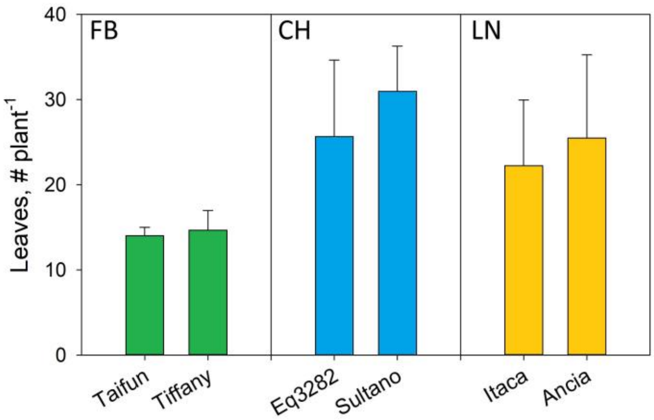

Figure 1.10. Number of leaves per plant measured in selected varieties of Chickpea (CH, blue bars), Faba bean (FB, blue bars) and lentil (LN, yellow bars) at flowering on 10/06/2021. No significant differences were assessed.

SLA values measured at flowering are represented in Figure 1.11. Faba bean had the highest SLA values (13-14m^2kg^{-1}), followed by lentil (10-13m^2kg^{-1}), then chickpea (9-10m^2kg^{-1}). No difference was assessed.

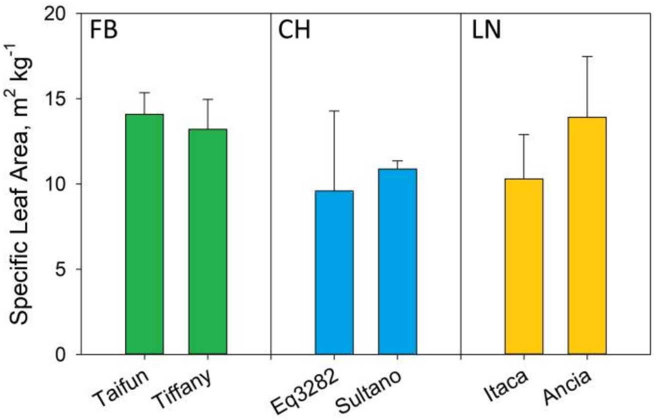

Figure 1.11. Specific leaf area measured in selected varieties of Chickpea (CH, blue bars), Faba bean (FB, blue bars) and lentil (LN, yellow bars) at flowering on 10/06/2021. No significant differences were assessed.

DW_{abv} values measured at flowering are represented in Figure 1.12. Cultivars were similar within each species and no statistical difference was assessed. Faba bean produced the higher biomass with ~2.3ton ha^{-1}, followed by lentil then chickpea, with ~1.2ton ha^{-1} and 0.9ton ha^{-1}, respectively.

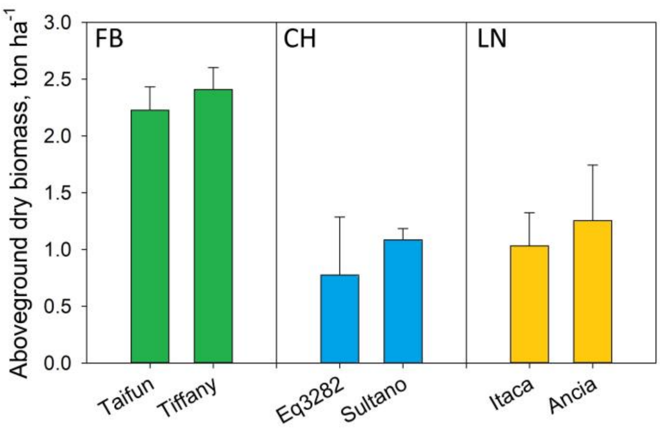

Figure 1.12. Aboveground dry biomass measured in selected varieties of Chickpea (CH, blue bars), Faba bean (FB, blue bars) and lentil (LN, yellow bars) at flowering on 10/06/2021. No significant differences were assessed.

LAI values measured at flowering in the selected varieties of chickpea, faba bean and lentil are graphically represented in Figure 1.13. Higher values were measured in faba bean, with approximatively $2m^2m^{-2}$, while in both chickpea and lentil mean values were lower than $1m^2m^{-2}$. Statistical differences among cultivars of the same species have not been assessed.

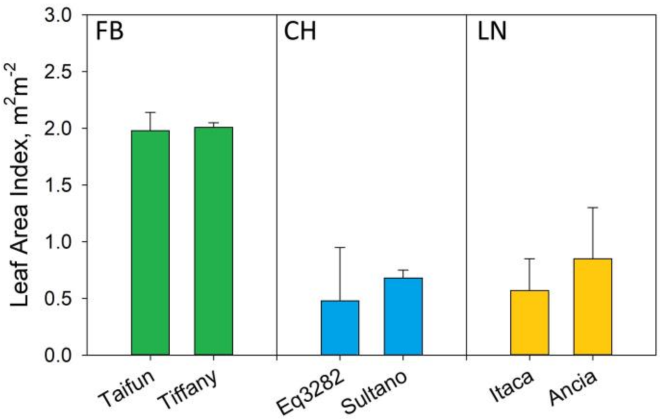

Figure 1.13. Leaf area index measured in selected varieties of Chickpea (CH, blue bars), Faba bean (FB, blue bars) and lentil (LN, yellow bars) at flowering on 03/06/2021. No significant differences were assessed.

3.4 Measurements at harvest

Mean values with corresponding standard deviations of parameters measured at harvest are reported in Table 1.7 and graphically represented in Figures 1.14-1.16.

DW_{abv} was similar between chickpea and faba bean (Figure 1.14). In chickpea, *Sultano* had the highest DW_{abv}, with 2.54 ± 1.18ton ha^{-1}, while *Eq.3279* exhibited the lowest one, 1.40 ± 0.34ton ha^{-1}). However, no statistical difference was assessed among the treatments. In faba bean, highest DW_{abv} was measured in *Tiffany*, with 3.25 ± 0.65ton ha^{-1}. Statistical differences ($p<0.01$) were assessed between *Tiffany* and four underperforming varieties: *Alexia*, *GL Emilia*, *Fanfare* and *Taifun*.

In terms of yield (Figure 1.15), faba bean varieties of *Tiffany* and *Famfare* were the most productive, while *GL Emilia* and *Taifun* yields significantly lower. Overall, regardless of the variety, yield of faba bean was generally low. In chickpea, *Sultano* and *Eq.3282* were the most productive, respectively with 1.71 ± 0.50ton ha^{-1} and 1.67 ± 0.44ton ha^{-1}. These treatments were significantly different from *Eq.3283 and Eq.3284,* which were the less performing. In intercropped treatments of *Sultano* we measured lower yields, approximatively 0.5ton ha^{-1} less than the pure variety, however no statistical difference was assessed.

In faba bean, HI was generally low (Figure 1.16). All *cvs* had mean values of HI lower than 0.3. Lowest values were in *Taifun* (0.14 ± 0.08), significantly different ($p < 0.01$) from *Alexia* and Fanfare which HI were 0.28 ± 0.09 and 0.30 ± 0.04, respectively. By the other side, all chickpea treatments performed well with average values ranging from 0.37 to 0.45. No statistical difference was assessed in chickpea.

Table 1.7. Mean values with corresponding standard deviation of yield, aboveground dry biomass of residues and harvest index measured at harvest in chickpea (CH) and faba bean (FB) at the end of the growing season 2021 in Udine. Statistical differences are denoted by different letters, otherwise not significant.

Species	**Treatment**	**Aboveground dry biomass of residues ton ha^{-1}**	**Yield ton ha^{-1}**	**Harvest index**
CP	Eq.3279	1.40 ± 0.34	1.13 ± 0.25 **AB**	0.45 ± 0.01
	Eq.3282	2.29 ± 0.52	1.67 ± 0.44 **B**	0.42 ± 0.04
	Eq.3283	1.67 ± 0.59	1.02 ± 0.40 **A**	0.38 ± 0.03
	Eq.3284	1.72 ± 0.08	1.03 ± 0.27 **A**	0.37 ± 0.06
	Sultano	2.54 ± 1.18	1.71 ± 0.50 **B**	0.41 ± 0.04
	Sultano+Eq.3282	1.82 ± 0.51	1.24 ± 0.22 **AB**	0.41 ± 0.03
	Sultano+Eq.3284	1.74 ± 0.12	1.20 ± 0.38 **AB**	0.40 ± 0.06
FB	Alexia	1.69 ± 0.65 **a**	0.64 ± 0.23 **ab**	0.28 ± 0.09 **a**
	GL Emilia	1.71 ± 0.82 **a**	0.35 ± 0.12 **a**	0.18 ± 0.09 **ab**
	Fanfare	1.66 ± 0.47 **a**	0.75 ± 0.36 **b**	0.30 ± 0.04 **a**
	Fuego	2.10 ± 0.78 **ab**	0.50 ± 0.04 **ab**	0.20 ± 0.06 **ab**
	Lynxs	2.12 ± 0.36 **ab**	0.62 ± 0.11 **ab**	0.23 ± 0.03 **ab**
	Taifun	1.56 ± 0.54 **a**	0.29 ± 0.22 **a**	0.14 ± 0.08 **b**
	Tiffany	3.25 ± 0.65 **b**	0.77 ± 0.24 **b**	0.20 ± 0.08 **ab**

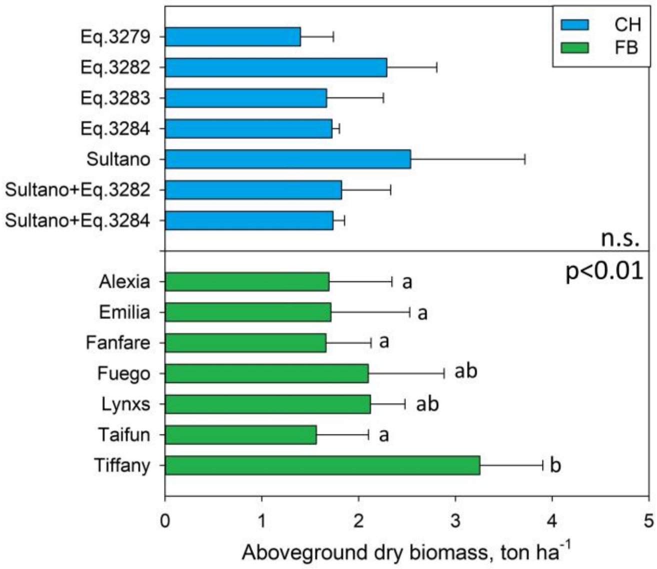

Figure 1.14. Aboveground dry biomass of residues, measured in Chickpea (CH, blue bars) and Faba bean (FB, green bars) treatments at harvest. Different letters denote significant differences. “n.s.” = not significant ($p > 0.05$).

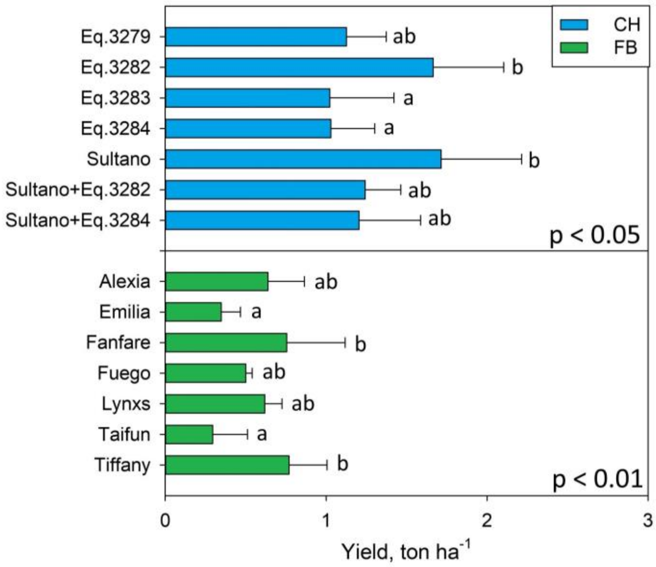

Figure 1.15. Yield measured in Chickpea (CH, blue bars) and Faba bean (FB, green bars) treatments at harvest. Different letters denote significant differences. “n.s.” = not significant ($p > 0.05$).

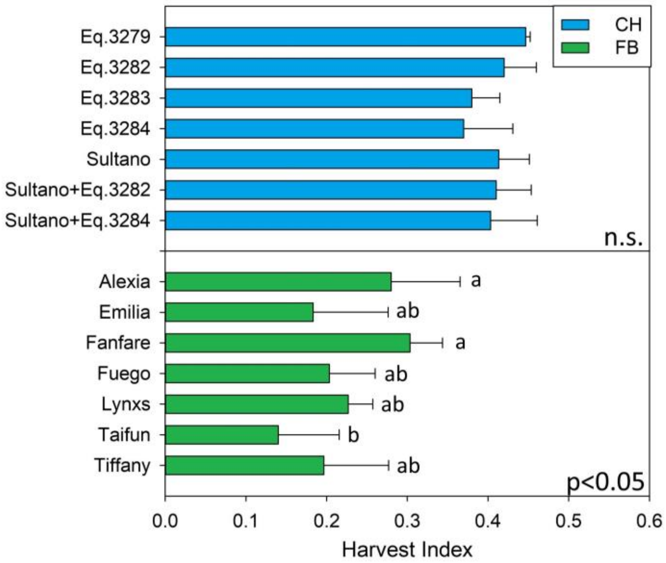

Figure 1.16. Harvest Index measured in Chickpea (CH, blue bars) and Faba bean (FB, green bars) treatments at harvest. Different letters denote significant differences. "n.s." = not significant ($p > 0.05$).

3.5 CHN analysis and protein content

Results of CHN analysis are reported in Table 1.8 and graphically represented in Figures 1.17-1.19.

Protein content of seeds (Figure 1.17) was mostly similar within different treatments of the same crop and no statistical difference was assessed. In chickpea, mean values of protein content (PC) ranged between 0.18g g^{-1} and 0.19g g^{-1}, while in faba bean from 0.27g g^{-1} to 0.32g g^{-1}.

Protein yield (Figure 1.18) in chickpea was higher in *Sultano* and *Eq.3282*, being respectively 313.2 ± 106.2kg ha^{-1} and 307.4 ± 89.1kg ha^{-1}. In all other treatments, average protein yields ranged from 190.0kg ha^{-1} in *Eq.3284* to 228 ± 48.4kg ha^{-1} in *Sultano* + *Eq.3282*, however we did not observe any statistical difference. In faba bean, protein yield was lower in *GL Emilia* and *Taifun*, with 101.3 ± 59.6kg ha^{-1} and 91.0 ± 64.4kg ha^{-1}, respectively. These were statistically different from the most productive varieties: *Fanfare* with 240.9 ± 111.8kg ha^{-1} and *Tiffany* with 223.8±47.4kg ha^{-1}.

Nitrogen stock of remaining crop residues (kgN ha^{-1}) (Figure 1.19) was generally low in both species. In chickpea, higher values were measured in Eq.3282, with 39.3 ± 10.9kg ha^{-1} and lower in *Eq.3279*, with 22.8 ± 5.9kg ha^{-1}. Despite this difference statistical differences were not assessed, probably due to the high variability of data. In faba bean the highest values were measured in *Lynxs* and *Tiffany*, with 43.3 ± 12.5kg ha^{-1} and 55.9 ± 14.1kg ha^{-1}, respectively. These were significantly different ($p < 0.05$) from *Taifun* 24.1 ± 6.1kg ha^{-1}.

Table 1.8. Mean values with corresponding standard deviation of protein content of seeds, protein yield and nitrogen stock of residuals measured in chickpea (CH) and faba bean (FB) at the end of the growing season 2021 in Udine. Statistical differences are denoted by different letters, otherwise not significant.

Species	**Treatment**	**Protein content** g g^{-1}	**Protein yield** kg ha^{-1}	**Nitrogen stock** kgN ha^{-1}
CP	Eq.3279	0.19 ± 0.01	210.8 ± 62.1	22.8 ± 5.9
	Eq.3282	0.18 ± 0.01	307.4 ± 89.1	39.3 ± 10.9
	Eq.3283	0.19 ± 0.01	197.3 ± 81.1	24.5 ± 11.3
	Eq.3284	0.19 ± 0.02	190.0 ± 43.8	23.7 ± 4.8
	Sultano	0.18 ± 0.01	313.2 ± 106.2	35.0 ± 23.2
	Sultano+Eq.3282	0.18 ± 0.01	228.6 ± 48.4	27.2 ± 15.0
	Sultano+Eq.3284	0.18 ± 0.01	221.4 ± 63.7	24.2 ± 5.2
FB	Alexia	0.28 ± 0.03	182.0 ± 67.0 **ab**	24.4 ± 15.1 **ab**
	GL Emilia	0.27 ± 0.10	101.3 ± 59.6 **a**	30.0 ± 16.3 **ab**
	Fanfare	0.32 ± 0.03	240.9 ± 111.8 **b**	26.9 ± 9.0 **ab**
	Fuego	0.28 ± 0.01	139.6 ± 12.7 **ab**	34.9 ± 9.7 **ab**
	Lynxs	0.29 ± 0.01	181.1 ± 24.2 **ab**	43.3 ± 12.5 **b**
	Taifun	0.31 ± 0.02	91.0 ± 64.4 **a**	24.1 ± 6.1 **a**
	Tiffany	0.30 ± 0.03	223.8 ± 47.4 **b**	55.9 ± 14.1 **b**

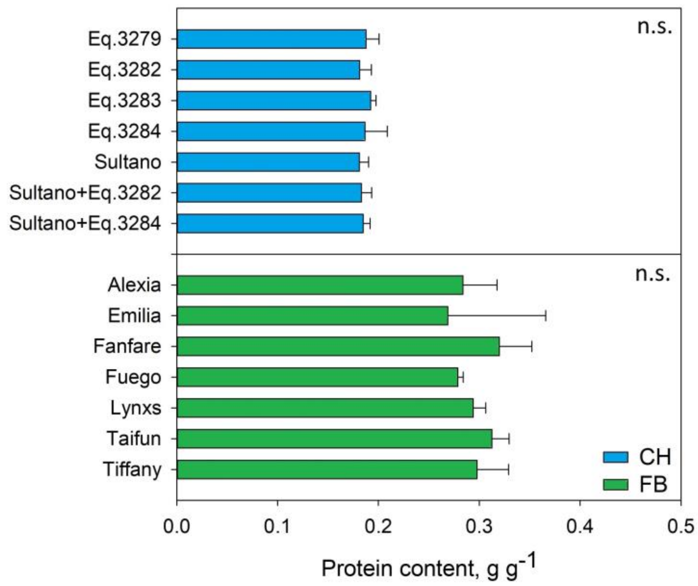

Figure 1.17. Protein content of seeds estimated for Chickpea (CH, blue bars) and Faba bean (FB, green bars) treatments. No statistical difference was assessed (n.s.).

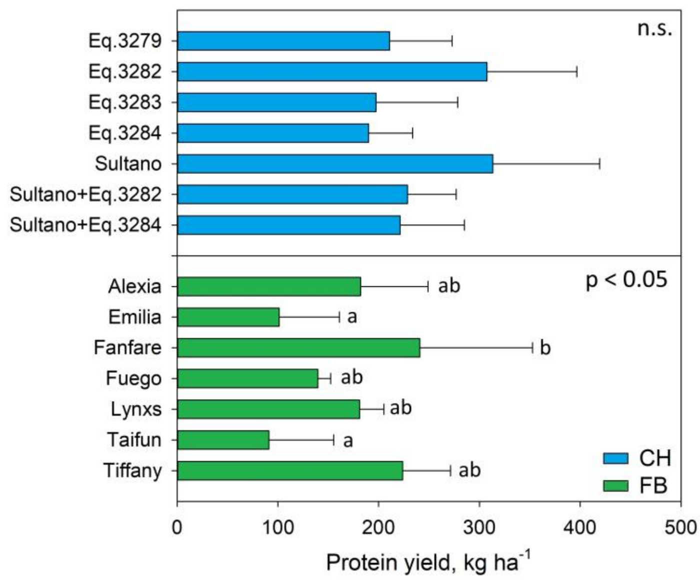

Figure 1.18. Protein yield estimated for Chickpea (CH, blue bars) and Faba bean (FB, green bars) treatments. No statistical difference was assessed (n.s.).

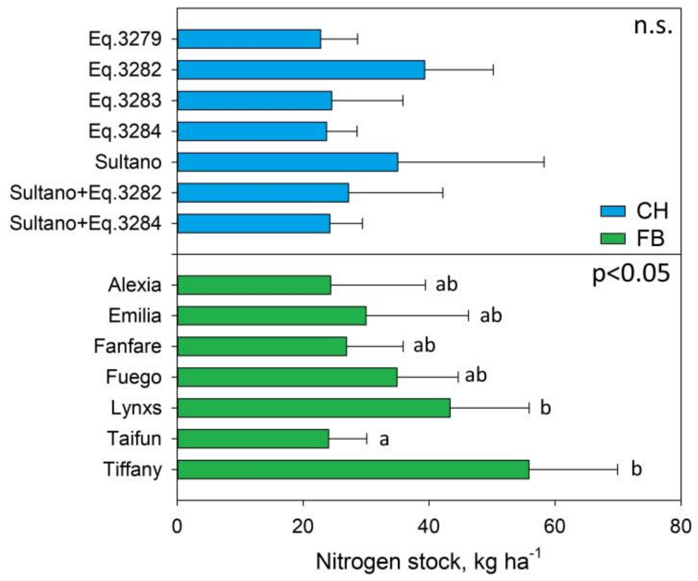

Figure 1.19. Nitrogen stock of crop residues measured in Chickpea (CH, blue bars) and Faba bean (FB, green bars) treatments. Different letters denote significant differences. "n.s." = not significant ($p > 0.05$).

3.6 Remote sensing of crops

Spectral signatures of crops obtained from reflectance data are represented in Figure 1.20. On 21/05/2021, during crops development, reflectance in the visible spectrum (VIS, 400-700nm) was mostly similar among species, with the exception of faba bean, which exhibited higher absorption in the red region and higher reflectance of NIR. On 04/06/2021, at flowering, spectral signature of all crops followed the same trend, however reflectance in the green and red region was slightly lower in faba bean. On 23/06/2021, at early pods maturation, spectral reflectance in the infrared region decreased in faba bean and lentil, while in chickpea it followed the same trend of the previous measurement. On 05/07/2021, at late pods maturation all crops exhibited a similar trend of low reflectance among all spectrum, typical of crops senescence.

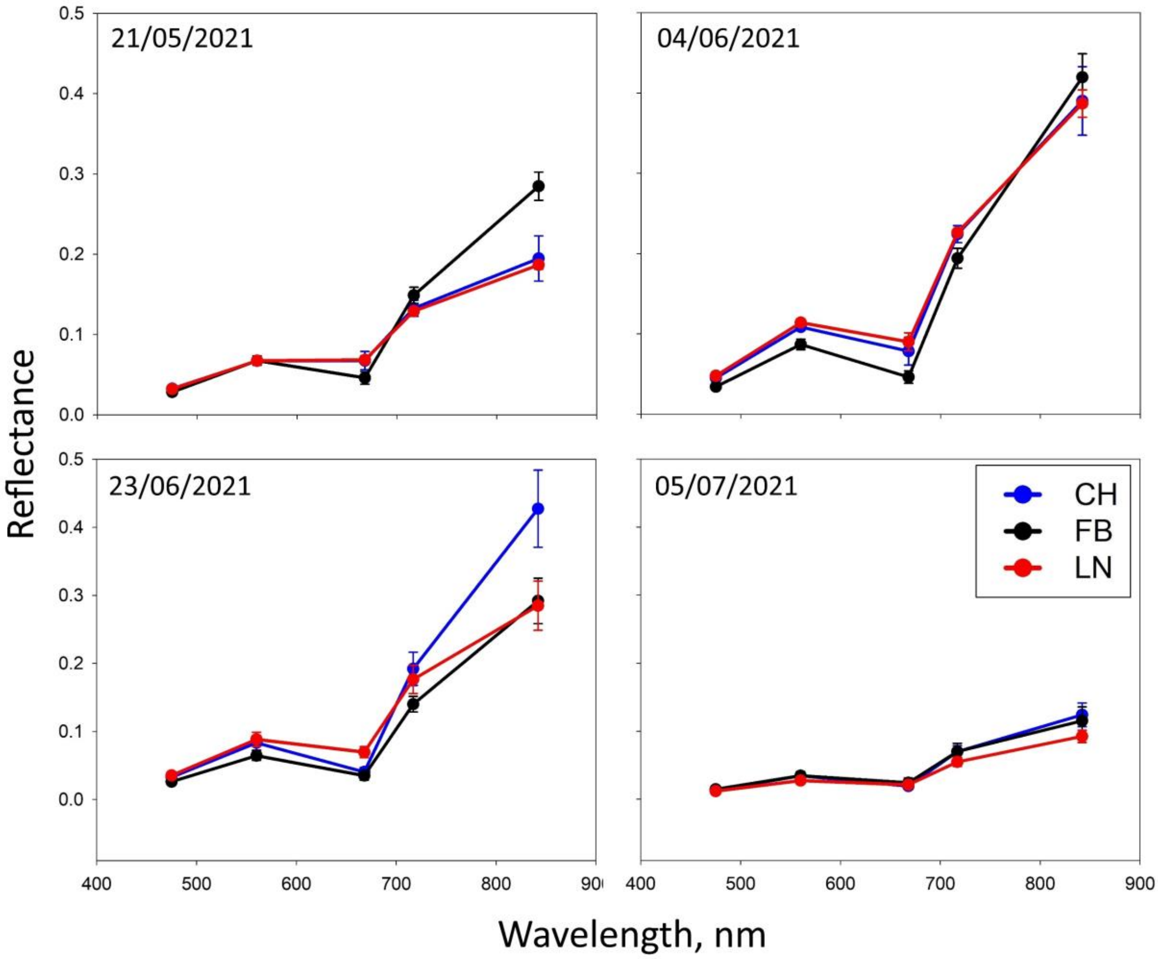

Figure 1.20. Spectral signature of Chickpea (CH), Faba bean (FB) and Lentil (LN) from multispectral data acquired on 4 dates during the growing season in Udine.

NDVI values measured during the growing season are listed in Table 1.9 and graphically represented in Figures 1.21-1.23. No statistical difference was assessed for any crop on any date. The highest values were recorded in faba bean and lentil approaching flowering stage while in chickpea, NDVI increased even after flowering through the end of June. Subsequently, NDVI decreased in all crops, as senescence progressed. Looking at standard deviation values and comparing 1st and 2nd date with 3rd and 4th, standard deviation values are higher. It is possible to comment saying that CVs were affected differently by diseases and weed cover (e.g. disease Botrytis affected more *Taifun* than *Tiffany*) and weed cover was different in different block and this interacted with the CVs effect. Nevertheless, we need to consider that the blocking factor is used to reduce spatial variability and assure the comparability among the treatments. Therefore, if weed cover is different among blocks, you can still compare the relative differences among the varieties.

Table 1.9. Mean values with corresponding standard deviation of Normalized Difference Vegetation Index (NDVI) acquired in chickpea (CH), faba bean (FB) and lentil (LN) on 4 dates during the growing season at: i) crops development – 21/05/2021; ii) flowering – 04/06/2021; iii) early pods maturation – 26/06/2021; iv) late pods maturation – 05/07/2021. No statistical difference was assessed ($p > 0.05$).

	NDVI				
Species	**Treatment**	**21 May '21**	**4 June '21**	**23 June '21**	**5 July '21**
CH	Eq.3279	0.64 ± 0.02	0.78 ± 0.02	0.80 ± 0.02	0.67 ± 0.03
	Eq.3282	0.62 ± 0.02	0.77 ± 0.01	0.86 ± 0.01	0.74 ± 0.05
	Eq.3283	0.62 ± 0.04	0.77 ± 0.02	0.83 ± 0.01	0.74 ± 0.06
	Eq.3284	0.64 ± 0.06	0.76 ± 0.04	0.82 ± 0.01	0.66 ± 0.10
	Sultano	0.59 ± 0.02	0.73 ± 0.02	0.85 ± 0.02	0.75 ± 0.06
	Sult + Eq.3282	0.61 ± 0.04	0.76 ± 0.05	0.84 ± 0.02	0.76 ± 0.06
	Sult + Eq.3284	0.66 ± 0.06	0.79 ± 0.04	0.85 ± 0.01	0.73 ± 0.04
FB	Alexia	0.68 ± 0.02	0.80 ± 0.00	0.78 ± 0.02	0.61 ± 0.06
	Emilia	0.71 ± 0.03	0.82 ± 0.01	0.76 ± 0.07	0.62 ± 0.08
	Fanfare	0.73 ± 0.03	0.83 ± 0.02	0.77 ± 0.07	0.64 ± 0.11
	Fuego	0.70 ± 0.06	0.80 ± 0.04	0.78 ± 0.06	0.62 ± 0.10
	Lynxs	0.72 ± 0.02	0.82 ± 0.02	0.78 ± 0.07	0.65 ± 0.12
	Taifun	0.72 ± 0.02	0.82 ± 0.02	0.78 ± 0.06	0.66 ± 0.10
	Tiffany	0.72 ± 0.03	0.83 ± 0.03	0.81 ± 0.05	0.72 ± 0.09
LN	Ancia	0.66 ± 0.03	0.81 ± 0.03	0.53 ± 0.06	0.59 ± 0.09
	Flora	0.69 ± 0.02	0.82 ± 0.02	0.59 ± 0.04	0.57 ± 0.07
	Itaca	0.67 ± 0.03	0.80 ± 0.04	0.55 ± 0.10	0.56 ± 0.11

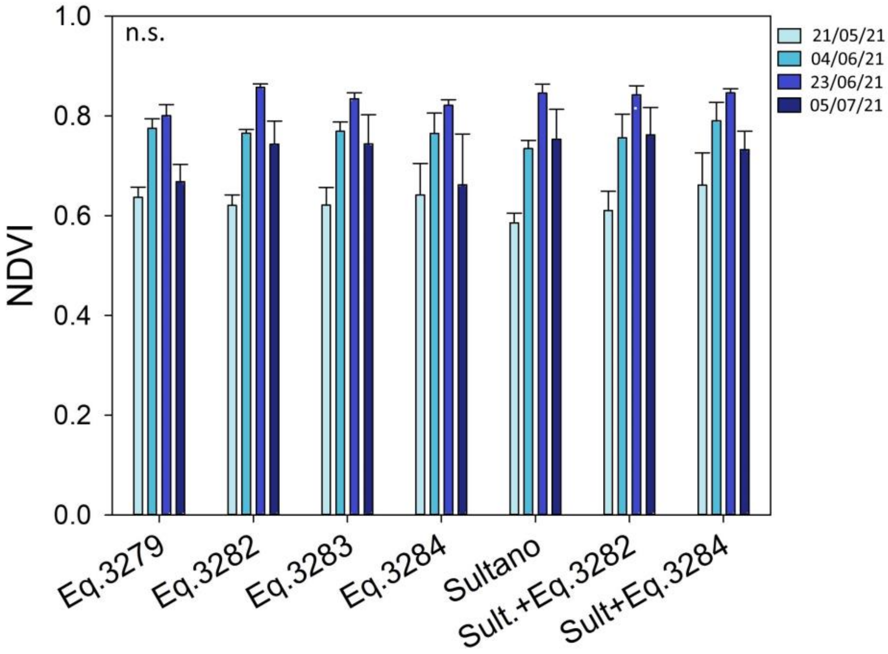

Figure 1.21. Normalized Difference Vegetation Index (NDVI) values measured in Chickpea treatments on 4 dates during the growing season in Udine. No statistical difference was assessed (n.s.).

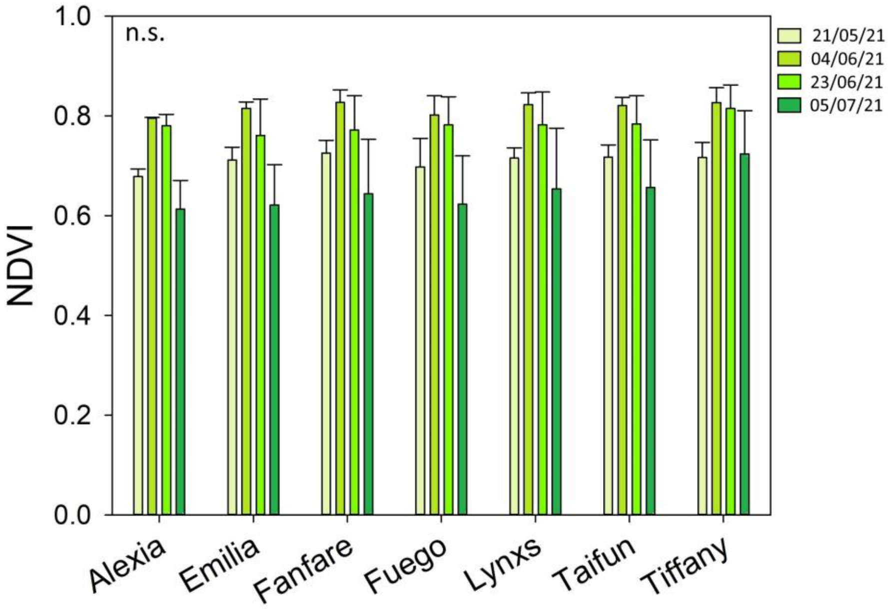

Figure 1.22. Normalized Difference Vegetation Index (NDVI) values measured in Faba bean treatments on 4 dates during the growing season in Udine. No statistical difference was assessed (n.s.).

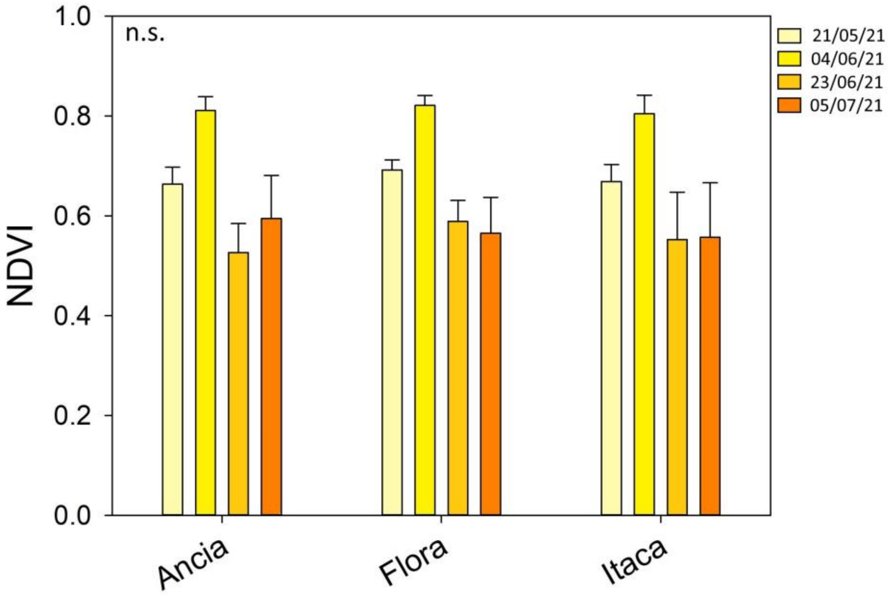

Figure 1.23. Normalized Difference Vegetation Index (NDVI) values measured in Lentil treatments on 4 dates during the growing season in Udine. No statistical difference was assessed (n.s.).

4 Discussion

In Italy, and in Europe, there is a lack of research into chickpea in general, and into the breeding of the species in particular (Zaccardelli et al. 2010). To improve production stability and grain quality, greater efforts should be made to validate cultivars of such crop. An example of this activity has been conducted by Zaccardelli et al. (2010), which carried out a genetic selection activity aimed to obtain chickpea varieties with higher protein yields and more suitable for mechanization.

In our study, all chickpea varieties performed similarly, and no pre-commercial variety performed better than the commercial variety of *Sultano*. *Sultano* has been validated as one of the best CV for the Italian region, especially south Italy where climatic conditions are generally more favorable for this crop (*e.g.* Pardo et al. 2000, Zaccardelli et al. 2010, 2013, Sellami et al. 2021). In our study, *Sultano* yield was estimated approximatively 1.7 t ha^{-1} with a protein content of 18% resulting in a protein yield of 313kg ha^{-1}. In a study conducted in 2012 in south Italy, the same variety yielded 2.6ton ha^{-1} with protein content of 20%, resulting in a protein yield of 540 kg ha^{-1} (Zaccardelli et al. 2013).

Faba bean is an important source of protein in the diets of many regions, especially in the Mediterranean area, however, in north of Europe faba bean production is mostly directed towards livestock feed. (Jensen et al. 2010). Faba bean is considered one of the most protein-rich pulses as its seeds have an average protein content of 29%, reaching up to 35% (Muktadir et al. 2020). Although, in the last 50 years its production area has significantly decreased, especially in Italy, also because of its susceptibility to drought and diseases (Jensen et al. 2010). Hence, the urgent need for breeding and selecting varieties adapted to regional situations (Maalouf et al. 2019). In this study all screened faba bean varieties had poor yields with higher values of 0.77ton ha^{-1} in *Tiffany* and lowest of 0.29ton ha^{-1} in *Taifun*. This result may be attributed to biotic stress. In fact, by visual assessment, faba bean plots were indiscriminately affected by diseases, in particular botrytis and coffee rust. Despite that, protein content of seeds was high in all tested varieties and ranged from 27% in *GL Emilia* to 32% in *Fanfare*. This result is quite promising as it confirms the suitability of this crop for facing the challenge of producing large quantities of raw material addressed to the production of ingredients for plant-based food. However, a high protein content is not a sufficient result if it is not coupled with high yield and grain quality.

Lentil was not harvested as flowers aborted and plants did not undergo seed formation. This problem may be attributed to weather conditions of mid-June, when a prolonged drought period coupled with high temperatures occurred. In fact, it is known that under heat stress (prolonged temperatures higher than 32°C), lentil flowers tend to abort and not produce seeds (Sehgal et al. 2021).

Multispectral data have been widely used coupled with environmental and genotypic traits for crop modeling, with interesting applications in breeding (Lake and Sadras 2016). Crops growth is traditionally assessed by destructive sampling (Zhang and Flottmann 2016) or with morphometric measurements integrating allometric relations (Vega et al. 2001). These methods are time consuming and, being destructive, they do not allow to monitor changes in plant growth over time (Sadras et al. 2013). Hence, spectral monitoring of crops has been widely adopted as an efficient tool to model crops development and the NDVI index has been demonstrated being a proxy of crop biomass (Seo et al., 2019), leaf area index (Kross et al., 2015), nitrogen uptake (Holzhauser et al., 2022), and yield (Gao et al., 2018). Despite that, limited work has been conducted on grain legumes, generally considered more challenging than cereals or oilseeds (Sadras et al. 2013). In this study we used multispectral data, in particular the NDVI index, to monitor plants development the growing season.

No difference was assessed among cultivars of the same species at any date. However, these data were particularly suitable for monitoring plants development and senescence. In May, NDVI was higher in faba bean than in the other crops. This result agrees with field-measured data, indicating faba bean as the species with higher plant height and aboveground biomass. At flowering NDVI was similar in all species and after that it is possible to observe different responses of crops in terms of stress/senescence. NDVI values decreased rapidly in lentil, indicating that crops may have suffered of heat stresses shortly after flowering. In faba bean it slowly decreased during June and reached the minimum at beginning of July as plant senescence occurred and fungal infections of leaves increased (*Botrytis* sspp.). In chickpea there was not a proper decrease neither in July. This result was expected as chickpea was the later species to enter senescence and was harvested at the end of July.

Our results highlight the suitability and adaptability of these crops in the Friuli Venezia Giulia region. Up to our knowledge, the investigated species have been poorly cropped in this territory, being reputed low-yielding crops generating reduced incomes for the farmers. Nevertheless, our findings support that these species, especially chickpea, may be suitable for being cropped by the local farmers.

The incorporation of minor crops aligns with the broader objectives outlined in the Common Agricultural Policy of the European Union. This policy encourages agricultural diversity, sustainability, and rural development by promoting crop diversification. Cultural diversification, coupled with organic farming practices, is recognized as a crucial strategy to enhance environmental sustainability and mitigate the risks associated with market volatility. Therefore, our study supports the potential integration of pulse crops, particularly chickpea, into the agricultural landscape of the region, aligning with the overarching goals of the Common Agricultural Policy.

5 Conclusions

This study provides one of the few pieces of evidence of pulse species and cultivars validation in North of Italy. We highlighted the suitability of these crops for this region where up to now they are scarcely cultivated. In particular, the most performing species resulted being chickpea, which to our knowledge is cropped by a restricted number of farmers (<5) being reputed a low yielding crop, generating low income. Our results strongly support the possibility to integrate these crops in the current agricultural rotations of the local farmers. Integration of minor crops in is considered within the broader framework of supporting agricultural diversity, sustainability and rural development stated by the Common Agricultural Policy of the European Union, as it promotes the diversification of crops. Cultural diversification, along with organic agriculture practises are recognised as pivotal strategies to enhance environmental sustainability and reduce the risk of market volatility.

Among all selected varieties of chickpea, *Sultano* seems to be the most suitable for this region.

In faba bean, all cultivars underperformed in terms of yield, however measured protein content of seeds was high. More experiments would be suggested to assess different optimization practices (e.g, sowing dates, selection of resistant varieties, irrigation management).

Due to stress-induced abortion of flowers, lentil did not produce grains. Further investigation is necessary to assess the suitability of this crop, already present mostly in southern regions of Italy.

Multispectral sensing of crops, in particular the NDVI index was an effective tool for monitoring crops development. Since the lack of literature for investigations using remote sensing on pulses Further studies may couple spectral sensing with field-measured parameters like diseases incidence and severity and water stress.

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Chapter 2. Cultivar Validation and Irrigation Strategy Assessment in Chickpea and Lentil under Anomalous Drought Conditions

Keywords: LAI, Yield, NDVI, Stress, Protein

1 Introduction

Chickpeas is the primary protein source for approximately 20% of the global population and ranks as the second largest pulse, following common bean. Worldwide, chickpea production exceeds 17 Mton per year and average yield depends on many factors like geographical area, sowing period, variety, and nutrient deficiency (Amede et al. 2002, Vanlauwe et al. 2010, Korbu et al. 2016, Abdulkadir et al. 2017, Tamene et al. 2017). However, drought stress has been addressed as the major limitation for this yield (Vadez et al. 2012).

Chickpea is mostly cultivated in geographical regions with distinct season variability like India, Pakistan, Turkey Ethiopia (Vadez et al. 2021). It is cultivated during the dry winter season (Majumdar 2011) and farmers generally rely on water stored in the soil during the rain reason (Hajjarpoot et al. 2018). Despite differences among regions, it has been observed that terminal water stress occurs in about 60% of the situations and it may reduce crop yield by about 70% (Hajjarpoot et al. 2018, Vadez et al. 2021). According to the observed correlation patterns between water use efficiency and grain yield, thanks to its vigorous root system, chickpea seems to be a crop adapted to mild drought (Palta and Turner 2019), however tolerance is strictly dependent on the variety (Krishnamurthy et al. 2013) and the vegetative phase at which drought occurs (Kashiwagi et al. 2013). As an example, according to the models developed by Vadez et al. (2012), it has been suggested that even a single irrigation event provided during the grain-filling phase, may increase chickpea yield by over 30%.

Lentil is a cool-season legume mostly cultivated in the Mediterranean and northern temperate regions (Tullu et al. 2011). It is the most important pulse after chickpea, however, its production represents 6% of the global pulse production and it is mostly located (~ 70%) in India, Turkey and Canada (Alexander 2015). Lentil is usually grown as a rainfed crop, it is considered as a stress resistant as it is able to face water deficit and temperature extremes during growth stages (Sehgal et al. 2021).

Lentil yield ranges between 900 and 1000kg ha-1 (FAO 2005), however it may drastically reduce when plants experience drought individually or in combination with high temperatures (affecting pollen viability) (Barnabas et al. 2008, Delabunty et al. 2015, Shegal et al. 2017). In fact, despite being adapted to drought conditions, water stress can reduce yield by 50% to 70% whether coupled with prolonged high temperatures (Delahunty et al. 2015, Mishra et al. 2016, Sita et al. 2017). Therefore, the Lentil strategy typically involves stress avoidance, inducing crop senescence and early maturity in response to excessive drought or heat stress (Shrestha et al. 2006).

The aim of this study is to evaluate the adaptability to extreme drought conditions experienced in Udine (NE Italy) of two cultivars of chickpea and lentil differing in terms of maturation period, also assessing yield and seeds quality. Furthermore, for one cultivar per species we tested the response to a gradient of irrigation conducted during the critical phase of grain-filling. For this purpose, we aim to combine the most used agronomical parameters, coupled with vegetation assessment by remote with multispectral and thermal data acquired by unmanned aerial system (UAS).

2 Materials and Methods

2.1 Study area

The experiment was conducted during the growing season 2022 in Udine (NE Italy) at the experimental farm “A. Servadei” of the University of Udine (46.03°N, 13.22°E), in a field that had previously been cultivated with soybean. Study area is shown in Figure 2.1.

Figure 2.1. Study area of the experimental trials conducted in the experimental farm of the University of Udine during the growing season of 2022.

Soil samples of the top layer (0-30cm) were carried out from 5 different points randomly selected in the experimental field. Soil samples were analyzed by Eurofins - Scientific Netherlands. Soil was classified as loam (43% sand, 21 % silt, and 25% clay), with 2.3 % of Soil Organic Carbon, 7.4 pH and 10 C/N. The soil has high microbial biomass (with low activity) and fungi/bacteria ratio (0.7) is good in the term of the balanced turnover of the SOC. In fact SOC balance indicates a steady state supply of C equal to 12.1tC/ha/year, assuming an annual mineralization rate of 2.9% of the actual SOC (69t ha^{-1} in 0-0.25m soil layer). Deficiencies of Mn are foreseen for legumes; Fe, Zn, P and K plant available are evaluated rather low. The total Soil Water Holding Capacity (SWHC) for 0.25m soil depth is 52 mm and irrigation requirements is calculated of 30mm equal to 40% of depleted SWHC.

2.2 Weather conditions

Weather conditions in Udine during the 2022 growing season were particularly warm and dry (Figure 2.1). Compared to long term data (Figure 2.2 and Table 2.1), June and July were warmer, with mean temperature of +2.7°C in June and +3.2°C in July. Moreover, 2022 marked an anomalous drought year, with cumulative precipitation from March 1st to July 31st of 237.1mm, 60% less than the average from the same period (592.3mm).

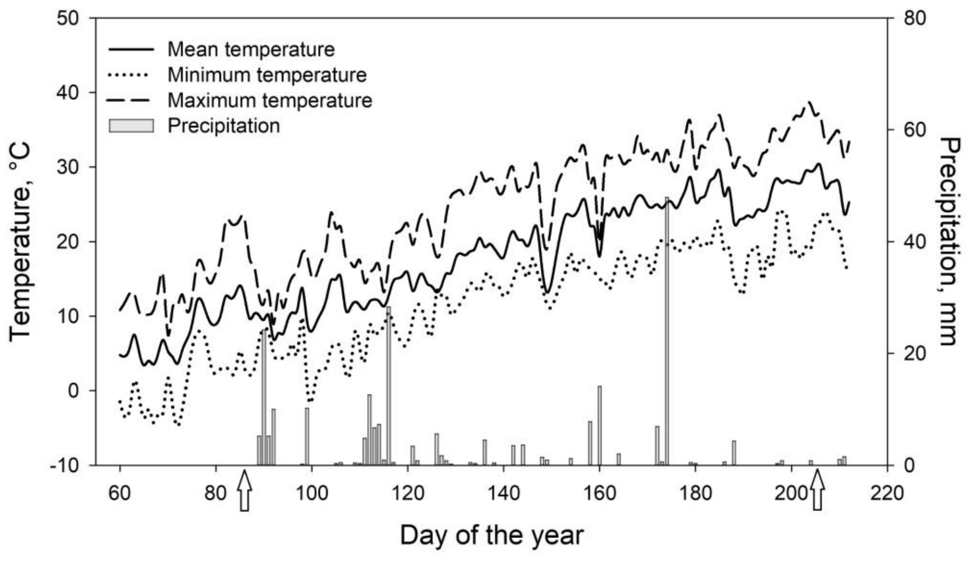

Figure 2.2. Weather conditions of the growing season in Udine during 2022. Sowing (March 29th and harvest (July 26th) are marked by arrows. Data collected by ARPA-OSMER weather station (Udine Sant'Osvaldo).

Table 2.1. Monthly comparison of mean temperature (Tmean), maximum temperature (Tmax),cumulative rainfall (Rain) and reference evapotranspiration (ET0) values for 2022 in Udine with average values of 2001 – 2021 period. Data collected by ARPA-OSMER weather station (Udine Sant'Osvaldo).

Month	**Average Tmean °C**	**Tmean °C**	**Average Tmax °C**	**Tmax °C**	**Average rain mm**	**Rain mm**	**Average ET0 mm**	**ET0 mm**
March	9.0	8.1	14.4	14.7	108.3	29.5	58.6	67.1
April	13.4	11.8	19.1	17.3	113.3	88.8	86.0	78.7
May	17.7	19.6	23.4	25.6	142.1	27.7	116.6	131.7
June	21.9	24.6	27.8	31.1	116.0	81.4	139.9	162.5
July	23.7	26.9	29.9	33.9	112.6	9.7	154.8	196.1
TOT					592.3	237.1	555.9	636.1

2.3 Experimental design and crop management

Chickpea (CH) and Lentil (LN) crops were tested considering two experimental factors: variety and irrigation amount. Two varieties were tested for each crop: Chickpea *cvs Sultano* and *Maragià*; lentil *cvs. Itaca* and *Elsa*. All varieties were provided by AgroService SpA, Italy.

Chickpea *Sultano* and lentil *Itaca* were tested under four different post-flowering irrigation volumes defined as an increasing fraction of Full Irrigation (FI) of 30mm as follows: i) No irrigation (NI); ii) 10mm equivalent to 1/3 of Full Irrigation (1/3 FI); iii) 20mm equivalent to 2/3 of FI (2/3 FI); iv) 30mm equivalent to Full Irrigation (FI). Full Irrigation of 30 mm is equivalent to 40% of the Soil Plant Available Water (i.e., the readily available soil water) in the 30cm upper soil layer. This amount was estimated by implementing a water retention soil model with soil characteristics and measured values of soil water content (see chapter 2). The irrigation dates were defined when the sum of daily reference crop Evapotranspiration (ET; Penman-Monteith equation in FAO56) was equal to 30mm. The irrigation dates resulted as it follows: June 16th, June 21st and June 28th.

A detailed scheme of treatments is reported in Table 2.2.

A total of 20 plots per species were sown, arranged according to a systematic experimental design replicated for 3+1 blocks, where all treatments were present in 3 out of 4 blocks, while the last block was composed by varietal treatments only. Chickpea was sown in 15.2m^2 plots (4 rows with a row-distance of 0.38m at a length of 10m) at target plant density 60 plants m^{-2}, while lentil was sown in 12m^2 plots (8 rows with a row-distance of 0.15m at a length of 10m) with 250 plants m^2.

Table 2.2. Summary of tested species with corresponding varieties, irrigation treatments, thousand seeds weight (TSW), germination percentage (G%), target plant densities and number of replicates.

<table>
<tr><th>Species</th><th>Variety</th><th>Irrigation treatment</th><th>TSW, g</th><th>G%</th><th>Target plant density # m^{-2}</th><th>Replicates</th></tr>
<tr><td rowspan="5">Chickpea</td><td>Maragià</td><td>NI</td><td>393.9</td><td>80</td><td rowspan="5">50</td><td>4</td></tr>
<tr><td rowspan="4">Sultano</td><td>NI</td><td rowspan="4">275.6</td><td rowspan="4">90</td><td>7</td></tr>
<tr><td>1/3 FI</td><td>3</td></tr>
<tr><td>2/3 FI</td><td>3</td></tr>
<tr><td>FI</td><td>3</td></tr>
<tr><td rowspan="5">Lentil</td><td>Elsa</td><td>NI</td><td>49.4</td><td>85</td><td rowspan="5">250</td><td>4</td></tr>
<tr><td rowspan="4">Itaca</td><td>NI</td><td rowspan="4">33.5</td><td rowspan="4">85</td><td>7</td></tr>
<tr><td>1/3 FI</td><td>3</td></tr>
<tr><td>2/3 FI</td><td>3</td></tr>
<tr><td>FI</td><td>3</td></tr>
</table>

Crops were sown on 28/03/2022. Sown seeds amount was defined according to values of thousand seeds weight (TSW, g), germination rate (G%) and targeted plant density (see Table 2.2). Target plant densities (plants m-^{2}) were chickpea: 50; lentil: 250. Sown seeds were inoculated with specific Rhizobia; chickpea was inoculated with *Mesorhizobium ciceri* and lentil with *Rhizobium leguminosarum*. Rhizobia, provided by Agrifutur S.r.l. (Alfianello-Brescia, IT). Emergence was completed on 19/04/2022, while flowering occurred differently according to the variety, from 23/05/2022 to 30/05/2022. Harvest differed according to species and irrigation treatments. lentil was harvested from 06/07/2022 to 26/07/2022, while chickpea from 18/07/2022 to 26/07/2022.

An emergency irrigation of 10mm was performed on all plots on 23/05/2023, while irrigation treatments were conducted on three dates in the second half of June: 16/06/2022, 21/06/2022, and 28/06/2022.

Crops were grown under organic practices; no fertilization was applied, and weeding was weekly performed from beginning of May to half June through hand hoeing.

2.4 Field measurements

Soil water content (SWC, m^2m^{-2}) was monitored and analyzed continuously in real time from 10/06/2022 to 20/07/2022. For this 6 ZL6 dataloggers with 22 EC-5 volumetric soil moisture sensors ((METER Group, Pullman, WA, USA)), were distributed at a depth of 15cm, among different irrigation treatments.

Three aboveground biomass samplings have been conducted during the growing season, on: 26/05/2022, 01/06/2022 and 15/06/2022. For each sampling we sampled an area of $0.38m^2$ in chickpea (2 rows with row-distance of 0.38m at a length of 0.5m) and $0.30m^2$ in lentil (4 rows with row-distance of 0.15m at a length of 0.5m). Aboveground fresh biomass (FW, kg m^{-1}) was measured immediately with a field scale. Samples were then oven-dried at 70°C for 72h to measure aboveground dry biomass (DW, kg m^{-2}). Crops dry matter content (DMC, g g^{-1}) was then calculated as the ratio between DW and FW.

Leaf Area Index (LAI, m^2m^{-2}) was monitored from flowering to harvest. A total of 6 measurements were conducted, on: 26/05/2022, 01/6/2022, 07/06/2022, 15/06/2022, 21/06/2022 and 28/06/2022. Measurements were done with the LI-COR LAI-2200C Plant Canopy Analizer. For each plot 2 sets of 10 measurements per row were conducted, following the diagonal transects' protocol for row crops specified by the producer.

Harvest was conducted differently according to the variety and the irrigation treatment. Harvests were done in the following order: lentil *cv* Elsa on 04/07/2022; lentil *cv Itaca* NI on 06/07/2022;

lentil 1/3 FI on 11/07/2022; chickpea *cvs Maragià, Sultano* NI and 1/3 FI on 18/07/2022; lentil and chickpea 2/3 FI and FI on 26/07/2022.

For each plot, plant density (PD, plants m^{-2}) was measured in a sampling area on $0.76m^2$ in chickpea (2 rows with row-distance of 0.38m at a length of 1m) and $0.3m^2$ in lentil (4 rows with row-distance of 0.15m at a length of 0.5m). A larger area of $1.52m^2$ in chickpea (2 rows with row-distance of 0.38m at a length of 2m) and $1.20m^2$ in lentil (4 rows with row-distance of 0.15m at a length of 2m) was then sampled to measure DW and yield. Harvest Index (HI) was then calculated as:

$$HI = \frac{yield}{yield + DW}$$

2.5 CHN analysis and protein content

Dry samples of crop residues and seeds were grounded into a fine powder with a ball mill to ensure uniformity in the sample. Nitrogen content (N_C, g g^{-1}) was then measured using Dumas' combustion using a CN Elemental Analyser (Vario Microcube, © Elementar) coupled to a stable isotope ratio mass spectrometer (IRMS; Isoprime 100, © Elementar).

A subsample of grounded samples was oven-dried at 120°C for 48h to determine the residual water content and subsequently correct the CHN measurements.

Protein content (PC, g g^{-1}) of seeds was then estimated as:

$$PC = N_c * 6.25$$

where N_c is the Nitrogen content of seeds (g g^{-1}) and 6.25 is the conversion factor to estimate the protein content based on the assumption that, on average, proteins contain approximately 16% Nitrogen.

Protein yield (ton ha^{-1}) was calculated as the product of yield for PC and Nitrogen stock (kg ha^{-1}) as DW for NC.

2.6 Remote sensing of crops

Multispectral data was periodically acquired with an unmanned aerial system (UAS) on a total of 7 dates during growing season, on: 02/05/2022, 26/05/2022, 01/06/2022, 08/06/2022, 15/06/2022, 30/06/2022 and 04/07/2022. Flights were conducted at 11:00 a.m. (LST) with a DJI P4 Multispectral drone equipped with 5 monochromatic sensors, acquiring reflected radiation in: Blue (450±16nm), Green (560±16nm), Red (650±16nm), Red-Edge (RE, 730±16nm) and Near-Infrared (NIR, 840±26nm). Pictures of a Parrot official calibrated reflectance panel were also acquired before and after each flight, in order to correct the acquired images on the day's lighting conditions and compare the results of different flights. Spectral data were mapped with spatial resolution of 2cm using Pix4D software. Georeferenced maps of the plots were created with R v2.0.1 software (R Core Team, 2021) with *raster*, *tiff* and *rgdal* libraries (Hijmans et al. 2013, Urbanek 2013, Bivand et al. 2021). In particular, the central area of each plot was cropped with vector masks of 0.76x6m in chickpea and 0.6x6m in lentil. Data were used to calculate the Normalized Difference Vegetation Index (NDVI, Rouse et al. 1974) as:

$$NDVI = \frac{(NIR - R)}{(NIR + R)}$$

To prevent possible biases due to inhomogeneous or incomplete soil cover between rows, pixels with NDVI value lower than 0.05 were excluded.

Crops' Thermal data were also acquired with a Parrot ANAFI thermal drone, equipped with a Forward Looking Infrared (FLIR) Lepton 3.5 module camera on: 15/06/2022, 30/06/2022 and 04/07/2022. Data acquisition was conducted immediately after multispectral data acquisition, and the geoprocessing was conducted with the same method.

2.7 Statistical analysis

All statistical analyses were conducted with the R v2.0.1 software (R Core Team 2021). Due to issues in data normality, homoscedasticity and inhomogeneity in data abundance, differences among treatments of the same species were tested with Kruskal-Wallis H test and Dunn's Test for Multiple Comparisons as *post hoc*.

3 Results

3.1 Soil water content

Trends in soil water content during the 2022 growing season are represented in Figures 2.4-2.5. After irrigation in chickpea, SWC was higher in the irrigation treatment at FI, while in lentil measured values were similar among all treatments.

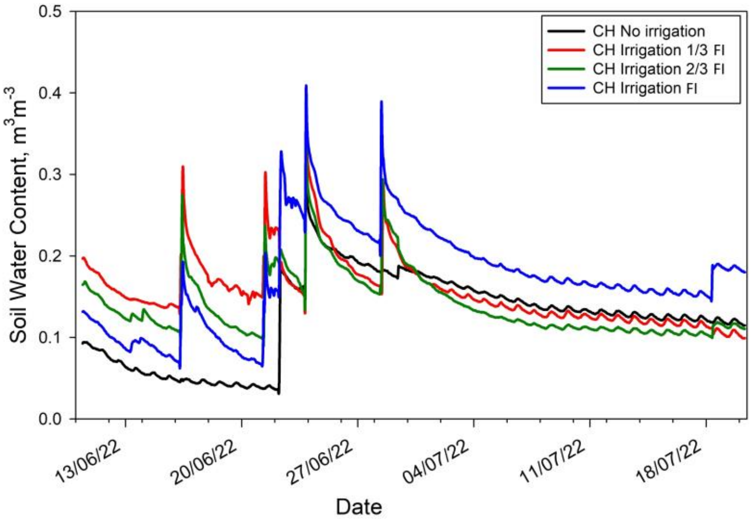

Figure 2.4. Soil water content (15cm) measured during the growing season 2022 in Udine, in fields cultivated with Chickpea (CH) under different irrigation treatments.

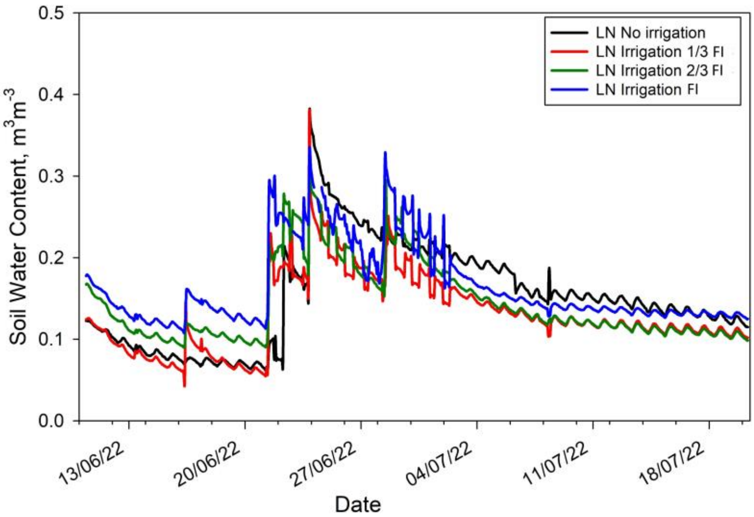

Figure 2.5. Soil water content (15cm) measured during the growing season 2022 in Udine, in fields cultivated with Lentil (LN) under different irrigation treatments.

3.2 Crop phenology and development

Crops phenological development stages with corresponding thermal sums are reported in Table 2.3. Phenological development was different among varieties. Among varietal treatments, chickpea *cv Maragià* and lentil *cv Elsa* reached flowering and harvest maturity earlier than chickpea *cv Sultano* and lentil *cv Itaca*. Since irrigation treatments were conducted after flowering, differences in phenological development occurred in terms of harvest maturity date, which was delayed as far as irrigation intensity increased.

Trends in aboveground dry biomass measured during the growing season are graphically represented in Figures 2.6-2.7. No statistical difference was assessed between different treatments both in chickpea and lentil, however, it has to be noted that all measurements were conducted before the start of irrigation. In both species, highest values of DW were reached on 15/06/2022. chickpea *cv Maragià* had a DW of 4.8±0.6ton ha^{-1} while *cv Sultano* ranged from 4.4±0.6ton ha^{-1} to 5.4±1.4ton ha^{-1}. In LN, cv *Elsa* had a DW of 4.6±0.4ton ha^{-1}, while *cv Itaca* ranged from 4.8±0.6ton ha^{-1} to 5.7±0.4ton ha^{-1}.

Trends of dry matter content measured during the growing season are graphically represented in Figures 2.8-2.9. No statistical difference was assessed. As for DW, all measurements of DMC were conducted before the start of irrigation, hence all plots from different irrigation treatments follow the same trend. In lentil, DMC was tendentially higher in *cv Elsa* then *Itaca,* however difference is not statistically significant.

Trends in LAI measured during the growing season are graphically represented in Figures 2.10-2.11. In chickpea, higher values were measured at flowering, on 07/06/2022, when LAI was 3.3±0.7m^2m^{-2} in *cv Maragià* and ranged from 3.0±0.6m^2m^{-2} to 3.2±0.4m^2m^{-2} in *cv Sultano*. After irrigation treatments started, LAI decreased below 3m^2m^{-2} in all treatments but irrigation 2/3 FI and irrigation FI, where trends in LAI remained stable. In lentil, higher values were measured after flowering, on 15/06/2022, when LAI was 4.1±0.5m^2m^{-2} in *cv Elsa* and ranged from 3.7±0.3m^2m^{-2} to 4.0±0.4m^2m^{-2} in *cv Itaca*.

Table 2.3. Overview of crops development during the growing season 2022 in Udine. For each phenological stage are reported the average date and corresponding Growing Degree Days (GDDs, base temperature = 0°C).

Species	**Cultivar**	**Irrigation treatment**	**Sowing**	**Emergence**		**Flowering**		**Harvest**	
			Date	**Date**	**GDDs (°C/d)**	**Date**	**GDDs (°C/d)**	**Date**	**GDDs (°C/d)**
Chickpea	Maragià	No Irrigation	28/03/2022	19/04/2022	226.2	28/05/2022	918.0	18/07/2022	2168.1
	Sultano	No Irrigation	28/03/2022	19/04/2022	226.2	30/05/2022	950.6	18/07/2022	2168.1
		1/3 FI	28/03/2022	19/04/2022	226.2	30/05/2022	950.6	18/07/2022	2168.1
		2/3 FI	28/03/2022	19/04/2022	226.2	30/05/2022	950.6	26/07/2022	2397.1
		FI	28/03/2022	19/04/2022	226.2	30/05/2022	950.6	26/07/2022	2397.1
Lentil	Elsa	No Irrigation	28/03/2022	19/04/2022	226.2	23/05/2022	809.1	04/07/2022	1813.9
	Itaca	No Irrigation	28/03/2022	19/04/2022	226.2	30/05/2022	950.6	06/07/2022	1866.8
		1/3 FI	28/03/2022	19/04/2022	226.2	30/05/2022	950.6	11/07/2022	1981.8
		2/3 FI	28/03/2022	19/04/2022	226.2	30/05/2022	950.6	26/07/2022	2397.1
		FI	28/03/2022	19/04/2022	226.2	30/05/2022	950.6	26/07/2022	2397.1

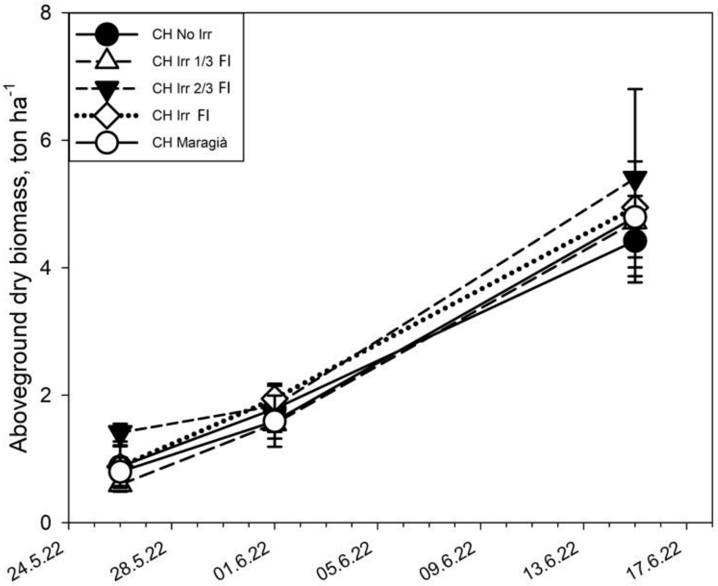

Figure 2.6. Aboveground dry biomass of chickpea (CH) treatments, measured during the 2022 growing season in Udine. No statistical difference was assessed.

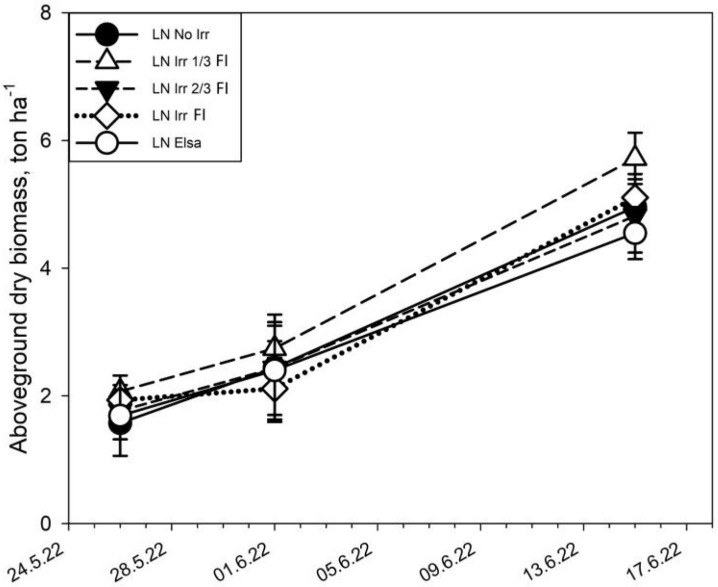

Figure 2.7. Aboveground dry biomass of lentil (LN) treatments, measured during the 2022 growing season in Udine. No statistical difference was assessed.

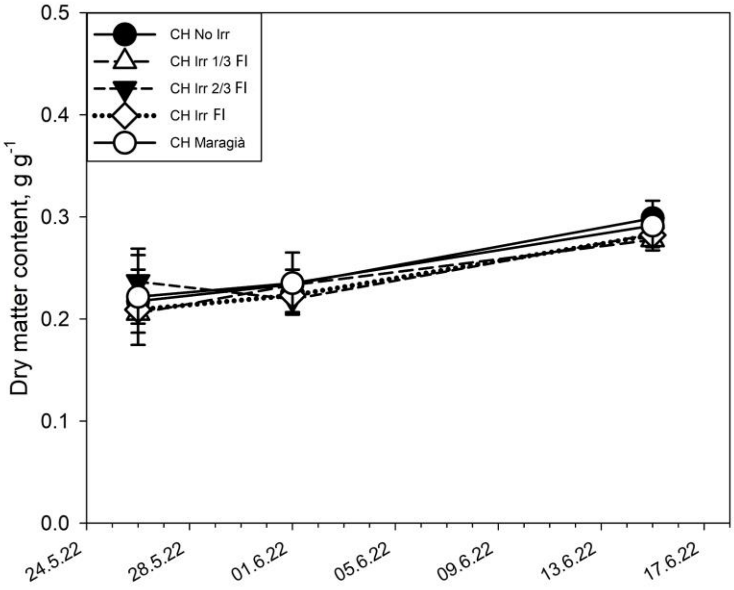

Figure 2.8. Dry matter content of chickpea (CH) treatments, measured during the 2022 growing season in Udine. No statistical difference was assessed.

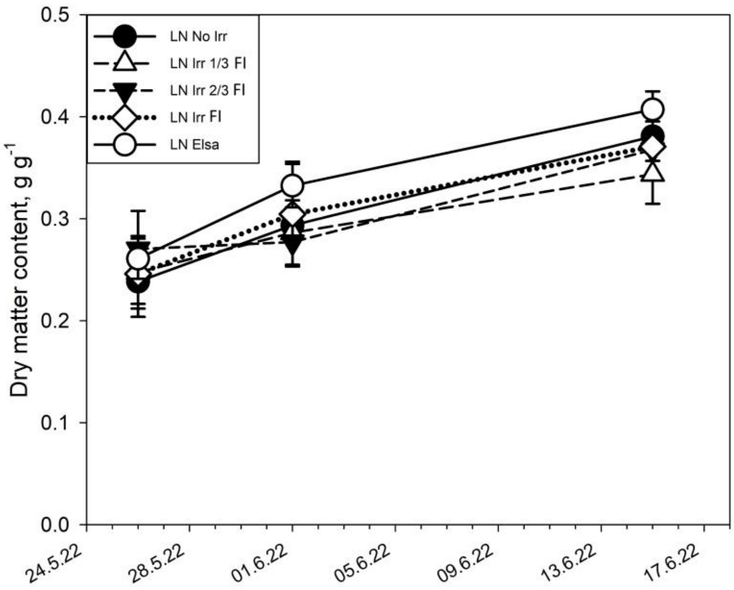

Figure 2.9. Dry matter content of lentil (LN) treatments, measured during the 2022 growing season in Udine. No statistical difference was assessed.

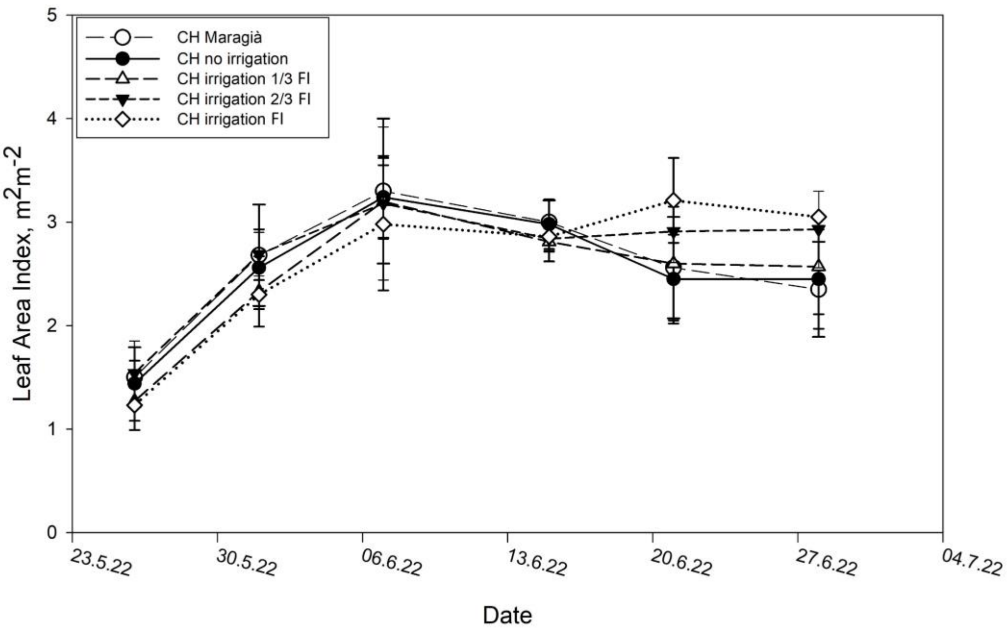

Figure 2.10. Leaf area index of chickpea (CH) treatments, measured during the 2022 growing season in Udine. No statistical difference was assessed. Vertical bars correspond to days when irrigation treatment was provided.

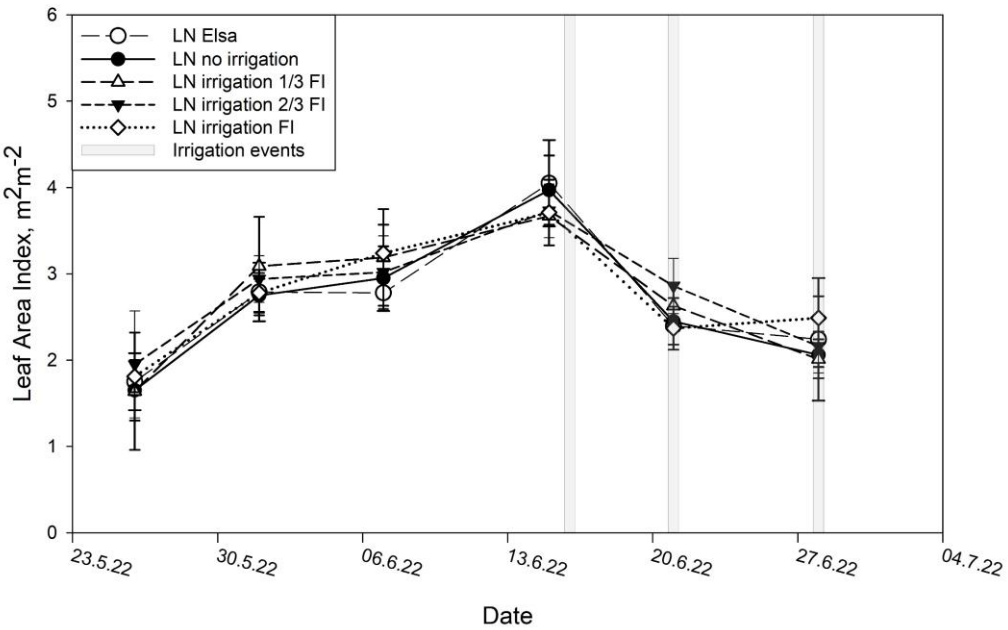

Figure 2.10. Leaf area index of lentil (LN) treatments, measured during the 2022 growing season in Udine. No statistical difference was assessed. Vertical bars correspond to days when irrigation treatment was provided.

3.3 Harvest

Plant density of crops is shown in Figure 2.11. Measured plant density corresponded to target plant densities (See Table 2.2). In chickpea, PD ranged from 50.9±7.7 plant m^{-2} (*Sultano* 1/3 FI) to 63.9±10.0 (*Sultano* NI), while in lentil from 232.5±67.8 (*Elsa*) to 287.8±35.3 (*Itaca* 1/3 FI). There was no statistical difference among treatments of the same species (p>0.05).

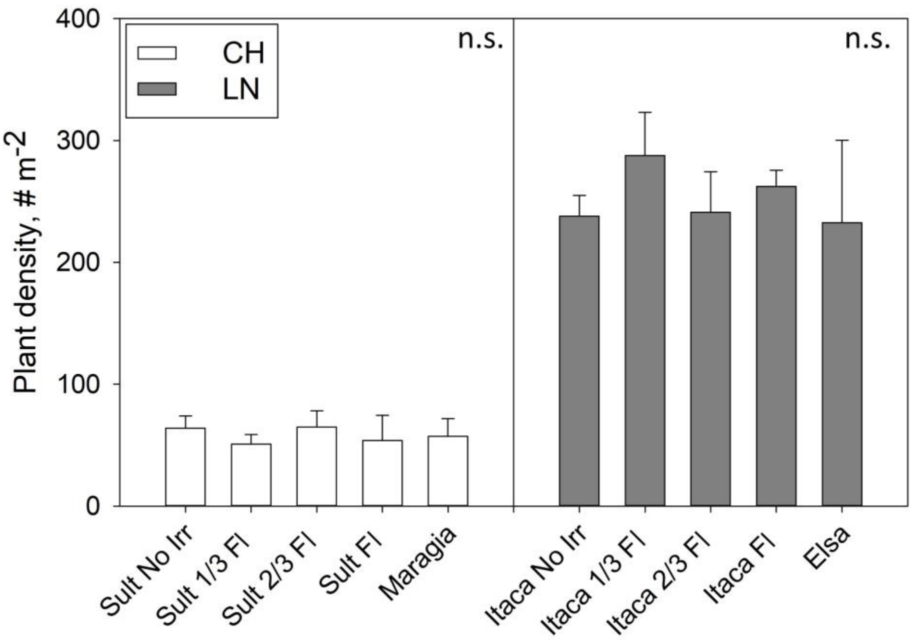

Figure 2.11. Plant density of chickpea (CH) and lentil (LN) treatments, measured at harvest at the end of the growing season 2022 in Udine. No statistical difference was assessed (n.s.).

Aboveground dry biomass measured at harvest is represented in Figure 2.12. For both chickpea and lentil, irrigation treatments were significantly higher than not irrigated ones. However, no difference occurred among different irrigations.

Yield measured at harvest is represented in Figure 2.13. Yield was tendentially higher in irrigated treatments, however, no statistical difference was assessed. In chickpea, yield was lower in *cv Sultano* not irrigated, with 2.0±0.4ton ha^{-1} and increased up to 2.8±0.2ton ha^{-1} in the irrigation at FI treatment. In lentil, lower values were measured in *cv Elsa*, with 0.9±0.1ton ha^{-1}, statistically different from *cv Itaca* under irrigation, which higher values were assessed in irrigation at 1/3 FI with 1.7±0.4ton ha^{-1}.

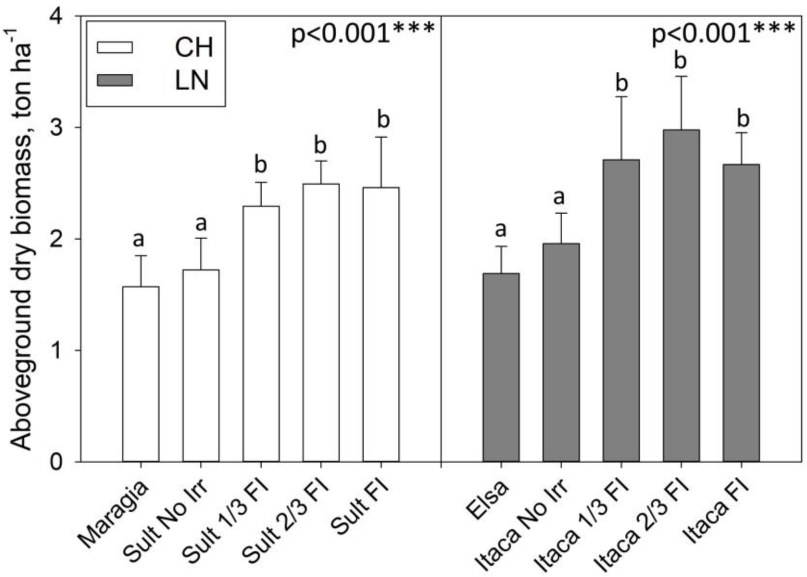

Figure 2.12. Aboveground dry biomass of chickpea (CH) and lentil (LN) treatments, measured at harvest at the end of the growing season 2022 in Udine. Statistical differences are denoted by different letters. *: p<0.05; **: p<0.01; ***:p < 0.001.

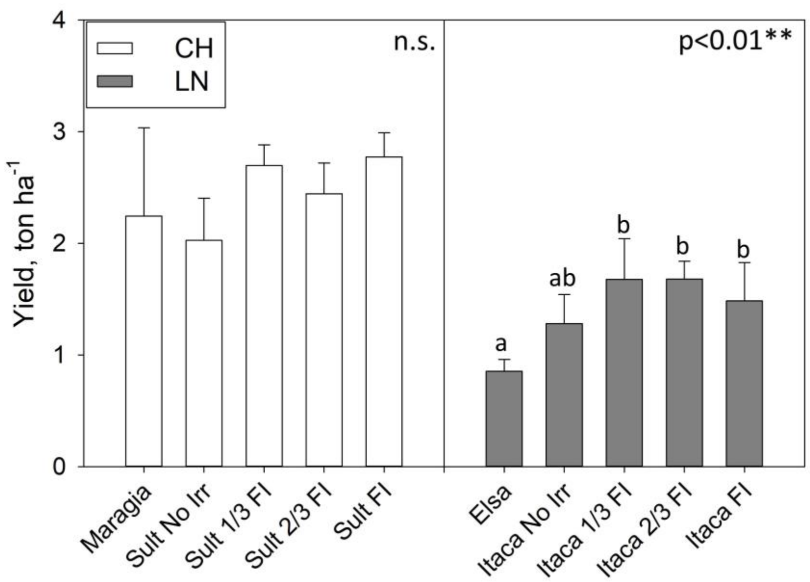

Figure 2.13. Yield of chickpea (CH) and lentil (LN) treatments, measured at harvest at the end of the growing season 2022 in Udine. Statistical differences are denoted by different letters. *: p<0.05; **: p<0.01; ***:p < 0.001; n.s.: not significant (p>0.05).

Harvest Index of chickpea and lentil treatments is reported in Figure 2.14. There were no statistical differences among treatments. In chickpea, HI ranged from 0.49±0.05 in *cv Sultano* irrigated 2/3 FI, to 0.58±0.05 in *cv Maragià*. In lentil, minimum values were observed in *cv Elsa*, with 0.34±0.04 and higher in *cv Itaca* not irrigated, with 0.41±0.03.

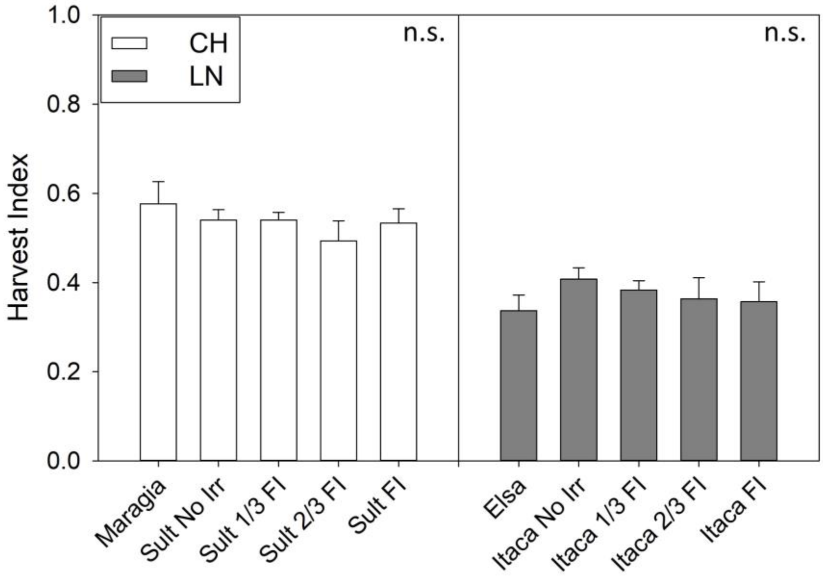

Figure 2.14. Harvest Index calculated for chickpea (CH) and lentil (LN) treatments grown in Udine during the growing season 2022. No statistical difference was assessed (n.s., $p>0.05$)

3.4 CHN analysis and protein content

Results of CHN analysis are reported in Table 2.4 and graphically represented in Figures 2.15-2.17. The protein content of seeds (Figure 2.15) was consistent across treatments of chickpea, which values ranged from 0.19±0.01g g^{-1} in *cv Sultano* irrigation 2/3 FI to 0.2±0.01g g^{-1} in the not-irrigated treatment of the same variety. On the contrary, significant differences were assessed in lentil, where protein content tended to decrease as the irrigation intensity increased. Minimum values were recorded in *cv Itaca* at FI with 0.23±0.01g g^{-1}, while highest value in both *cvs Itaca* and *Elsa* not irrigated, with 0.26±0.01g g^{-1}.

Protein yield (Figure 2.16) was mostly similar among treatments in chickpea and no statistical difference was assessed. Lowest protein yield was measured in *cvs Sultano* and *Maragià* with respectively 0.43±0.11ton ha^{-1} and 0.43±0.08ton ha^{-1}, while higher value was measured in *cv Sultano* irrigated FI, with 0.56±0.07ton ha^{-1}. In lentil, despite differences in protein content, protein yield was mostly driven by grain yield of different treatments. The *cv Elsa* was statistically different from all the other treatments, while no difference was assessed among irrigation treatments. Protein yield of *Elsa* was 0.22±0.04ton ha^{-1}, while in *Itaca* it ranged from 0.35±0.04ton ha^{-1} in the NI treatment, to 0.42±0.07ton ha^{-1} in 1/3 FI.

In both species, Nitrogen stock (Figure 2.17) increase according to the irrigation intensity. In chickpea lower values were recorded in the NI treatments, with 15.1±3.5kg ha^{-1} in *Maragià* and 15.9±2.4kg ha^{-1} in *Sultano*. These were statistically different from irrigation treatments at 2/3 FI and FI, with 23.5±0.4kg ha^{-1} and 24.1±6.1kg ha^{-1}, respectively. In lentil values were generally higher than chickpea. Lowest Nitrogen stock ranged from 24.4±3.5kg ha^{-1} in n *cv Elsa*, to 44.4±8.5kg ha^{-1} in *Itaca* 2/3 FI.

Table 2.4. Mean values with corresponding standard deviation of protein content of seeds, protein yield and nitrogen stock of residuals measured in chickpea (CH) and lentil (LN) at the end of the growing season 2022 in Udine. Statistical differences are denoted by different letters, otherwise not significant.

Species	**Treatment**	**Protein content** g g^{-1}	**Protein Yield** ton ha^{-1}	**Nitrogen stock** kg/ha
CH	Maragià	0.20±0.02	0.43±0.11	15.1±3.5 **A**
	Sultano NI	0.21±0.01	0.43±0.08	15.9±2.4 **A**
	Sultano 1/3 FI	0.20±0.01	0.53±0.03	18.0±1.0 **AB**
	Sultano 2/3 FI	0.19±0.01	0.47±0.05	23.5±0.4 **B**
	Sultano FI	0.20±0.01	0.56±0.07	24.1±6.1 **B**
LN	Elsa	0.26±0.01 **a**	0.22±0.04 **a**	24.4±3.5 **a**
	Itaca NI	0.26±0.01 **a**	0.35±0.04 **b**	25.0±4.7 **a**
	Itaca 1/3 FI	0.25±0.02 **ab**	0.42±0.07 **b**	34.8±8.1 **b**
	Itaca 2/3 FI	0.24±0.01 **b**	0.40±0.04 **b**	44.4±8.5 **b**
	Itaca FI	0.23±0.01 **b**	0.34±0.09 **b**	41.0±6.3 **b**

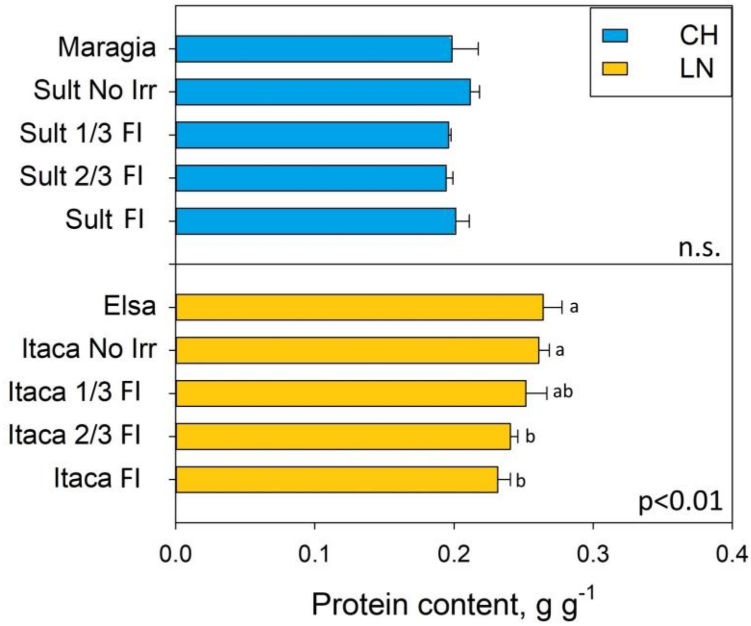

Figure 2.15. Protein content of seeds estimated for chickpea (CH, blue bars) and lentil (LN, yellow bars) treatments. No statistical difference was assessed (n.s.).

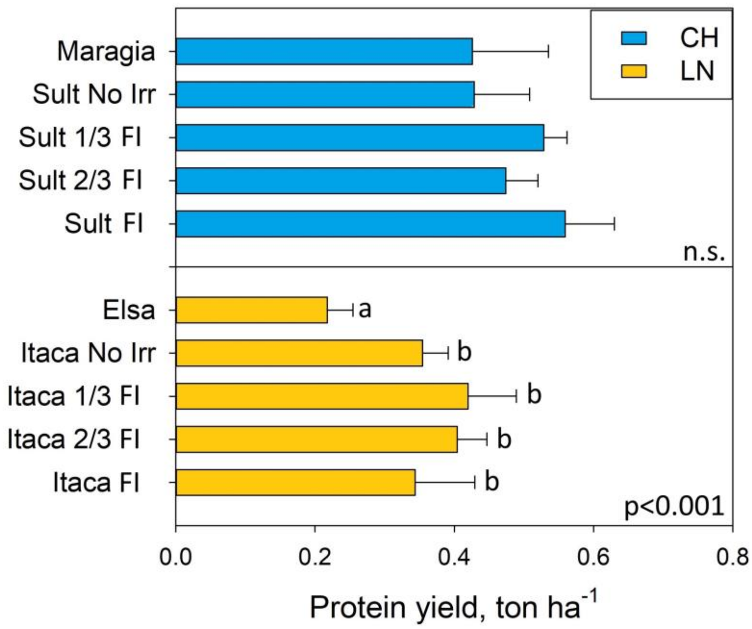

Figure 2.16. Protein yield estimated for chickpea (CH, blue bars) and lentil (LN, yellow bars) treatments. Different letters denote significant differences. “n.s.” = not significant ($p > 0.05$).

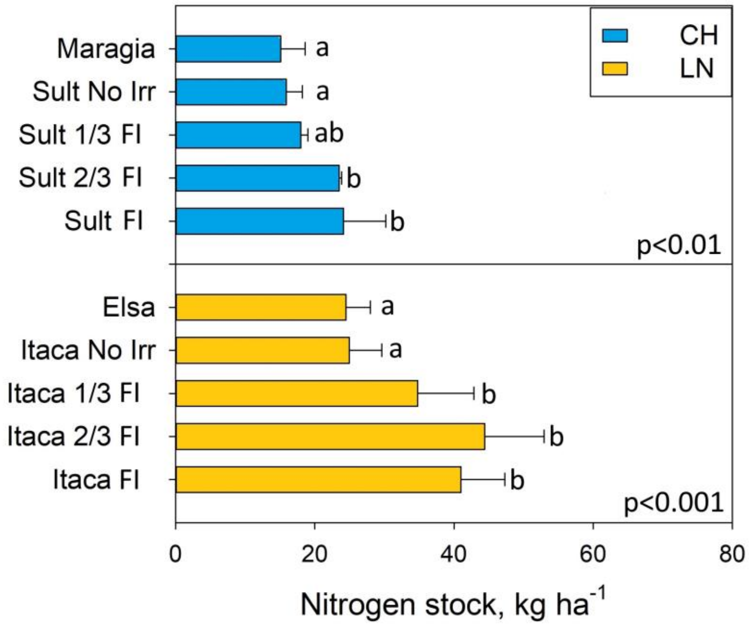

Figure 2.17. Nitrogen stock of crop residues measured in chickpea (CH, blue bars) and lentil (LN, yellow bars) treatments. Different letters denote significant differences.

3.5 Remote sensing of crops

Values of NDVI measured during the growing season are listed in Table 2.4 and graphically represented in Figures 2.18-2.19. In chickpea, NDVI increased until it reached a plateau of approximately 0.80 around flowering (26/05/2022). Subsequently, the values slowly incremented and reached the maximum of 0.85 in all treatments on 15/06/2022. After irrigation started trend in NDVI decreased according to the irrigation intensity. Statistical differences were assessed both on 30/06/2022 and 04/07/2022. In particular, NI treatments of both *cvs Sultano* and *Maragià* were significantly different from irrigation treatments at 2/3 FI and FI. Moreover, irrigation at FI was statistically different from irrigation 2/3 FI and maintained an average NDVI of 0.75±0.03, still close to values at flowering. In lentil, NDVI values followed the same trends of chickpea. A plateau of around 0.8 was reached at flowering and maintained among the whole growing up to the beginning of irrigation. Decrease in NDVI began at first in *cv Elsa*, which was significantly different from the other treatments already on15/06/2022. After irrigation, decrease in values was less pronounced with higher irrigation intensities. NI treatments were significantly lower than irrigation treatments and on 04/07/2022, *Itaca* 2/3 FI and FI were even significantly different from 1/3 FI.

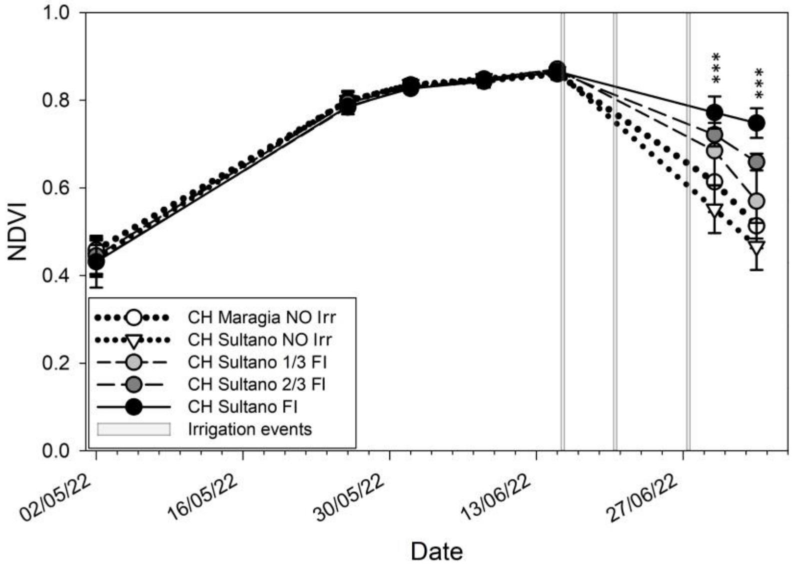

Figure 2.18. Normalized Difference Vegetation Index (NDVI) of chickpea (CH) treatments, measured during the 2022 growing season in Udine. Vertical bars correspond to days when irrigation treatment was provided. *: p<0.05; **: p<0.01; ***: p<0.001, otherwise not significant.

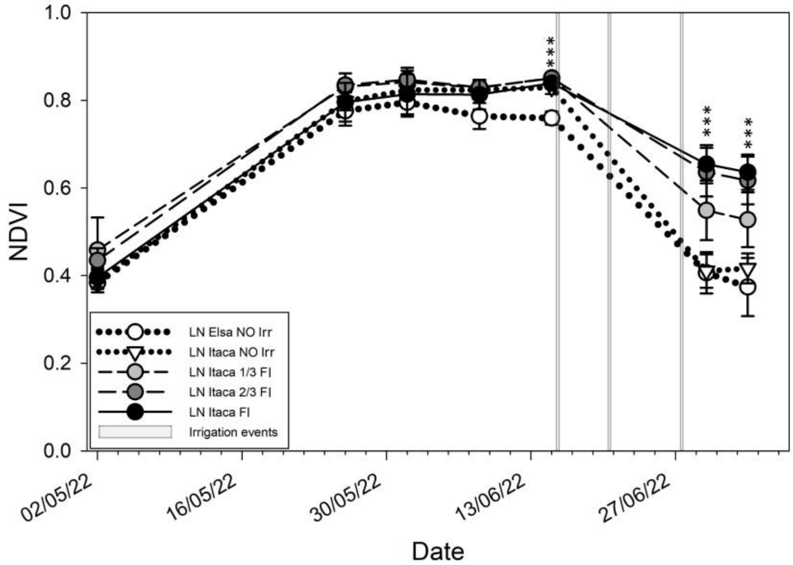

Figure 2.19. Normalized Difference Vegetation Index (NDVI) of lentil (LN) treatments, measured during the 2022 growing season in Udine. Vertical bars correspond to days when irrigation treatment was provided. *: p<0.05; **: p<0.01; ***: p<0.001, otherwise not significant.

Table 2.4. Mean values with corresponding standard deviation of Normalized Difference Vegetation Index (NDV), measured in chickpea (CH) and lentil (LN) treatments during the growing season 2022 in Udine. Statistical differences are denoted by different letters, otherwise not significant.

Species	**Treatment**	**NDVI**						
		02/05/22	**26/05/22**	**01/06/22**	**08/06/22**	**15/06/22**	**30/06/22**	**04/07/22**
CH	Maragià	0.46±0.03	0.80±0.02	0.83±0.01	0.85±0.01	0.86±0.01	0.61±0.07 **A**	0.51±0.05 **A**
	Sultano NI	0.44±0.04	0.80±0.02	0.84±0.01	0.85±0.01	0.87±0.01	0.55±0.05 **A**	0.47±0.05 **A**
	Sultano 1/3 FI	0.43±0.04	0.78±0.02	0.83±0.01	0.85±0.00	0.87±0.01	0.68±0.08 **AB**	0.57±0.09 **AB**
	Sultano 2/3 FI	0.45±0.04	0.79±0.01	0.83±0.01	0.84±0.01	0.86±0.00	0.72±0.03 **B**	0.66±0.02 **B**
	Sultano FI	0.43±0.06	0.79±0.00	0.83±0.00	0.84±0.01	0.87±0.01	0.77±0.04 **B**	0.75±0.03 **C**
LN	Elsa	0.38±0.01	0.78±0.03	0.80±0.03	0.76±0.03 a	0.76±0.02 **a**	0.41±0.05 **a**	0.37±0.07 **a**
	Itaca NI	0.39±0.03	0.80±0.02	0.82±0.01	0.82±0.01 b	0.83±0.02 **b**	0.41±0.04 **a**	0.42±0.03 **a**
	Itaca 1/3 FI	0.46±0.07	0.83±0.03	0.84±0.03	0.83±0.02 b	0.85±0.01 **b**	0.55±0.07 **ab**	0.53±0.06 **b**
	Itaca 2/3 FI	0.43±0.03	0.83±0.01	0.85±0.02	0.83±0.02 b	0.85±0.01 **b**	0.64±0.06 **b**	0.62±0.05 **c**
	Itaca FI	0.40±0.02	0.80±0.04	0.81±0.05	0.81±0.03 b	0.84±0.01 **b**	0.65±0.04 **b**	0.64±0.04 **c**

Mean values and corresponding standard deviations of the difference between remotely sensed canopy temperature and air temperature (ΔT, °C) are listed in Table 2.5 and graphically represented in Figures 2.20-2.21. Data of lentil *cv Itaca* irrigated FI were discarded due to anomalous values abundance in UAS output. First measurement was conducted before starting the irrigation treatments and the last one (04/07/2022) six days after last irrigation. This period was particularly dry and hot, cumulative rainfall was 56.4mm, divided in two rainfall events of 47.9mm (23/06/2022) and 6.9mm (21/06/2022), while the remaining precipitation occurred in 5 different days with precipitation from 0.1mm to 0.6mm. Average minimum, mean and maximum temperatures were respectively 19.4°C, 26.2°C and 32.3°C. In chickpea (Figure 2.20), there was no difference before the start of irrigation, while the gap between irrigated and not irrigated treatments increased and ΔT in NI treatments were significantly higher than the irrigated ones. In lentil (Figure 2.21), differences between *Elsa* and *Itaca* occurred already before irrigation was provided. Then, ΔT of irrigated treatments were significantly lower than the NI ones.

Table 2.5. Mean values with corresponding standard deviation of the difference between remptely sensed canopy temperature and air temperature (ΔT, °C), measured in chickpea (CH) and lentil (LN) treatments during the growing season 2022 in Udine. Statistical differences are denoted by different letters, otherwise not significant.

Species	**Treatment**	**ΔT, °C**		
		15/06/2022	**30/06/2022**	**04/07/2022**
CH	Maragià	-1.1±0.7	4.0±3.2 **AB**	6.9±1.7 **A**
	Sultano NI	-1.0±0.9	5.8±2.4 **A**	7.1±1.1 **A**
	Sultano 1/3 FI	-1.8±0.5	2.6±1.5 **AB**	3.5±1.5 **B**
	Sultano 2/3 FI	-1.0±0.3	0.8±0.8 **B**	0.4±1.0 **B**
	Sultano FI	-1.0±0.7	-0.2±0.4 **B**	0.1±1.3 **B**
LN	Elsa	5.2±0.8 **a**	11.1±1.6 **a**	11.3±0.5 **a**
	Itaca NI	4.21±0.6 **ab**	10.8±1.8 **a**	10.8±1.7 **a**
	Itaca 1/3 FI	3.7±0.5 **b**	5.8±1.2 **b**	8.4±1.6 **b**
	Itaca 2/3 FI	3.1±0.3 **b**	3.1±0.5 **c**	6.1±0.8 **b**
	Itaca FI	3.9±0.1 **b**	2.0±0.9 **c**	-

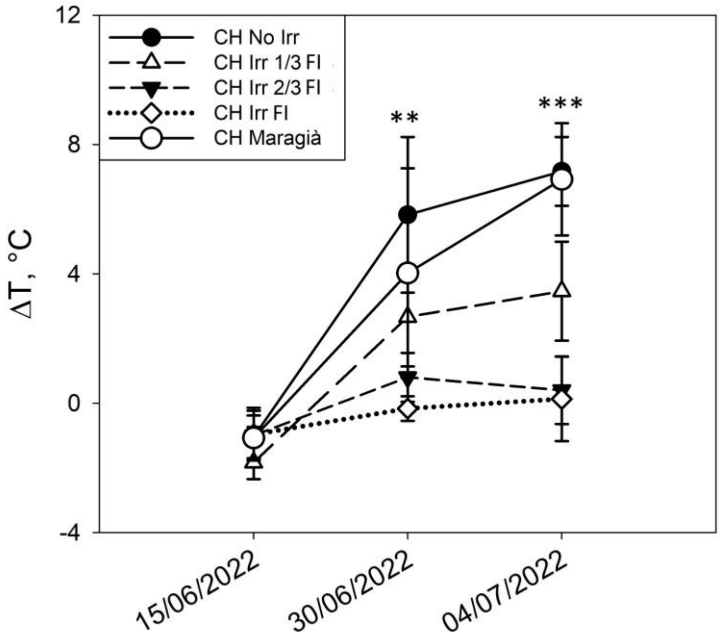

Figure 2.20. Difference between remotely sensed canopy temperature and air temperature (ΔT, °C) of chickpea (CH) treatments during the 2022 growing season in Udine. Vertical bars correspond to days when irrigation treatment was provided. *: p<0.05; **: p<0.01; ***: p<0.001, otherwise not significant.

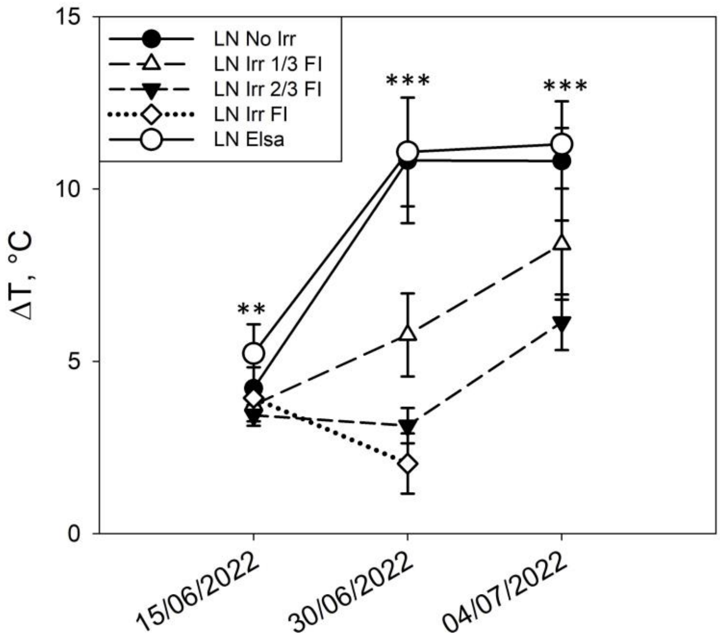

Figure 2.21. Difference between remotely sensed canopy temperature and air temperature (ΔT, °C) of lentil (LN) treatments during the 2022 growing season in Udine. Vertical bars correspond to days when irrigation treatment was provided. *: p<0.05; **: p<0.01; ***: p<0.001.

4 Discussion

This study evaluates the performance of chickpea and lentil grown during the 2022 growing season in Udine, a year marked by extraordinary drought conditions. From sowing to harvest, cumulative precipitation was 234mm and the mean temperature was 19.8°C, with maximum temperatures exceeding 30°C on 46 out of the 121 days of cycle. Irrigation treatments started on 16 June after 20 and 24 days flowering chickpea and lentil respectively (i.e., during the grain-filling phase). There is no consensus if the drought occurring after flowering is the most critical for these crops (Sehgal et al. 2021, Vadez et al. 2021) or not (Sadras and Calderini 2020).

Compared to the previous year 2021 (see Chapter 1), our results are quite promising and support the hypothesis that these crops are suitable for North of Italy, as they can ensure a satisfactory harvest even facing anomalous drought conditions. In fact, even if yields were slightly lower in growing season 2022 compared to 2021, no difference was observed between the no-irrigated and irrigated treatments.

In chickpea, all treatments had yield higher than 2t ha^{-1} with high values of HI (>0.5) and protein content (~0.20) and showed a typical and stable TSW for each CV tested. None of the yield factors measured in cv *Sultano* (i.e. Plant density, TSW and seeds/plant not discussed here) was affected by the tested irrigation treatments. These results support the observations of the previous year except for the slightly lower yield, which may be attributed to the experienced stress before flowering and irrigation. An evident effect of irrigation was the delay of maturity observed for the 2/3FI and FI irrigated treatments. In fact, under drought conditions, chickpea tends to implement an avoidance strategy by reducing the duration of the seed formation phase (Devasirvatham and Tan 2018). This hypothesis is supported by the lower DW_{ABV} of the not-irrigated chickpea at harvest.

In irrigated treatments, it was observed an elongation of the flowering period, inducing new flowers and pods formation. This caused a phenomenon of seed maturity shifting, leading to negative impacts in the quality of the harvest. This result confirms the observations of Mbarek et al. (2012) which tested various irrigation treatments and on eight varieties of Kabuli chickpea in Tunisia. By their result, they observed that despite being flowering regulated by genotype (Ellis et al. 1994), photoperiod and temperature (Roberts et al. 1995), irrigation amount may play a crucial role in flowering and maturity phases duration, with significant impacts in terms of seed quality production (Mbarek and Boujelben 2012).

Despite the drought conditions of the 2022 growing season, not-irrigated lentil reached harvest maturity with a satisfying production. Average yields of not irrigated lentil were 0.85ton ha^{-1} in *Elsa* and 1.28 t ha^{-1} in *Itaca*, reaching up to 1.68 t ha^{-1} with irrigation. However, no difference was observed between not irrigated and irrigated treatments of *Itaca*. These results are quite promising, especially considering that in the previous year lentil was not harvested as drought conditions caused flowering abortion and did not produce grains (see Chapter 1). Moreover, irrigation had a notable effect on delaying the canopy senescence (see NDVI in fig. 2.19) and the harvest maturity. Not-irrigated *Itaca* was harvested on 06/07/2022 at 1867°C d^{-1}, while treatments at 2/3 FI and FI were harvested on 26/07/2023 at 2397°C d^{-1}. This result and NDVI confirm that the drought-resistance strategy of lentil typically involves drought or heat stress avoidance strategy by inducing crop senescence and early maturity (Shrestha et al. 2006). The irrigation did not affect any of the yield components factors measured in cv *Itaca* (i.e. Plant density, TSW and seeds/plant not discussed here).

A noteworthy result is the protein content of lentil seeds. Higher values (26%,) were measured in the NI treatment of *Itaca*, while with irrigation, the protein content decreased up to 23% in the FI treatment. These values of protein contents range from 20.6% to 31.4% and support evidences reported in literature (e.g., Jarpa-Parra 2018). The decrease in protein content with increasing irrigation can be attributed to its effect of delaying protein accumulation while new seeds are setting-up. This irrigation effect on protein content was not observed for chickpea (see table 2.4). This phenomenon has been already reported for grain legumes (Farah et al. 1988, Silim et al.

1993, Thomson et al. 1997, and it is generally seen as a positive impact, as it usually leads to higher yields (Silim et al. 1993). Effectively, also in our study, even if it was not significant, it was observed an increase in yield which also leaded to a higher protein yield. However, it has to be taken into consideration that irrigation had a significant impact in terms of seed quality production, when a low protein content is a concern for the following steps of seed processing (e.g., protein extraction).

As already stated in Chapter 1, scientific literature lacks evidence about using remote sensing data on grain legumes, as they are generally considered more challenging than other crops like cereals or oilseeds (Sadras et al. 2013). In this study, we used multispectral (NDVI index) and thermal data, to monitor crops performance during the growing season. For both crops it is possible to observe a divergence in NDVI trends among irrigation treatments. In fact, it is possible to observe varying senescence patterns caused by irrigation, with NDVI values decreasing faster in treatments receiving lower irrigation volumes. The suitability of MSP data and in particular of the NDVI index for detecting crop maturity monitoring has been demonstrated for several crops (Xie and Yand 2020), however evidence on chickpea and lentil are still few (Sankaran et al. 2015). Detecting senescence is however an important parameter to investigate, with important application in breeding activity (*e.g.* Lanke and Sadras 2016) and crop modeling (Lindsey et al. 2020). A detailed analysis of these data and their relationship with agronomic variables is reported in Chapter 3.

Remotely sensed thermal data provided interesting insights for assessing varying levels of water stress among the treatments. In fact, the difference between canopy and air temperature (ΔT), significantly increased in the non-irrigated treatments, with ΔT being lower in the plots that received the higher irrigation amounts. This result agrees with many studies that have evaluated remotely sensed thermal data as a promising technology for monitoring and assessing crop water status (Khanal et al. 2017). Ezenne et al. (2019) used frequently measured thermal data to calculate a Crop Water Stress Index (CWSI) for monitoring and assessing crop water status. In a recent study (Olivera-Guerra et al. 2020) long term series of remotely sensed thermal data by UAS and satellite, were used to implement an evapotranspiration model for estimating timing and amount of irrigation for crops. These data were coupled with derived water stress coefficient (Kc) to produce a very accurate model (R = 0.95). The results presented here may not be sufficient for modeling purposes. However, they offer valuable initial insights into the suitability of this technology for grain legumes, particularly chickpea and lentil, where literature is still limited.

5 Conclusions

This study provides new evidence about irrigation of chickpea and lentil in North of Italy. The aim of this study was to evaluate the adaptability to extreme drought conditions experienced in NE Italy of chickpea and lentil. This was obtained by testing the performance of such crops treated under a gradient of irrigation conducted during the critical phase of grain-filling. This research was valuable for understanding the water requirements and drought tolerance of these crops, particularly in this region where the increasing occurrence of drought conditions during the summer period is a major concern.

Our results support the adaptability of chickpea and lentil for this region (see chapter 1) and support the suitability of introducing these species in the agricultural rotations of the local farmers. In fact, despite the experienced conditions of prolonged drought of year 2022, coupled with high temperatures, both species performed well, and it has not been observed a statistical difference of yield between not-irrigated and irrigated treatments.

Chickpea yielded around 2.3ton ha^{-1} with an average protein content of 20% resulting in an overall protein yield of 480kg ha^{-1}. Lentil yielded around 1.4ton ha^{-1} with an average protein content of 25% resulting in an overall protein yield of 350kg ha^{-1}.

In both crops, irrigation influenced crop phenology by extending the duration of the flowering seed setting and ripening stages.

Only in lentil, this phenomenon resulted in a decrease of seed protein content as irrigation amounts increased.

Use of remote sensing data was effective in monitoring crops development. The NDVI index and remotely sensed canopy temperature were efficient in detecting differences among treatments in term of senescence and water stress respectively. Future studies are suggested for relating such data with more accurate ground truth measurements of soil water status and crop responses.

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Chapted 3. UAV remote sensing of agronomic parameters and yield in chickpea and lentil

Keywords: grain legume, vegetation index, biomass, LAI, UAV

Abstract

Grain legumes cropping has been purposed as a pivotal practise for facing future issues in terms of food security and agroecosystem stability. Despite the importance of such cultures, literature lack of knowledge in monitoring grain legumes performance with remote sensing data. Hence, this study investigates these aspects in Chickpea (CH) and Lentil (LN), grown in Udine (Italy) during the growing season 2022. Crop dry biomass (BM_{AG}), dry matter content (DMC) and leaf area index (LAI) were correlated with multispectral data acquired by unmanned aerial vehicle (UAV) on seven dates during the growing season. Near-infrared (NIR) band performed as the best proxy of LAI, while for DMC, best correlation was obtained with Normalized Difference Chlorophyll index (NDI). BM_{AG} also was correlated with NDI, and correlation strength improved by implementing the cumulative elaboration of the index. Cumulative indices performed also as proxies of yield; best index was Modified Green-Red Vegetation Index (MGRVI).

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1 Introduction

The foreseen increase in drought occurrence and water scarcity due to climate change is expected to cause substantial reductions in crop yield, with consequent impacts for food availability and the economic system (Mabhaudhi *et al.*, 2019). Since their high nutritional values, grain legumes have been proposed as a sustainable substitute for animal derived protein to promote food security and foster other agroecosystem services (Tilman *et al.*, 2002). Chickpea and Lentil are currently two of the most important pulses cropped worldwide (De Ron *et al.*, 2017). Chickpea is ranked third in the global pulses production. Its use has widely increased in recent decades reaching a global production of over 17 Mt in 2018 (Grasso *et al.*, 2022). Lentil is the fourth most important grain legume worldwide. Its success is due to the low input requirements in terms of water and nutrients. Its seeds are rich in nutritional components, with a protein content that can reach up to 30% (Romano *et al.*, 2021). Despite the high potential of these crops in facing future issues for farming worldwide, the actual production is highly specialized and still relies on a restricted number of species for animal feed (FAO, 2021). Hence, the need to develop novel management strategies, in order to achieve competitive cropping systems.

For this purpose, remotely sensed data has been widely adopted, with particular concern to crop modelling, which plays a fundamental role in policymaking and land management (Kasampalis *et al.*, 2018). Indeed, remote sensing data have been used as proxies of many physiological and agronomic parameters like crop biomass (Seo *et al.*, 2019), leaf area index (Kross *et al.*, 2015), nitrogen uptake (Holzhauser *et al.*, 2022), and yield (Gao *et al.*, 2018). In particular, the increasing availability of times-series data enabled the calculation of cumulative vegetation indices, increasing the accuracy in parameters prediction. Mkhabela *et al.* (2005), used decadal Normalized Difference Vegetation Index (NDVI) from satellite data, to forecast maize's yield. Their results highlighted that most accurate predictions have been assessed using data from two months before harvest. Similar results have been obtained in a further study by the same authors, where they used the same method to forecast yield of barley, canola, field peas and spring wheat, grown on the Canadian Prairies (Mkhabela *et al.*, 2011). Recently, Panek and Gozdowski (2021) investigated the possibility of predicting wheat and barley yield, with cumulative NDVI data from 2000 to 2018, in 20 European countries. Their results suggest that the use of cumulative vegetation indices allows the assessment of more reliable predictions for grain yields.

Despite the use of remote sensing data which has been widely used in some of the most cropped species (*e.g.* maize – Mkhabela *et al.*, 2005; rice – Huang *et al.*, 2013; wheat - Kancheva *et al.*, 2007), evidences on pulse crops are poor and mainly focused on soybean (Kross *et al.*, 2015, Gao *et al.*, 2018, Seo *et al.*, 2019). Hence, the objectives of this study were to investigate the possibility to correlate remotely sensed data with crop biomass, dry matter content, leaf area index and yield, in chickpea and lentil grown under different irrigation management.

2 Materials and Methods

2.1 Experimental design

The experiment was conducted during the growing season 2022 in Udine (NE Italy), at the experimental farm of the University of Udine (46.03N, 13.22E), in a field that had previously been cultivated with soybean. Chickpea (CH, *Cicer arietinum*) cv. *Sultano* and Lentil (LN, *Lens culinaris*) cv. *Itaca* were sown on 28/03/2022. Both varieties were provided by AgroService SpA, Italy. Chickpea was sown in 15.2m^2 plots (4 rows with a row-distance of 0.38m at a length of 10m) at target plant density 60 plants/m^2, while lentil was sown in 12m^2 plots (8 rows with a row-distance of 0.15m at a length of 10m) with 250 plants/m^2.

Plots were arranged according to a systematic experimental design replicated for three blocks. A total of 12 plots per species were sown, in order to compare 4 irrigation treatments: *i*) Not irrigated; *ii*) Irrigated with 10 mm; *iii*) 20 mm; *iv*) 30mm. The 30mm irrigation volume is equivalent to full recovery of Field Capacity (FI) soil moisture in the 30 cm upper layer of soil when the sum of daily reference crop Evapotranspiration (ET; Penman-Monteith equation in FAO56) is equal to 30mm. Each treatment was replicated for 3 plots. First irrigation was done on 16/05/2022 at 20 and 24 days after beginning of flowering of lentil and chickpea, respectively. The other two irrigations were on 21/06/2022 and 28/06/2022. Emergence for both crops was fully completed on 19/04/2022. Hand hoeing weeding was conducted weekly, to prevent possible biases in the spectral response of crops due to weeds presence. Harvest differed according to species and irrigation treatments. lentil was harvested from 06/07/2022 to 26/07/2022, while chickpea from 18/07/2022 to 26/07/2022.

2.2 Multispectral data

Multispectral data were acquired on seven dates during the growing season: 02/05/2022, 26/05/2022, 01/06/2022, 08/06/2022, 15/06/2022, 30/06/2022, 04/07/2022. Flights were conducted at 11:00 a.m. (LST) with a DJI P4 Multispectral drone equipped with 5 monochromatic sensors, acquiring reflected radiation in: Blue (450±16nm), Green (560±16nm), Red (650±16nm), Red-Edge (RE, 730±16nm) and Near-Infrared (NIR, 840±26nm). Spectral data were mapped with spatial resolution of 2cm using Pix4D software. Georeferenced maps of the plots were created with R software by cropping the central area of each plot with a 0.76x6m mask in chickpea and 0.6x6m mask in lentil. To prevent possible biases due to inhomogeneous or incomplete soil cover between rows, pixels with NDVI value lower than 0.05 were excluded.

Multispectral data were used to calculate the most used multispectral (MSP) and RGB Vegetation Indices (VIs) as proxies of productivity, crop biomass and green biomass. (Table 3.1). Then, cumulative indices (CVIs), were calculated as the integral of the time dependent spline function of each VI.

Table 3.1. List of vegetation indices tested, with corresponding abbreviations and equations.

Classification	**Index**	**Label**	**Equation**	**Reference**
Single bands	Blue	B		
	Green	G		
	Red	R		
	Red Edge	RE		
	Near-Infrared	NIR		
Multispectral indices	Simple ratio	RVI	RVI = R / NIR	Pearson and Miller, 1972
	Normalized Difference Vegetation Index	NDVI	NDVI= (NIR – R) / (NIR + R)	Rouse *et al*., 1974
	Green NDVI	GNDVI	GNDVI = (NIR – G) / (NIR + G)	Gitelson *et al.*, 1996
	Normalized Difference Chlorophyll Index	NDI	NDI = (NIR – RE) / (NIR + RE)	Gitelson and Merzlyak, 1994
	Soil-Adjusted Vegetation Index	SAVI	SAVI = (1+L) * (NIR – R) / (NIR + R + L)	Huete *et al*., 1988
RGB indices	Excess Green Vegetation Index	ExG	ExG = (2*G)-R-B	Woebbecke *et al., 1995*
	Excess Red Vegetation Index	ExR	ExR = (1.4*R-G)/(R+G+B)	Meyer and Neto, 2008
	Excess Green minus Red Vegetation Index	ExGR	ExGR = ExG – ExR	Neto, 2004
	Green-Red Vegetation Index	GRVI	GRVI = (G-R)/(G+R)	Tucker, 1979
	Modified GRVI	MGRVI	MGRVI = $(G^2 – R^2)/(G^2 + R^2)$	Bendig *et al.*, 2015

2.3 Crop Measurements

Above ground crops biomass (BM_{AG}, g m^{-2}) has been assessed immediately after UAV flights on three dates: 26/05/2022, 01/06/2022 and 15/06/2022. From the central rows of each plot an area of 0.38m^2 in chickpea and 0.3m^2 in lentil was harvested for BM_{AG}. The fresh weight (FW, g) was measured in field with a portable balance and then oven-dried at 70°C for 72h to measure the dry weight (DW, g). Dry matter content (DMC, g g^{-1}) was calculated as the ratio of DW to FW.

Grain yields were determined on the central rows of each plot for an area of 1.5- 2 m^2 at harvest dates. The not irrigated and 10mm irrigation treatments of both species were harvested at the earliest dates.

Leaf Area Index (LAI) was measured with LAI-2200C Plant Canopy Analyzer (LI-COR Biosciences, Lincoln, Nebraska, USA) as the mean of 10 measurements per plot. LAI was assessed on five out of seven flight-dates: 26/05/2022, 01/06/2022, 08/06/2022, 15/06/2022 and 30/06/2022.

2.4 Statistical analysis

Statistical analyses were conducted with R software. Due to normality, homoscedasticity and independence issues, relationship between measured variables and indices were tested separately for each species, by Spearman's rank correlations, according to the methodology of Marusig *et al.* (2020). In particular, single correlations were tested between measured agronomical parameters and VI or CVI averages at the plot level. Being a non-cumulative variable, DMC was correlated with VIs only. BM_{AG} and LAI were correlated with both VIs and CVIs, while yield with CVIs only (calculated till the last UAV-flight, *i.e.*, 04/07/2022). Critical value for significance (α) was set to 0.05.

3 Results

Best Spearman's rank correlations between remotely sensed VIs or CVIs and measured agronomical parameters are summarized in Table 3.2 and represented in Figure 3.1.

BM_{AG} had the highest correlation coefficients and significances with VIs. In particular, the Normalized Difference Chlorophyll Index (NDI) calculated at each date was most correlated with BM_{AG}. The cumulated data (cNDI) increased the correlation strength up to 0.90 in lentil (Figure 3.1a,b). NDI also performed as the best proxy for DMC, with correlation coefficients higher than 0.70 (Figure 3.1c),

Table 3.2. The most significant Spearman's rank correlation coefficients (ρ) between measured variables and vegetation indices (VIs) or cumulative vegetation indices (CVIs). Significance: *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; in parenthesis number of data; n.s. = not significant; n.a. = not applicable.

Measured Variable	Vis	CVIs
BM_{AG}	**NDI**	**cNDI**
CH	0.83***	0.87***
LN	0.87***	0.90***
DMC	**NDI**	
CH	0.79***	n.a.
LN	0.72***	n.a.
LAI	**NIR**	**cNIR**
CH	0.59***	n.s.
LN	0.64***	n.s.
Yield		**cMGRVI**
CH	n.a.	0.65**
LN	n.a.	0.75**

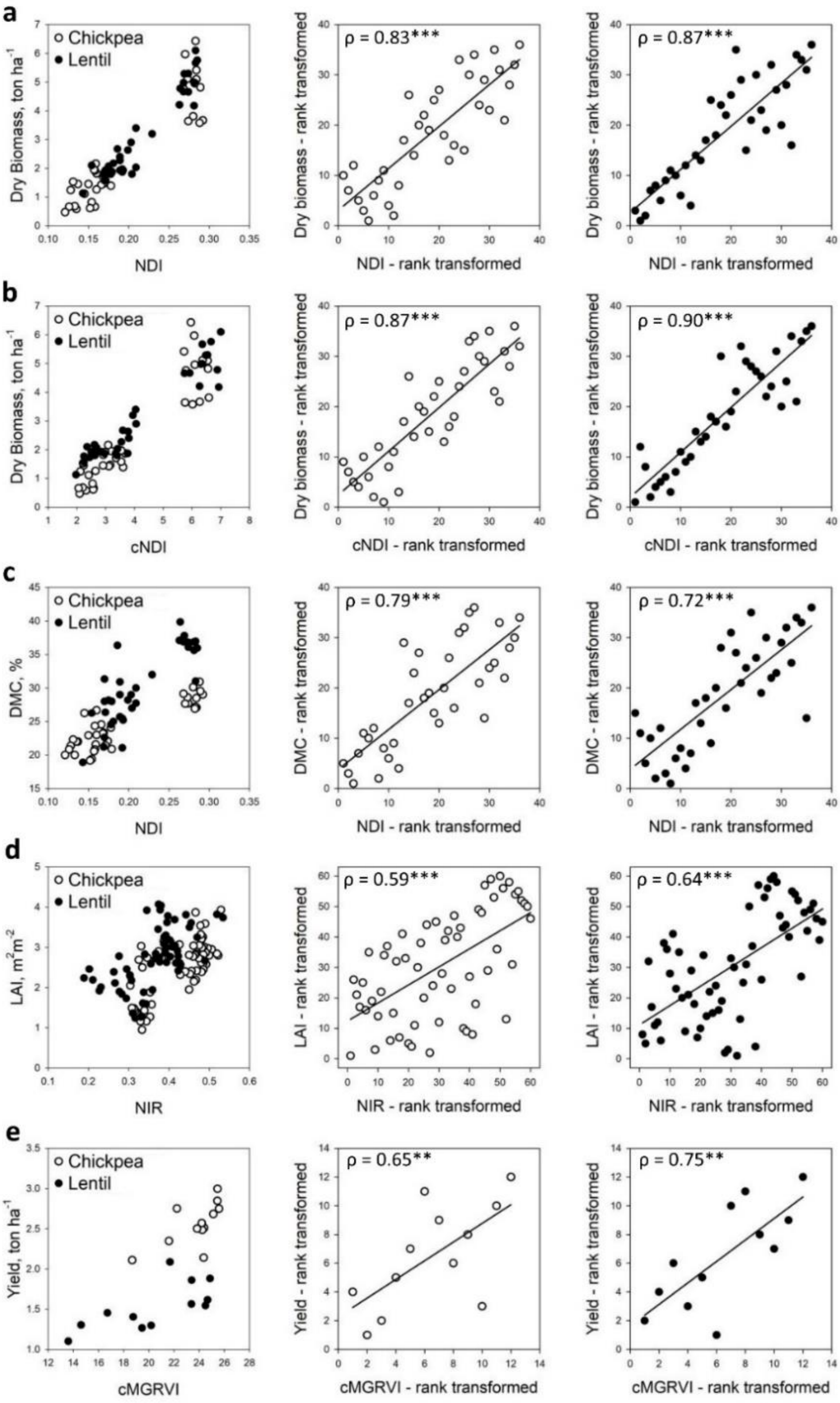

Figure 3.1. Best correlations assessed between remotely sensed and field-measured agronomical parameters with respective Spearman's correlation coefficients (ρ) and statistical significance: a) dry biomass vs Normalized Difference Chlorophyll Index (NDI); b) dry biomass vs cumulative NDI (cNDI); c) dry matter content (DMC) vs NDI; d) leaf area index (LAI) vs NIR band; e) yield vs cumulative Modified Green-Red Vegetation Index (cMGRVI). *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

Best correlation with LAI for both species, was assessed with the near-infrared (NIR) band alone. In fact, correlations with other VIs were lower or not significant. Moreover, no statistical significant correlation was assessed for LAI with any CVI (Table 3.2).

CVIs were successfully related to crops yield. Best results were obtained with cumulative Modified Green-Red Vegetation Index (cMGRVI), an RGB derived VIs. Correlations between indices and yield were the ones with higher discrepancy of results between chickpea ($\rho = 0.65$) and lentil ($\rho = 0.75$). This result may be due to the reduced data amount (12 data, one per plot), or to the longer timespan between the last UAV-flight (04/07/2022) and harvest, especially in irrigated plots, where harvest maturity was reached 22 days later.

4 Discussion

Estimation of agronomic parameters with remote sensing data is nowadays a widespread practice for the most widely cropped species (Huang *et al.*, 2013, Kross *et al.*, 2015, Panek and Godzowski, 2021). However, relationships between field-measured variables and remotely sensed ones are generally assessed without considering time-series variability (Holzhauser *et al.*, 2022) or rely on a restricted selection of indices, (*e.g.*, the Normalized Difference Vegetation Index) (Seo *et al.*, 2019). The data suggest that some VIs data elaboration as cumulative index may improve the estimation of cumulative variables like biomass and yield. For this, it is suggested that the choice of the type of VI may differ according to the intrinsic property (cumulative or not cumulative) of the variable of interest like above-ground biomass (BM_{AG}) or yield and LAI respectively.

Normalized Difference Chlorophyll Index (NDI) performed as the best predictor for both BM_{AG} and DMC. NDI is in fact calculated as a normalized difference of NIR and Red Edge (RE) bands, which are related to canopy structure and water status, respectively (Sun *et al.*, 2019, Holzhauser *et al.*, 2022). Moreover, even if single-date correlations were considerable, the use of cumulative NDI (cNDI), improved correlation with biomass in both species. Similar results have been reported in the literature (*e.g.* Gao *et al.*, 2018, Seo *et al.*, 2019). However, this methodology is still poorly adopted.

In contrast, LAI was not correlated with any cumulated vegetation index (CVI). In fact, canopy growth was generally limited after flowering and green LAI tended to decrease during the maturation phase, due to leaf senescence and loss, or accelerated by drought or other stresses. Moreover, best correlations between LAI and remotely sensed information were achieved with NIR band alone. This result is in accordance with an investigation conducted by Kira *et al.* (2016), which coupled LAI and hyperspectral reflectance measurements in corn and soybean for

8 years, suggesting that NIR band is one of the most informative and suitable bands to be used as a proxy of leaf and canopy structure.

Stronger correlations between CVIs and yield were assessed in lentil then chickpea. This result may be due to the longer timespan that occurred between the last UAV flight and the harvest date. In fact, chickpea was generally harvested later than lentil, and especially in the irrigated plots, the treatment significantly delayed crop senescence. Similar issues have been reported in various studies investigating the same topic, reporting indeed that better yield forecasts should be assessed using data from at least the last months before harvest (Mkhabela *et al.*, 2005, Mkhabela *et al.*, 2011, Gao *et al.*, 2018). However, significant correlations were assessed with cumulative Modified Green-Red Vegetation Index (cMGRVI).

5 Conclusions

This paper provides additional evidence about the possibility to monitor crop biomass, dry matter content, leaf area index and yield, in chickpea and lentil, with remotely sensed data provided by UAV. The Normalized Difference Chlorophyll Index performed as the best predictor of dry-matter content throughout the growing season.

Significant correlations were also assessed between remotely sensed data with LAI and the Near-Infrared band.

The cumulative vegetation indices are strongly correlated with cumulative variables like crop biomass and yield.

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Chapter 4. Intercropping of Grain Legumes with Buckwheat (*Fagopyrum esculentum*, L) as a tool for Weed Control and Sustainable Crop Production

Keywords: Chickpea, Lentil, Allelopathy, Competition, Yield, Agroecology

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1 Introduction

Climate change effects are foreseen to negatively affect crop yield and yield stability, with consequent impacts in terms of food security. Hence, the current agricultural system, based on poorly diversified cropping is no more reputed a reliable approach for sustainable food production (Raseduzzaman and Jensen 2017). A more suitable and resilient approach consists in crop diversification. Among the diversification practices, a particular interest is devoted to intercropping, practice based on the cultivation of multiple crops in the same field (Brooker et al. 2015). Intercropping does not only diversify the income for the farmers, but it has also been demonstrated to improve soil health (Yang et al. 2020), increase the resource use efficiency (Jensen et al. 2020) and water utilization (Yin et al. 2020), promote weed suppression (Gu et al. 2021) and enhance yield stability (Raseduzzaman and Jensen 2017).

Intercropping has been widely investigated, as it has been demonstrated be an effective strategy for mitigating weeds competition as intercropped plants are more effective at competing for resources (Gu et al. 2021). Furthermore, in intercropping systems, weed management is frequently achieved by incorporating companion species with allelopathic properties. These species are able to produce and release natural compounds, known as allelochemicals, into their environment. These allelochemicals can have both stimulatory and inhibitory effects on the growth and development of other nearby plants (Jabran et al. 2015). Some widespread allelopathic crops are barley, brassica, buckwheat, mustard, oat, rice, rye and sunflower (Jabran 2017). Intercropping of grain legumes with allelopathic crops has been demonstrated being successful in many consociations like chickpea and wheat (Banik et al. 2006), as well as lentil with wheat and oat (Fernandez et al. 2015).

In this study, we focused on buckwheat (*Fagopyrum esculentum*) a widespread crop cultivated in a wide range of geographical regions thanks to its adaptability to different climates and growing conditions (Ahmet et al. 2014), as a companion crop for grain legumes. Because of its allelopathic properties, buckwheat had been traditionally used by farmers for weed management, however, despite its weed suppressing activity has been widely investigated, the mechanisms of action have not been clearly identified yet (Falquet et al. 2015). It has been widely demonstrated that buckwheat residues incorporation in soil has a significant effect on weeds suppression (Kumar et al. 2008, 2009a, 2009b, Szwed et al. 2019). Up to our knowledge, quantification of allelochemicals of buckwheat has been conducted only for plant residues, seed flour and soils with incorporated plant residues (Table 4.1). Literature about allelopathic effects promoted by root exudates production is scarce (e.g. Biszczak et al. 2020, Cheriere et al. 2020) or denies this hypothesis (Wirth and Geller 2016). Moreover, literature on buckwheat as a companion crop for grain legumes in intercropping is limited (Wang et al. 2012, Biszczak et al. 2020, Cheriere et al. 2020, 2023).

In this study, we examined the interactions between buckwheat, weeds, and crops, with particular focus on grain legumes. We conducted multiple trials, varying in complexity, to evaluate how buckwheat interacts with the other species. The hypothesis of this study is that allelopathic substances produced by buckwheat are released into the environment in the form of root exudates and that these have a significant inhibitory effect on weeds while causing minimal harm to crops.

2 Materials and Methods

Buckwheat capability to produce allelochemicals has been widely documented (Table 4.1), however, examples of intercropping with this species are scarce and the allelopathic effect in consociation has been poorly investigated (Table 4.2).

To test the capability of buckwheat to produce and discharge allelochemicals into the environment to suppress weed growth, we set up a multiple level experiment: i) An in-vitro germination assay to test the impact of buckwheat water extracts on weed and crop seeds; ii) A pot experiment to determine whether the potential plant-suppression effects identified in the germination experiment may be observed in soil conditions; iii) A field trial to assess the weed control effectiveness of buckwheat both in the inter-row spaces and within the rows of grain legumes.

Table 4.1. List of allelochemicals detected in buckwheat. For each compound we specified the class, the measured average concentration, the analyzed matrix, and the reference.

Class	**Compound**	**Concentration**	**Matrix**	**Reference**
Alkaloid	2-piperidinemethanol	Not specified	Plant residues	Iqbal et al 2003
	4-piperidone	Not specified	Plant residues	Iqbal et al 2003
	Fagomine	Not specified	Plant residues	Iqbal et al 2003
Benzenoids	Iso-vanilic acid	Not specified	Plant residues	Szwed et al 2020
Dihydroxybenzoic acid	Protocatechuic acid	Not specified	Plant residues	Szwed et al 2020
	Vanillic acid	Not specified	Plant residues	Szwed et al 2020
Fatty acid	Arachidic acid	Not specified	Plant residues	Tsuzuki et al. 1987
		18 mg/g	Flour	Bonfaccia et al 2003
	Behenic acid	Not specified	Plant residues	Tsuzuki et al. 1987
		11 mg/g	Flour	Bonfaccia et al 2003
	Palmitic acid	Not specified	Plant residues	Tsuzuki et al. 1987
		156 mg/g	Flour	Bonfaccia et al 2003
	Stearic acid	Not specified	Plant residues	Tsuzuki et al. 1987
		20 mg/g	Flour	Bonfaccia et al 2003
Flavonoid	(-)-Epicatechin	0.98 mg/g	Plant residues	Golisz et al. 2007
	(+)-Catechin	0.22 mg/g	Plant residues	Iqbal et al 2003
	Apigenin	11 mg/g	Soil with incorporated residues	Szwed et al 2020
	Kaempferol	Not specified	Plant residues	Szwed et al 2020
	Luteolin	Not specified	Plant residues	Szwed et al 2020
	Quercetin	0.5 mg/g	Plant residues	Golisz et al 2008
	Rutin	50 mg/g	Plant residues	Golisz et al 2008
Hydroxycinnamic acid	m-Coumaric acid	75 mg/g	Soil with incorporated residues	Szwed et al 2020
	o-Coumaric acid	185 mg/g	Soil with incorporated residues	Szwed et al 2020
	p-Coumaric acid	79 mg/g	Soil with incorporated residues	Szwed et al 2020
Phenolic acid	Caffeic acid	0.11 mg/g	Plant residues	Golisz et al. 2007
	Chlorogenic acid	1.79 mg/g	Plant residues	Golisz et al. 2007
	Ferulic acid	0.05 mg/g	Plant residues	Golisz et al 2008
	Gallic acid	0.42 mg/g	Plant residues	Golisz et al. 2007
	Syringic acid	Not specified	Plant residues	Szwed et al 2020
Phenylpropanoid	Sinapic acid	Not specified	Plant residues	Szwed et al 2020

Table 4.2. List of the papers reporting field trials investigating intercropping with buckwheat.

Companion crops	Intercropping type	Aim	Reference
Alfalfa	row intercropping	Enchance/Optimize productivity	Başaran et al. 2018
	row intercropping	Enchance/Optimize productivity	Basan et al. 2020
	strip cropping	Enchance/Optimize productivity	Gao et al. 2022
bell pepper	strip cropping	Integrated Pest Management	Bickerton et al. 2012
Broccoli	strip cropping	Integrated Pest Management	Ponti et al. 2007
Cabbage	strip cropping	Integrated Pest Management	Al-Doghairi et al. 2004
	strip cropping	Integrated Pest Management	Nilsson et al. 2012
	strip cropping	Integrated Pest Management	Pandey et al. 2019
Cotton	strip cropping	Integrated Pest Management	Li et al. 2019
Fenugreek	row intercropping	Enchance/Optimize productivity	Salehi et al. 2017
	row intercropping	Enchance/Optimize productivity	Salehi et al. 2018
	row intercropping	Enchance/Optimize productivity	Salehi et al. 2019
Lentil	mixed cropping	Weed control	Wang et al. 2012
Maize	strip-relay intercropping	Enchance/Optimize productivity	Zhongmin et al. 1990
Onion	mixed cropping	Integrated Pest Management	Trdan et al. 2006
Potato	strip-relay intercropping	Enchance/Optimize productivity	Zhongmin et al. 1990
Soybean	row and mixed intercropping	weed control	Cheriere et al. 2020
	strip-relay intercropping	Weed control	Biszczak et al. 2020
	row intercropping	Enchance/Optimize productivity	Ponte et al. 2022
	intercropping	Weed control	Cheriere et al. 2023
Squash	strip cropping	Integrated Pest Management	Razze et al. 2016
Sunflower	row intercropping	Weed control	Latify et al. 2017
Zucchini	cover crop	Integrated Pest Management	Manandhar et al. 2009
	cover crop	Integrated Pest Management	Manandhar et al. 2011

2.1 Buckwheat water extracts effect on seeds germination

2.1.1 Experimental Design

A germination test in petri dishes has been carried out in spring 2023, to test the allelopathic effect of buckwheat extracts on seed germination of various crops and weeds. A total of 11 species were tested, 5 crops: chickpea (*Cicer arietinum*, CH), lentil (*Lens culinaris*, LN), soybean (*Glycine max*, SB), quinoa (*Chenopodium quinoa*, QN) and barley (*Hordeum vulgare*, BR); 4 weeds: red pigweed (*Amaranthus retroflexus*, AR), cockspur (*Echinochloa crus-galli*, EC), couch grass (*Cynodon dactylon*, CD) and foxtail millet (*Setaria italica*, SI); 2 model species: tobacco (*Nicotiana tabacum*, TB) and garden cress (*Lepidium sativum*, CR). A detailed summary of species with corresponding information is reported in Table 4.3. Seeds of crops and model species were provided by Agrifutur S.r.l. (Alfianello-Brescia, IT) or by the seeds stock of the experimental farm "A. Servadei" of the University of Udine, while weeds' seeds were collected in the fields of the experimental farms on 07/10/ 2022 and conserved in a dark and dry environment after a two-weeks vernalization.

Each species was tested under three different treatments: control (CNT); buckwheat water extracts at concentration 1:10 (1g of buckwheat residues and 10ml of water – d10); buckwheat water extracts at concentration 1:5 (1g of buckwheat residues and 5ml of water – d05). For detailed information about buckwheat extracts see section 2.1.2.

Germination tests were conducted in sterile petri dishes with diameter of 90mm or 150mm, according to the seed size. For each petri dish we tested 15 seeds soaked in 10ml of water/extract poured onto filter paper. For each combination crop *x* treatment, we tested five replicates. The seeds were germinated in a growth chamber at the laboratories of the University of Udine. The incubation period lasted for 7 days with a 12-hour light-dark alternation, maintained at 25°C and 20°C, respectively.

Table 4.3. List of investigated species in the germination tests with corresponding abbreviations and thousand seeds weight (TSW).

Common name	**Scientific name**	**Abbreviation**	**TSW, g**
Chickpea	*Cicer arietinum*	CH	467
Lentil	*Lens culinaris*	LN	47
Soybean	*Glycine max*	SB	159
Quinoa	*Chenopodium quinoa*	QN	3.2
Orzo	*Hordeum vulgare*	BR	49.2
Red Pigweed	*Amaranthus retroflexus*	AR	0.43
Cockspur	*Echinochloa crus-galli*	EC	1.63
Couchgrass	*Cynodon dactylon*	CD	0.68
Foxtail millet	*Setaria italica*	SI	2.03
Tabacco	*Nicotiana tabacum*	TB	0.3
Garden cress	*Lepidium sativum*	CR	0.35

2.1.2 Buckwheat extracts preparation

Buckwheat extracts were obtained by adapting the protocol of Carrubba *et al.* (2020). Buckwheat plants were collected on 20/10/2022, in the fields of a farm situated in Buttrio (Udine, Italy, 45.98° N, 13.39°E), when plants were at full bloom. We collected the entire plants, including the root system and the retained soil layer. Plant material was dried in oven at 60°C for 72h and then ground in multiple steps, from blade mill to ball mill. The obtained product was stored in paper bags in dark conditions at room temperature until its use.

Water extracts were prepared by soaking 200g of product in 1l of distilled water (weight/volume ratio of 1:5) and stirring at 150 rounds per minute for 5h. Water extracts were strained with filter paper and refrigerated at 4°C until used. Water extract at 1:10 rate was obtained by diluting the prepared extract (1:5) with an equivalent volume of pure water.

2.1.3 Measurements

Measurements were conducted according to the protocols of Emino and Warman (2004). After 7 days of incubation, we counted the number of seeds germinated (N_G) and estimated the germination percentage ($G_{\%}$, %). Seeds were then photographed on graph paper and primary root length (L_R) measured with the ImageJ v1.54d software (Schindelin et al. 2012). We calculated the Relative Seed Germination (RSG, %) and the Relative Root Growth (RRG, %) as:

$$RSG = \frac{N_G}{Mean\ N_G\ in\ control\ (pure\ water)} \times 100$$

$$RRG = \frac{L_R}{Mean\ L_R\ in\ control\ (pure\ water)} \times 100$$

The Germination Index (GI, %) was then calculated as:

$$GI = \frac{RSG\ \times RRG}{100}$$

2.1.4. Allelochemical analysis

Samples of ground buckwheat and a d05 solution were analyzed using high-performance liquid chromatography to assess the content of two of the most abundant allelochemicals found in this species according to literature: Quercetin and Rutin. The sample of ground buckwheat (200mg) was extracted with 15ml of CH_3OH:H_2O (80:20), agitated using a vortex, sonicated for 20 minutes, centrifuged for 20 minutes at 6000 rpm, and filtered through syringe filters (PTFE 0.2μm, 25 mm). Samples of d05 solution were only filtered through syringe filters and directly injected in HPLC. The quantification was carried out using an external standard calibration curve. The chromatographic conditions were as follows: flow rate: 0.8ml/min; mobile phase: CH_3OH:H_3PO_4 0.4% (50:50); injection volume: 25μl; column: Spherisorb ODS 2 5μm (Waters) maintained at 30°C; Detector: a diode-array detector (PDA) set at 360nm. For each matrix, 3 replicates were analyzed.

2.1.5 Statistical analysis

Statistical analysis was conducted with the R v2.0.1 software (R Core Team, 2021). After data normality and homoscedasticity check, differences among treatments of the same species were tested by one way analysis of variance (ANOVA) and Tukey's Honest Significance Difference test as *post hoc*.

2.2 Plants-consociation effect in a controlled environment – A greenhouse experiment

2.2.1 Esperimental Design

We set up a pot experiment aimed to: 1) test whether buckwheat may provide weed-control services by allelopathy or competition for resources; 2) evaluate if possible plant-suppression effects reported by the germination experiment can be observed in soil.

The experiment was conducted in a greenhouse at the facilities of the University of Udine during spring 2023. Crops' seeds were provided by Agrifutur S.r.l., while weeds seeds were previously collected in field (see section 2.1.1). A total of four species were tested, two crops: chickpea *cv Sultano* and lentil *cv Itaca*; and two weeds: velvetleaf (*Abutilon theophrasti* – AT) and red pigweed (*Amaranthus retroflwxus* – AR). Each species was tested under three experimental treatments: monoculture (MC), intercropped with buckwheat (IC) and grown in staircase device (SD), an experimental tool specifically designed to separate the effect of allelopathy from competition by watering plants with percolated water from pots containing pure buckwheat stands (Mahé et al. 2022). All treatments were tested for four replicates and placed according to a randomized block design (Figure 4.1). Since the experimental protocol included three destructive samplings, the MC and IC treatments, were replicated three times, while for SD it was not possible due to logistical issues in making the devices itself and a single block was set.. Due to insufficient space in the greenhouse for the staircase device, pots for SD treatment (both buckwheat and investigated species) were placed in a different area of the greenhouse, covered by a permanent shading net.

Sowing was conducted on 21/04/2023. Seeds were sown in 13.5l pots, with surface area of 225cm^2 (15cm x 15cm). Pots were filled with clay-loam sifted soil (clay 28%, silt 30% and sand 42%), with pH 5.01, organic Carbon 13.8mg g^{-1}, total Nitrogen content 0.16%, organic matter 2.4%, cation exchange capacity 15.09meq/100g and Phosphorus concentration 20mg kg^{-1}. buckwheat, lentil, pigweed, and velvetleaf were sown at a density of 4 plants per pot in pure stands (MC and SD), and at 2 plants per plot when intercropped (IC). Chickpea was sown at 2 plants per pot in the pure stands and one plant per pot when intercropped.

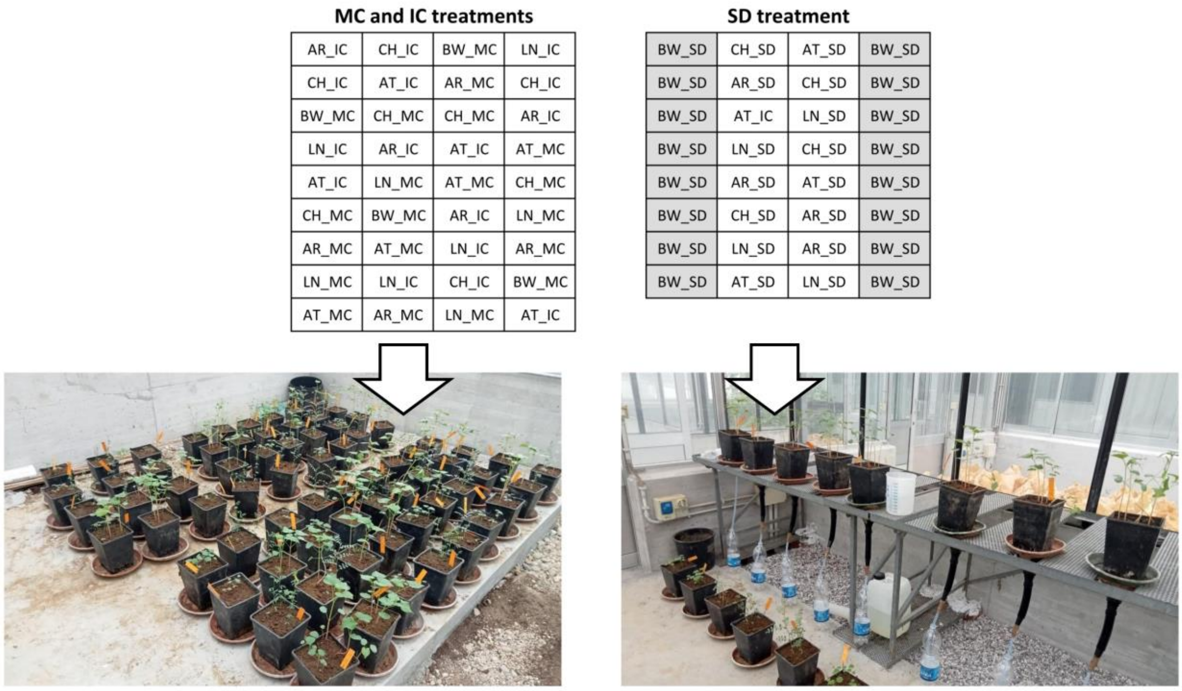

Figure 4.1. Experimental design of chickpea (CH), lentil (LN), red rigweed (AR) and velvetleaf (AT) tested under monoculture (MC), intercropped (IC) with buckwheat (BW) and with staircase device (SD). Grey boxes denote BW pots placed on top of the SD.

Emergence occurred on 24/04/2023 in chickpea and lentil, and on 26/04/2023 in *A. retroflexus* and *A. theophrasti*. Plants were then irrigated 3 times per week with 0.5l per pot. In SD, pure stands of buckwheat were grown in pots with perforated saucers. Plants were fully irrigated (~1-1.5l per pot) and leached water was collected into a sink through a funnel with a filter and then used for irrigation with the same volumes of the other treatments. Temperature in the greenhouse was hourly monitored from 02/05/2023 until the end of the experiment, with an EL-USB datalogger (Laskar Electronics, Whiteparish, UK). The datalogger was placed in a semi-open box to prevent overheating due to irradiation. Temperature between sowing and datalogger installation was estimated with a polynomial regression model, based on the greenhouse temperature as a function of the air temperature recorded by the ARPA-OSMER weather station of Udine Sant'Osvaldo (R^2 = 0.90).

The pots were consistently weeded to avoid potential biases arising from the presence of weeds not under investigation.

2.2.2 Measurements

A total of three samplings were conducted, based on buckwheat phenology: on 16/05/2023 at fifth full leaf development; on 06/06/2023 at full bloom; on 26/06/2023, at seeding onset. SD treatment was sampled only on last sampling. At each sampling, plants were removed from the soil and roots carefully cleaned. Plants height (H_P, cm) and primary root length (L_R, cm) were measured, then root system was separated from the shoot and dried in oven at 60°C for 72h to measure aboveground dry biomass (DW_{ABV}, g plant^{-1}) and root dry biomass (DW_R, g plant^{-1}). Growth rate (GR, cm d^{-1}) and the Competitive Balance Index (CBI, Wilson 1988) were then calculated as:

$$GR = \frac{H_P at\ t_2 - H_P\ at\ t_1}{number\ of\ days}$$

$$CBI_x = \ln(\frac{\frac{DW_{ABV}\ of\ x\ in\ IC}{mean\ DW_{ABV}\ of\ x\ in\ MC}}{\frac{DW_{ABV}\ of\ BW\ in\ IC}{mean\ DW_{ABV}\ of\ BW\ in\ MC}})$$

where x is the single species.

Furthermore, at the last irrigation and at harvest, samples of leached water and soil were collected and subsequently frozen at -20°C. Samples will be analysed using high-performance liquid chromatography (HPLC) to determine the content in Rutin and Quercetin, produced by BW.

2.2.3. Allelochemical analysis

During the final irrigation, samples of leached water were collected and refrigerated. Additionally, soil samples were collected from each pot during the final sampling and refrigerated alongside the leached water samples. The leached water samples were analyzed using high-performance liquid chromatography (HPLC) to determine the content of Quercetin and Rutin. Analysis of the soil samples has not been conducted at this time. The HPLC analysis followed the protocol described in section 2.1.4.

2.2.4 Statistical analysis

Statistical analysis was conducted with the R software. For the first two samplings, after data check for normality and homoscedasticity, orthogonal contrasts were tested with one way ANOVA, to test differences between treatments among the same species, while one way ANOVA and Tukey's Honest Significant Difference test as *post hoc* were used for testing differences among buckwheat treatments. On the last measurement, one way ANOVA and Tukey's test were used for each species.

2.3 Crops intercropping for weed management – A field experiment

2.3.1 Study area and experimental design

The trials were carried out during the growing season 2023 at the experimental farm "A. Servadei" of the University of Udine (Udine, IT, Figure 4.2.), in a field next to were trials in 2022 took place (refer to Chapter 2 section 2.1), previously cultivated with maize.

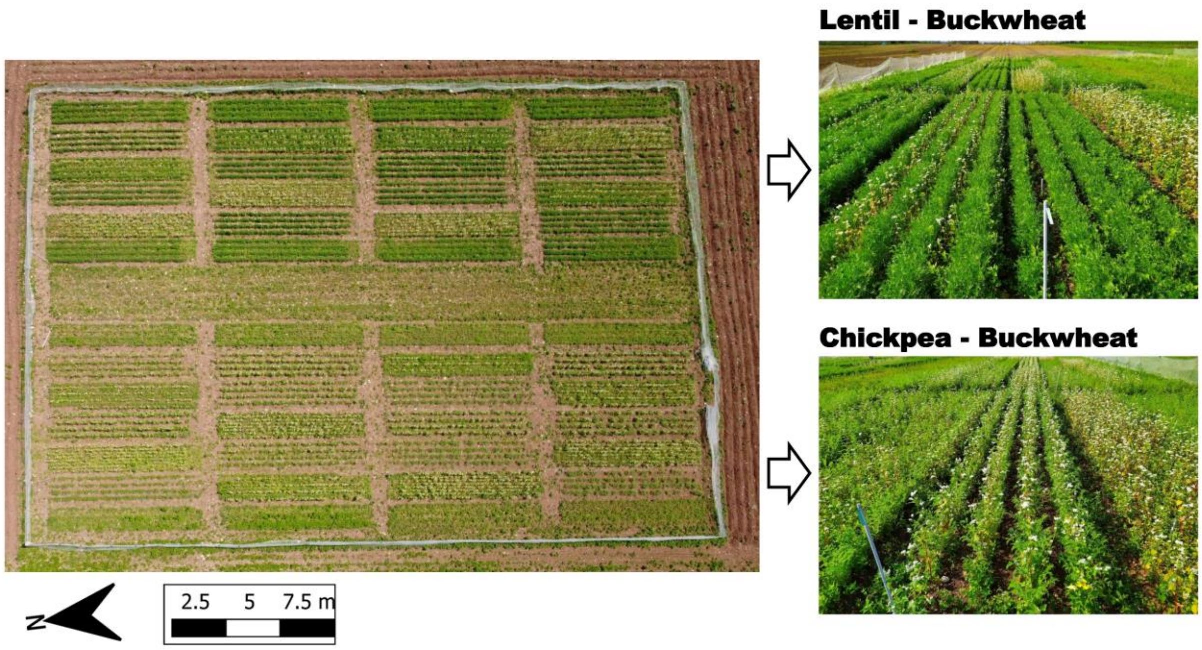

Figure 4.2. Study area of the field trials 2023 on intercropping buckwheat with lentil and chickpea.

Chickpea *cv Sultano* and lentil *cv Itaca* were tested in consociation with buckwheat *cv Panda*. Crops were tested under different intercropping layouts: 1) alternate rows (AR), where crop and buckwheat were sown in separate rows; within-row intercropping (WR), where crops were sown in the same row. Plots of both legume and buckwheat in monoculture were also included as controls (MC). In the WR layout, buckwheat was sown at two different plant densities: i) 25% of the monoculture sowing density, tested in both chickpea and lentil; ii) 50% of monoculture sowing density, tested in chickpea only. A detailed summary of the experimental treatments is reported in Table 4.2.

Crops were sown on 29/03/2023. Crops were sown in two separate adjacent areas, following a completely randomized plot design (Figure 4.2) with four replicated per treatment. Plot area was 10.88m^2, divided in 4 rows with a row-distance of 0.34m at a length of 8m in MC and WR, and 8 rows with a row-distance of 0.17m at a length of 8m in AR. Since the spatial separation of crops, buckwheat treatment as monoculture was replicated two times, one for each area. Crops in monoculture were sown at a plant density of 45 plants m^{-2} in chickpea, 120 plants m^{-2} in lentil and 200 plants m^{-2} in buckwheat. Plant density of buckwheat was halved in the AR and scaled accordingly in the WR (see Table 4.4).

Table 4.4. Summary of the experimental design tested in Udine during the season 2023. For each treatment we specified the plant density (PD) of legume (chickpea or lentil) and buckwheat.

Species	**Variety**	**Treatment / Intercrop**	**ID**	**PD of legume # m^{-2}**	**PD of buckwheat # m^{-2}**
Chickpea	*Sultano*	Control/Monoculture	CH	40	-
		Alternate rows	$CHBW_{AR}$	40	100
		Within-row at 25%	$CHBW_{25\%}$	40	50
		Within-row at 50%	$CHBW_{50\%}$	40	100
Lentil	*Itaca*	Control/Monoculture	LN	120	-
		Alternate rows	$LNBW_{AR}$	120	100
		Within-row at 25%	$LNBW_{25\%}$	120	50
Buckwheat	*Panda*	Control/Monoculture	BW	-	200

For all crops emergence was completed on 10/04/2023. Flowering occurred on 10/05/2023 in buckwheat, on 01/06/2023 in LN and on 06/06/2023 in CH. LN was harvested on 10/07/2023, while chickpea on 24/07/2023. Crops were grown under organic agriculture practices. No irrigation or fertilization was provided, and weeding was performed once on 05/05/2023 by manual hoeing, only in pure legumes and WR treatments (*i.e*. all plots with row distance 0.34m)

2.3.2 Weather condition

Weather of 2023 in Udine is represented in Figure 4.3 and monthly mean values are reported in Table 4.3. The weather conditions of the 2023 season in Udine were quite representative of the average climate, except for the month of July, when rainfall occurrence was particularly abundant, reaching a cumulative of 251.5mm, higher than the average of 112.6mm (Table 4.5).

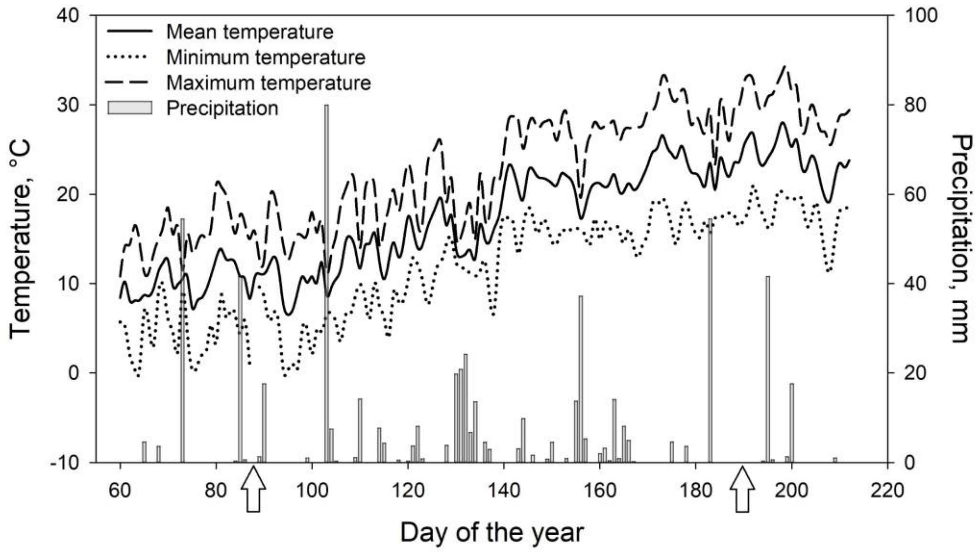

Figure 4.3. Weather conditions of the growing season 2023 in Udine. Sowing and harvest are marked by arrows. Data collected by ARPA-OSMER weather station (Udine Sant'Osvaldo).

Table 4.5. Monthly comparison of mean temperature and cumulative precipitation of 2023 in Udine with average values from data collected between 2001 and 2021. Data collected by ARPA-OSMER weather station (Udine Sant'Osvaldo).

Month	**Mean temperature in 2023 °C**	**Average mean temperature from 2001 to 2021 °C**	**Cumulative precipitation in 2022 mm**	**Average cumulative precipitation from 2001 to 2021 mm**
March	10.3	9.0	124.3	108.3
April	11.9	13.4	117.2	113.3
May	18.0	17.7	128.7	142.1
June	22.1	21.9	91.7	116.0
July	23.7	23.7	251.5	112.6
Total			713.4	593.2

2.3.3 Field measurements

Prior to hoeing, on 04/05/2023 a vegetation sampling has been conducted. For each plot, two quadrats of 0.35m^2 (0.35m x 0.7m) were randomly placed. For each quadrat we identified weed species and estimated the percentage of soil cover for both weeds and crops. A second vegetation sampling was performed one month later, on 07/06/2023, to evaluate the effectiveness of weeding or not, combined with the treatment. Data collected was used to calculate the Shannon Diversity Index (H index) as:

$$H\ index = \sum_{i=1}^{s} p_i \ln{(p_i)}$$

where p_i is the proportion of the i^{th} species within the sampled community.

Then, on the same date, at crops flowering, H_P was measured on 5 plants per plot, and one of the quadrats per plot was sampled, to measure DW_{ABV} lentil, chickpea, buckwheat and weeds, after drying the plants in oven at 70°C for 72h.

At harvest, two different samplings were conducted. A first sampling was conducted on the second quadrat, with the same protocol used at flowering. Dry samples were threshed to estimate DW_{ABV} and yield and calculate the Harvest Index (HI), as:

$$HI = \frac{Yield}{DW_{ABV} + Yield}$$

A second sampling was conducted on a more representative area of 1.36m^2 (0.68m x 2m). Plants were sampled and air-dried in a polytunnel for a weed and then dried to measure yield.

2.3.4 Statistical analysis

Statistical analyses were conducted with the R software (R Core Team 2021). After data check for normality and homoscedasticity, statistical differences among treatments were assessed separately for each crop (chickpea, lentil, buckwheat) with One Way Analysis of Variance (ANOVA) and Tukey Honest Significance Difference test as *post hoc*.

3 Results

3.1 Germination tests

Results of germination tests are summarized in Tables 4.6 and 4.7. Due to excessive presence of molds, for tobacco it was not possible to measure L_R and estimate Relative Root Growth (RRG) and calculate the Germination Index (GI), however it was still possible to count germinated seeds and calculate Relative Seed Germination (RSG).

Statistically significant reductions in germination percentage ($G_{\%}$) of seeds have been observed in most of the species. The only species where no difference in $G_{\%}$ or RSG were *C. dactylon* and *E. crus-galli* for weeds, and soybean for crops. Higher reductions were observed in *A. retroflexus*, chickpea, cress and tobacco, where in d05 treatment no seed germinated. On the contrary, *S. italica* was the only species where root length (L_R) and RRG were not significantly affected by the treatments. Hence, by looking at the overall result, differences in GI were assessed in all species and there seem to be a general and indiscriminate effect of germination inhibition by buckwheat extracts on all species. This effect seems to not be related to seed weight (Figure 4.4). The only exception was observed in quinoa, where despite the significant reduction in seeds germination, with the d10 treatment L_R was higher than control, however difference was not statistically significant.

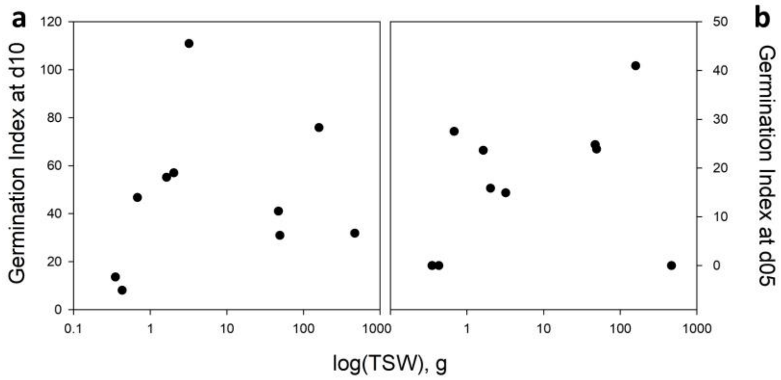

Figure 4.4. Scatter plot of mean values of germination index as a function of thousand seed weight (TSW). **a)** germination index measured at d10 treatment; **b**) germination index measured at d05 treatment.

Table 4.6. Mean values with corresponding standard deviations of germination percentage and root length measured during the experimentation. Statistical differences are denoted by different letters, otherwise not specified. Significance: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; n.s.: not significant.

Category	Species	Treatment	Germination %		Root length cm	
Weed	*A retroflexus*	Control	52.0 ± 20.8 **a**	***	24.0 ± 6.7 **a**	***
		d10	12.0 ± 13.7 **b**		5.9 ± 6.4 **b**	
		d05	0.0 ± 0.0 **c**		0.0 ± 0.0 **c**	
	C. dactylon	Control	92.0 ± 3.0	n.s.	27.0 ± 3.1 **a**	***
		d10	78.7 ± 9.9		14.9 ± 1.3 **b**	
		d05	77.3 ± 17.4		8.9 ± 3.1 **c**	
	E. crus-galli	Control	73.3 ± 4.7	n.s.	30.9 ± 2.6 **a**	***
		d10	64.0 ± 10.1		19.1 ± 5.7 **b**	
		d05	42.7 ± 31.1		9.7 ± 5.7 **c**	
	S. italica	Control	45.3 ± 25.6 **a**	*	5.9 ± 1.7	n.s.
		d10	24.0 ± 23.9 **b**		5.2 ± 3.2	
		d05	8.0 ± 5.6 **c**		4.2 ± 2.6	
Crop	Barley	Control	81.3 ± 7.3 **ab**	*	67.2 ± 12.2 **a**	***
		d10	94.7 ± 7.3 **a**		17.8 ± 1.7 **b**	
		d05	68.0 ± 18.5 **b**		18.8 ± 4.3 **b**	
	Chickpea	Control	52.0 ± 14.5 **a**	***	31.5 ± 11.4 **a**	**
		d10	21.3 ± 19.1 **b**		23.6 ± 19.3 **b**	
		d05	0.0 ± 0.0 **c**		0.0 ± 0.0 **c**	
	Lentil	Control	92.0 ± 3.0 **a**	**	72.0 ± 3.1 **a**	***
		d10	70.7 ± 13.8 **b**		38.7 ± 4.7 **b**	
		d05	61.3 ± 15.2 **b**		26.8 ± 5.1 **b**	
	Quinoa	Control	54.7 ± 13.7 **a**	*	11.6 ± 2.4 **ab**	*
		d10	36.0 ± 7.6 **b**		19.6 ± 3.9 **a**	
		d05	6.7 ± 6.7 **c**		8.5 ± 7.8 **b**	
	Soybean	Control	76.0 ± 10.1	n.s.	35.2 ± 12.0 **a**	*
		d10	72.0 ± 14.5		26.8 ± 9.9 **ab**	
		d05	64.0 ± 13.8		16.8 ± 3.1 **b**	
Model species	Garden cress	Control	97.3 ± 3.7 **a**	***	79.1 ± 24.0 **a**	***
		d10	84.0 ± 10.1 **b**		12.4 ± 1.7 **b**	
		d05	0.0 ± 0.0 **c**		0.0 ± 0.0 **c**	
	Tobacco	Control	74.7 ± 7.3 **a**	***	-	
		d10	37.3 ± 23.9 **b**		-	
		d05	0.0 ± 0.0 **c**		-	

Table 4.7. Mean values with corresponding standard deviations of Relative Seed Germination (RSG), Relative Root Growth (RRG) and Germination Index (GI), measured during the experimentation. Statistical differences are denoted by different letters, otherwise not specified.

Category	Species	Treatment	RSG %		RRG %		GI %	
Weed	*A retroflexus*	Control	100.0 ± 39.9 **a**	***	100.0 ± 28.0 **a**	***	104.7 ± 51.0 **a**	***
		d10	23.1 ± 26.3 **b**		24.5 ± 26.5 **b**		8.0 ± 7.7 **b**	
		d05	0.0 ± 0.0 **c**		0.0 ± 0.0 **c**		0.0 ± 0.0 **c**	
	C. dactylon	Control	100.0 ± 3.2	n.s.	100.0 ± 11.5 **a**	***	100.3 ± 14.1 **a**	***
		d10	85.5 ± 10.7		55.0 ± 4.9 **b**		46.7 ± 4.4 **b**	
		d05	84.1 ± 18.9		32.9 ± 11.6 **c**		27.5 ± 9.2 **c**	
	E. crus-galli	Control	100.0 ± 6.4	n.s.	100.0 ± 8.2 **a**	***	100.1 ± 11.0 **a**	***
		d10	87.3 ± 13.8		61.8 ± 18.5 **b**		55.1 ± 23.3 **b**	
		d05	58.2 ± 42.4		31.4 ± 18.5 **c**		23.6 ± 18.9 **c**	
	S. italica	Control	100.0 ± 56.4 **a**	*	100.0 ± 28.7	n.s.	97.1 ± 57.5 **a**	*
		d10	52.9 ± 52.6 **ab**		88.4 ± 54.5		57.0 ± 52.6 **ab**	
		d05	17.6 ± 12.3 **b**		70.8 ± 43.6		15.9 ± 12.7 **b**	
Crop	Barley	Control	100.0 ± 9.0 **ab**	*	100.0 ± 18.1 **a**	***	100.6 ± 23.8 **a**	***
		d10	116.4 ± 9.0 **a**		26.5 ± 2.5 **b**		30.9 ± 3.8 **b**	
		d05	83.6 ± 22.7 **b**		28.0 ± 6.5 **b**		23.9 ± 10.9 **b**	
	Chickpea	Control	100.0 ± 27.8 **a**	***	100.0 ± 36.3 **a**	***	98.7 ± 40.5 **a**	***
		d10	41.0 ± 36.7 **b**		75.0 ± 61.4 **b**		31.8 ± 26.5 **b**	
		d05	0.0 ± 0.0 **c**		0.0 ± 0.0 **c**		0.0 ± 0.0 **c**	
	Lentil	Control	100.0 ± 3.2 **a**	**	100.0 ± 4.3 **a**	***	100.0 ± 6.1 **a**	***
		d10	76.8 ± 15.0 **b**		53.8 ± 6.5 **b**		41.0 ± 8.2 **b**	
		d05	66.7 ± 16.5 **b**		37.2 ± 7.0 **c**		24.8 ± 8.5 **c**	
	Quinoa	Control	100.0 ± 25.0 **a**	***	100.0 ± 20.7	n.s.	97.2 ± 20.7 **a**	*
		d10	65.9 ± 13.9 **b**		169.3 ± 33.8		110.9 ± 26.4 **a**	
		d05	12.2 ± 12.2 **c**		73.3 ± 66.9		14.9 ± 15.0 **b**	
	Soybean	Control	100.0 ± 13.3	n.s.	100.0 ± 34.2 **a**	*	97.9 ± 26.7 **a**	*
		d10	94.7 ± 19.0		76.3 ± 28.0 **ab**		75.9 ± 41.6 **ab**	
		d05	84.2 ± 18.2		47.7 ± 8.9 **b**		40.9 ± 13.9 **b**	
Model species	Garden cress	Control	100.0 ± 3.8 **a**	***	100.0 ± 30.3 **a**	***	100.2 ± 31.9 **a**	***
		d10	86.3 ± 10.4 **b**		15.7 ± 2.1 **b**		13.6 ± 2.6 **b**	
		d05	0.0 ± 0.0 **c**		0.0 ± 0.0 **c**		0.0 ± 0.0 **c**	
	Tobacco	Control	100.0 ± 9.8 **a**	***	-		-	
		d10	50.0 ± 31.9 **b**		-		-	
		d05	0.0 ± 0.0 **c**		-		-	

3.2 Greenhouse experimentation results

Greenhouse temperature during the growing season was generally high (Figure 4.5). Mean temperature was 26.5°C and maximum temperature reached 53.5°C. Out of the total measured data, the temperature was higher than 25°C for 49.2% of the time, higher than 30°C for 30%, and higher than 40°C for 10.3% of the time (approximatively two and a half hours per day). Even if periodically and abundantly watered, plants did probably suffer from heat stress and buckwheat and *A. retroflexus* were the only species which did flowering.

Measurements were conducted according to buckwheat phenology, at fifth full leaf development when growing degree (GDDs) days reached 541°C d^{-1} (Base temperature = 0°C), full buckwheat bloom at 1139°C d^{-1} and seeding onset at 1713°C d^{-1}.

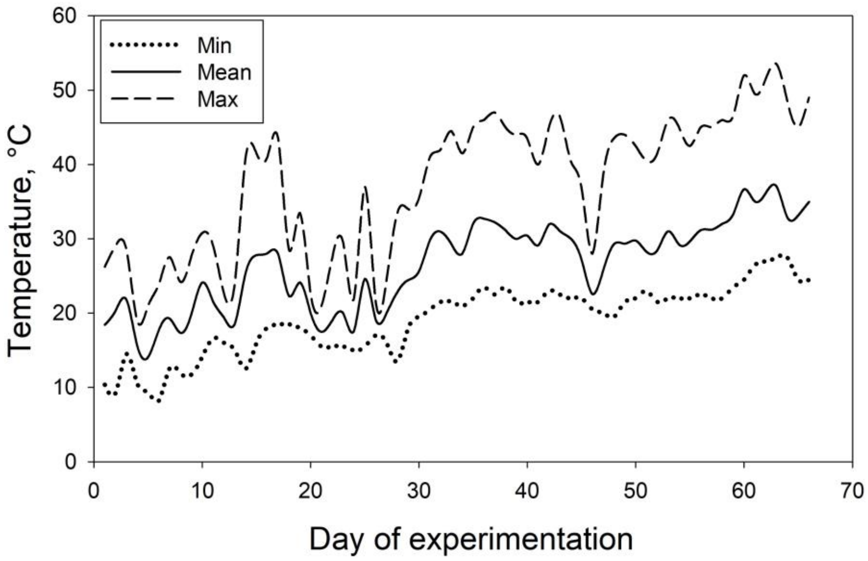

Figure 4.5. Minimum, mean and maximum temperature measured in the greenhouse during the experiment.

Plants heights at different measurements are reported in Table 4.8. Significant differences in plant height (H_P) were assessed at first sampling in chickpea and buckwheat, however plants were so small that such differences might be neglected. At second sampling, differences were assessed in chickpea and *A. theophrasti*. In chickpea, plants in monoculture (MC) were approximatively 47% higher than in intercropping (IC), while in *A. hteophrasti*, plants in MC were 35% higher than in IC. At the last sampling, differences in chickpea were no more assessed. Final H_P of lentil in IC was statistically lower than in MC and *A. theophrasti* was the only species where plants in staircase device (SD) treatment were less developed than the others. However, this result might be due to the effect of the shading net where plants of SD were placed.

Table 4.8. Plants height measured in buckwheat (BW), chickpea (CH), lentil (LN), *A. retroflexus* (AR) and *A. theophrasti* (AT) under three different treatments: monoculture (MC), intercropping (IC) and staircase device (SD). Statistical differences assessed by Tukey's *post hoc* test are specified by letters. Significance: *: p < 0.05; **: p < 0.01; ***: p < 0.001; n.s.: not significant.

Species	**Treatment**	**Plant height, cm**					
		16/05/23		**06/06/23**		**26/06/23**	
CH	MC	22.5 ± 2.0	**	54.7 ± 6.1	***	60.4 ± 5.3	n.s.
	IC	19.0 ± 2.2		37.0 ± 3.8		47.4 ± 14.5	
	SD					56.1 ± 6.3	
LN	MC	20.1 ± 0.8	n.s.	36.5 ± 5.7	n.s.	44.3 ± 3.2 **a**	***
	IC	19.1 ± 2.5		29.5 ± 4.9		29.8 ± 3.2 **b**	
	SD					42.0 ± 1.8 **a**	
AR	MC	4.7 ± 0.7	n.s.	34.5 ± 7.6	n.s.	52.6 ± 7.5	n.s.
	IC	5.9 ± 1.1		27.3 ± 1.8		43.0 ± 15.6	
	SD					44.6 ± 6.8	
AT	MC	6.8 ± 1.1	n.s.	41.6 ± 4.6	**	60.6 ± 3.4 **a**	***
	IC	8.0 ± 1.3		30.9 ± 6.9		57.4 ± 2.7 **a**	
	SD					47.3 ± 3.4 **b**	
BW	MC	22.9 ± 3.9 **a**	**	62.1 ± 5.3	n.s.	84.2 ± 14.1	n.s.
	IC with CH	25.4 ± 3.6 **a**		66.4 ± 4.9		95.3 ± 6.4	
	IC with AR	33.6 ± 1.9 **b**		66.1 ± 7.0		91.3 ± 20.9	
	IC with LN	25.8 ± 1.3 **a**		64.3 ± 3.5		95.9 ± 6.6	
	IC with AT	24.8 ± 5.5 **a**		67.3 ± 5.7		83.6 ± 9.8	
	SD					87.4 ± 20.4	

Measured root lengths are reported in Table 4.9. Differences were assessed only at last measurement in chickpea and buckwheat. In chickpea, plants of SD treatment had significantly deeper roots, 17.0 ± 3.0cm in SD versus 10.9 ± 0.8cm in monoculture. This result may be due to the predominantly heliophilous nature of chickpea, being forced to grow in a shaded environment. In this condition the plant interprets reduced light as a signal that it is in competition with neighboring plants for access to limited light resource.

Table 4.9. Root length measured in buckwheat (BW), chickpea (CH), lentil (LN), *A. retroflexus* (AR) and *A. theophrasti* (AT) under three different treatments: monoculture (MC), intercropping (IC) and staircase device (SD). Statistical differences assessed by Tukey's *post hoc* test are specified by letters. Significance: *: p < 0.05; **: p < 0.01; ***: p < 0.001; n.s.: not significant.

Species	**Treatment**	**Root length, cm**					
		16/05/23		**06/06/23**		**26/06/23**	
CH	MC	11.1 ± 1.5	n.s.	16.1 ± 0.3	n.s.	10.9 ± 0.8 **a**	**
	IC	10.0 ± 1.8		15.3 ± 3.2		10.1 ± 1.5 **a**	
	SD					17.0 ± 3.0 **b**	
LN	MC	11.3 ± 0.7	n.s.	12.3 ± 1.2	n.s.	16.2 ± 2.2	n.s.
	IC	11.1 ± 2.4		10.2 ± 1.1		13.3 ± 0.7	
	SD					15.7 ± 1.9	
AR	MC	5.8 ± 0.7	n.s.	14.9 ± 0.7	n.s.	19.6 ± 3.6	n.s.
	IC	6.3 ± 0.6		12.8 ± 1.1		17.6 ± 2.8	
	SD					19.8 ± 3.8	
AT	MC	8.6 ± 1.0	n.s.	13.3 ± 1.6	n.s.	17.2 ± 2.1	n.s.
	IC	7.8 ± 0.6		13.0 ± 2.2		17.2 ± 3.2	
	SD					17.4 ± 2.8	
BW	MC	7.3 ± 0.3	n.s.	10.9 ± 0.8	n.s.	9.0 ± 0.6 **a**	*
	IC with CH	9.3 ± 0.6		10.5 ± 4.4		8.6 ± 2.2 **a**	
	IC with AR	8.4 ± 2.1		10.2 ± 0.7		14.8 ± 2.2 **b**	
	IC with LN	9.6 ± 1.4		10.4 ± 0.4		12.2 ± 2.3 **ab**	
	IC with AT	8.3 ± 0.6		11.7 ± 2.4		13.2 ± 4.1 **ab**	
	SD					12.4 ± 1.8 **ab**	

Measured values of aboveground dry biomass (DW_{ABV}) are listed in Table 4.10. At first sampling, statistical differences occurred only among buckwheat treatments, as DW_{ABV} of buckwheat in MC was significantly lower than in IC, however this condition did not occur in the following samplings. In all other species, DW_{ABV} in MC was significantly higher than in IC, with the exception of *A. retroflexus*, when difference was not statistically significant on 26/06/23. DW_{ABV} of plants under SD treatment were lower in chickpea and *A. theophrasti*. However, as already noticed, this result may be due to the shading of plants under SD treatment.

Table 4.10. Aboveground dry biomass measured in buckwheat (BW), chickpea (CH), lentil (LN), *A. retroflexus* (AR) and *A. theophrasti* (AT) under three different treatments: monoculture (MC), intercropping (IC) and staircase device (SD). Statistical differences assessed by Tukey's *post hoc* test are specified by letters. Significance: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; n.s.: not significant.

Species	**Treatment**	**Aboveground dry biomass, g plant^{-1}**					
		16/05/23		**06/06/23**		**26/06/23**	
CH	MC	0.19 ± 0.04	n.s.	1.4 ± 0.4	***	3.3 ± 0.8 **a**	*
	IC	0.14 ± 0.05		0.8 ± 0.1		2.0 ± 0.4 **b**	
	SD					2.0 ± 0.5 **b**	
LN	MC	0.08 ± 0.01	n.s.	0.5 ± 0.1	***	1.4 ± 0.3 **a**	***
	IC	0.07 ± 0.02		0.2 ± 0.0		0.4 ± 0.0 **b**	
	SD					1.1 ± 0.1 **a**	
AR	MC	0.02 ± 0.01	n.s.	1.3 ± 0.3	**	4.0 ± 1.5	n.s.
	IC	0.04 ± 0.02		0.6 ± 0.0		2.5 ± 1.1	
	SD					3.4 ± 0.7	
AT	MC	0.07 ± 0.02	n.s.	1.0 ± 0.1	*	4.5 ± 0.5 **a**	***
	IC	0.06 ± 0.02		0.5 ± 0.2		3.9 ± 0.2 **a**	
	SD					2.8 ± 0.3 **b**	
BW	MC	0.18 ± 0.08 **a**	***	1.3 ± 0.3	n.s.	2.7 ± 0.7	n.s.
	IC with CH	0.35 ± 0.08 **b**		2.2 ± 0.4		3.9 ± 0.7	
	IC with AR	0.54 ± 0.06 **b**		2.0 ± 0.4		3.3 ± 0.6	
	IC with LN	0.29 ± 0.08 **b**		1.9 ± 0.5		3.8 ± 0.5	
	IC with AT	0.30 ± 0.09 **b**		2.0 ± 0.5		2.6 ± 0.5	
	SD					2.5 ± 0.4	

Measured values of root dry biomass (DW_R) are reported in Table 4.11. Values measured in buckwheat on 06/06/23 were omitted because of anomalous values, probably due to issues in roots cleaning with consequent bias of soil still attached to roots. Statistical differences on 06/06/23 were assessed in *A. retroflexus* only, however these did not occur at the following sampling. At the last sampling, under IC treatment DW_R was significantly lower in lentil, while for *A. theophrasti* there was a reduction in the SD treatment.

Table 4.11. Root dry biomass measured in buckwheat (BW), chickpea (CH), lentil (LN), *A. retroflexus* (AR) and *A. theophrasti* (AT) under three different treatments: monoculture (MC), intercropping (IC) and staircase device (SD). Statistical differences assessed by Tukey’s *post hoc* test are specified by letters. Significance: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; n.s.: not significant.

Species	**Treatment**	**Root dry biomass, g plant^{-1}**					
		16/05/23		**06/06/23**		**26/06/23**	
CH	MC	0.13 ± 0.05	n.s.	0.24 ± 0.09	n.s.	0.27 ± 0.02	n.s.
	IC	0.18 ± 0.08		0.15 ± 0.06		0.21 ± 0.03	
	SD					0.24 ± 0.03	
LN	MC	0.04 ± 0.01	n.s.	0.07 ± 0.02	n.s.	0.11 ± 0.03 **a**	**
	IC	0.03 ± 0.01		0.05 ± 0.00		0.05 ± 0.01 **b**	
	SD					0.11 ± 0.01 **a**	
AR	MC	0.02 ± 0.01	n.s.	0.32 ± 0.12	**	0.74 ± 0.14	n.s.
	IC	0.01 ± 0.01		0.17 ± 0.05		0.68 ± 0.24	
	SD					0.61 ± 0.16	
AT	MC	0.02 ± 0.01	n.s.	0.15 ± 0.04	n.s.	0.53 ± 0.05 **a**	*
	IC	0.02 ± 0.01		0.18 ± 0.04		0.49 ± 0.09 **ab**	
	SD					0.36 ± 0.06 **b**	
BW	MC	0.05 ± 0.02	n.s.	-		0.27 ± 0.07	n.s.
	IC with CH	0.08 ± 0.02		-		0.30 ± 0.07	
	IC with AR	0.16 ± 0.15		-		0.24 ± 0.02	
	IC with LN	0.06 ± 0.01		-		0.33 ± 0.01	
	IC with AT	0.09 ± 0.04		-		0.27 ± 0.04	
	SD					0.24 ± 0.03	

Competitive Balance Index (CBI) calculated from DW_{ABV} of various species in IC, is graphically represented in Figure 4.6. Among all the growing season, highest CBI values were measured in buckwheat, which acted like the most competitive species (CBI > 0). This result is interesting if compared to previous data. In fact, buckwheat was the species with generally the lowest values of L_R and DW_{ABV}. Moreover, due to heat stress, buckwheat plants tended to be prostrate, so we exclude a competitive effect due to shading of the companion species.

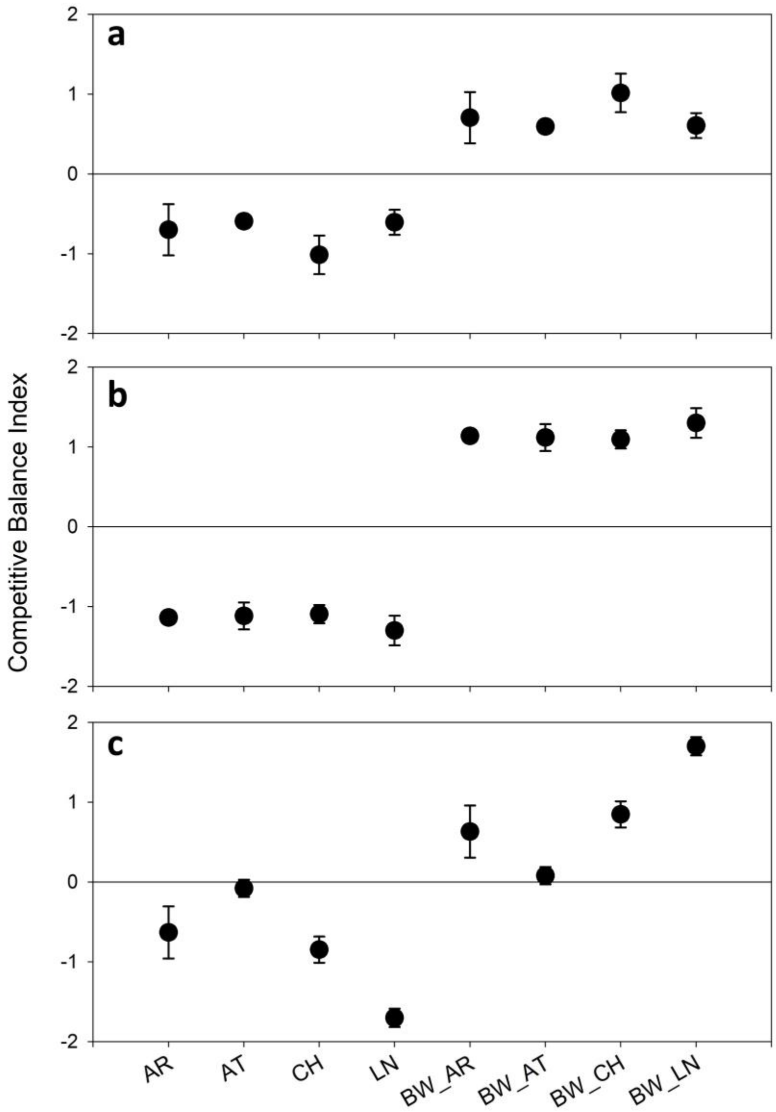

Figure 4.6. Competitive Balance Index of *A. retroflexus* (AR), *A. theophrasti* (AT), chickpea (CH) and lentil (LN) calculated from aboveground dry biomass measured on **a)** 16/05/2023; **b)** 06/06/2023; **c)** 26/06/2023.

Calculated growth rate (GR) values are reported in Table 4.12. Since H_P in SD treatment was measured only at last sampling, it was not possible to include that treatment. Furthermore, the calculation of GR was assessed on mean data for each treatment as plants underwent into destructive samplings, no confidence interval or statistic was assessed.

In general, it is possible to observe the four tested species, GR values are lower in IC than in MC. This is particularly evident in the second considered period (GR_{T1-T2}), where also CBI values were the lowest.

Table 4.12. Growth rate (GR) from sowing to 16/05/23 (GR_{T0-T1}), from 16/05/23 to 06/06/23 (GR_{T1-T2}) and from 06/06/23 to 26/06/23 (GR_{T2-T3}) calculated from measured plant heights of buckwheat (BW), chickpea (CH), lentil (LN), *A. retroflexus* (AR) and *A. theophrasti* (AT) under monoculture (MC) and intercropping (IC) treatments.

Species	**Treatment**	**GR_{T0-T1}, cm d^{-1}**	**GR_{T1-T2}, cm d^{-1}**	**GR_{T2-T3}, cm d^{-1}**
CH	MC	1.25	1.53	0.28
	IC	1.06	0.86	0.52
LN	MC	1.12	0.78	0.39
	IC	1.06	0.49	0.02
AR	MC	0.26	1.42	0.91
	IC	0.33	1.02	0.79
AT	MC	0.38	1.66	0.95
	IC	0.44	1.09	1.33
BW	MC	1.27	1.87	1.10
	IC with CH	1.87	1.55	1.26
	IC with AR	1.38	2.02	0.82
	IC with LN	1.41	1.96	1.44
	IC with AT	1.43	1.83	1.58

3.3 Field trials results

Measured plant densities are reported in Table 4.13. No crop reached the target plant density (PD) (see Table 4.2) and despite the sowing density of seeds was equal, germination was higher in chickpea and lentil in monoculture. Worst germination occurred in buckwheat, where we recorded a plant density of approximatively 25% of the target one. Moreover, in chickpea under treatment within-row (WR) at 50%, plant densituy of buckwheat was lower than at 25%.

Table 4.13. Plant density of chickpea (CH), lentil (LN) and buckwheat (BW) measured at flowering.

Experiment	Treatment / Intercrop	ID	PD of legume # m^{-2}	PD of buckwheat # m^{-2}
Chickpea	Control/Monoculture	CH	35.4 ± 4.2	-
	Alternate rows	$CHBW_{AR}$	29.4 ± 7.8	24.9 ± 7.7
	Within-row at 25%	$CHBW_{25\%}$	31.5 ± 5.9	31.5 ± 5.0
	Within-row at 50%	$CHBW_{50\%}$	29.4 ± 6.7	24.9 ± 7.4
	BW Control/Monoculture	BW	-	50.4 ± 11.2
Lentil	Control/Monoculture	LN	91.4 ± 14.5	-
	Alternate rows	$LNBW_{AR}$	77.7 ± 12.1	28.7 ± 6.13
	Within-row at 25%	$LNBW_{25\%}$	75.6 ± 9.6	19.3 ± 3.8
	BW Control/Monoculture	BW	-	59.5 ± 7.5

A total of 20 weeds were identified during the vegetation samplings. The five most common weeds and their percentage of occurrence are reported in Table 4.14, while percentage of soil cover of the two most common ones (*Chenopodium album* and *Galinsoga parviflora*) among treatments are listed in Table 4.15. Significant differences were assessed for *C. album* only in lentil experiments in the post-weeding assessment, where abundance was higher in buckwheat in monoculture. A similar result occurred for *G. parviflora*, which abundance was significantly higher in buckwheat in monoculture in the chickpea experiment.

Table 4.14. Percentage of occurrence of the most prevalent weeds in plots of chickpea (CH) and lentil (LN) experimental areas.

Species	Percentage of occurrence			
	Before hoeing		After hoeing	
	CH experiment	LN experiment	CH experiment	LN experiment
Chenopodium album	100%	100%	92.5%	100%
Galinsoga parviflora	97.5%	93.7%	100%	90.6%
Stellaria media	80%	59.4%	652.5%	56.2%
Lamium purpureum	80%	71.8%	50%	40.6%
Viola arvensis	75%	40.6%	67.5%	56.2%

Table 4.15. Mean values with standard deviation of estimated percentage of soil cover of *Chenopodium album* and *Galinsoga parviflora* among different treatments of chickpea (CH) and lentil (LN) experiments. Significance: *: p < 0.05; **: p < 0.01; ***: p < 0.001; n.s.: not significant.

Experiment	**Treatment**	**Percentage of soil cover, %**							
		Chenopodium album				*Galinsoga parviflora*			
		Before Hoeing	Sign	After Hoeing	Sign	Before Hoeing	Sign	After Hoeing	Sign
CH	CH	1.7 ± 0.6	n.s.	3.9 ± 3.7	n.s.	1.9 ± 0.8	n.s.	10.6 ± 10.9 **a**	**
	$CHBW_{AR}$ *	3.7 ± 3.6		20.8 ± 24.3		1.7 ± 1.6		13.3 ± 23.3 **a**	
	$CHBW_{50\%}$	3.4 ± 3.1		7.50± 6.9		1.6 ± 1.1		3.8 ± 2.5 **a**	
	$CHBW_{25\%}$	2.7 ± 1.4		4.4 ± 2.8		1.5 ± 1.0		5.3 ± 6.0 **a**	
	BW *	2.1 ± 0.7		11.6 ± 13.0		1.8 ± 1.2		36.0 ± 29.3 **b**	
LN	LN	13.6 ± 8.9	n.s.	14.1 ± 10.7 **a**	**	3.4 ± 10.4	n.s.	2.8 ± 9.0	n.s.
	$LNBW_{AR}$ *	5.4 ± 9.0		11.6 ± 8.5 **a**		2.5 ± 3.3		10.6 ± 12.2	
	$LNBW_{25\%}$	6.4 ± 4.1		12.5 ± 9.6 **a**		4.5 ± 6.0		6.1 ± 8.5	
	BW *	11.9 ± 6.1		29.9 ± 8.4 **b**		0.7 ± 0.7		10.2 ± 5.6	

***** Treatments where hoeing was not conducted.

Shanno Diversity index (H) of weed values are reported in Table 4.16. Differences were assessed only in chickpea, where diversity was significantly lower in the WR treatment at 50%. After weeding, no difference was more assessed. In lentil no difference was observed both before and after weeding.

Aboveground dry biomass (DW_{ABV}) at flowering is reported in Table 4.17. In chickpea, crop biomass was significantly higher in monoculture (MC) and reached the minimum in $CHBW_{50\%}$, where also lower plant density has also been assessed. Buckwheat biomass in chickpea was higher in MC, and reached the minimum in the alternate-row (AR) layout. Weeds biomass was significantly higher in AR and pure buckwheat, and gradually decreased reaching the minimum in pure chickpea and $CHBW_{50\%}$. In lentil, no statistical difference was assessed except for weed biomass, where DW_{ABV} of weeds was significantly higher in pure buckwheat and minimum values were measured in pure lentil.

Measured plant height values at flowering are reported in Table 4.18. Statistical differences were assessed in chickpea, where plant of the AR treatment were significantly shorter than the other treatments. No statistical difference was assessed in lentil or in buckwheat among all treatments.

Table 4.16. Mean values with standard deviation of Shannon Diversity Index (H) calculated on weed comunitites of different treatments of chickpea (CH) and lentil (LN) experiments. Significance: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; n.s.: not significant.

Experiment	Treatment	Weed H index			
		Before Hoeing	**Sign**	**After Hoeing**	**Sign**
CH	CH	1.45 ± 0.18 **a**	*	1.02 ± 0.39	n.s.
	$CHBW_{AR}$ *	1.21 ± 0.39 **ab**		0.99 ± 0.59	
	$CHBW_{25\%}$	1.20 ± 0.22 **ab**		1.12 ± 0.43	
	$CHBW_{50\%}$	0.93 ± 0.36 **b**		0.94 ± 0.30	
	BW *	1.20 ± 0.31 **ab**		0.88 ± 0.41	
LN	LN	0.60 ± 0.27	n.s.	0.72 ± 0.34	n.s.
	$LNBW_{AR}$	1.02 ± 0.42		0.99 ± 0.21	
	$LNBW_{25\%}$	1.02 ± 0.34		0.90 ± 0.35	
	BW	0.70 ± 0.44		1.03 ± 0.20	

***** Treatments where hoeing was not conducted.

Table 4.17. Mean values with standard deviation of aboveground dry biomass (DW_{ABV}) of crop (chickpea or lentil), buckwheat (BW) and weeds, measured at flowering in different treatments of chickpea (CH) and lentil (LN) experiments. Significance: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; n.s.: not significant.

Experiment	Treatment	Crop DW_{ABV} g m^{-2}	Sign	BW DW_{ABV} g m^{-2}	Sign	Weeds DW_{ABV} g m^{-2}	Sign
CH	CH	50.8 ± 2.3 **a**	*	-	*	27.0 ± 7.4 **bc**	**
	$CHBW_{AR}$	34.6 ± 15.5 **ab**		10.2 ± 3.6 **b**		56.7 ± 19.1 **a**	
	$CHBW_{25\%}$	34.0 ± 10.7 **ab**		17.8 ± 10.4 **ab**		31.6 ± 11.1 **bc**	
	$CHBW_{50\%}$	27.5 ± 5.6 **b**		30.3 ± 11.0 **ab**		23.8 ± 7.6 **c**	
	BW	-		35.4 ± 12.3 **a**		50.2 ± 11.7 **ab**	
LN	LN	113.6 ± 11.2	n.s.	-	n.s.	21.9 ± 2.9 **b**	***
	$LNBW_{AR}$	87.7 ± 15.4		13.7 ± 7.7		35.9 ± 6.02 **b**	
	$LNBW_{25\%}$	62.9 ± 44.2		13.5 ± 7.5		26.9 ± 14.1 **b**	
	BW	-		21.0 ± 0.3		68.5 ± 17.5 **a**	

Table 4.18. Mean values with standard deviation of plant height measured at flowering in different treatments of chickpea (CH) and lentil (LN) experiments. Significance: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; n.s.: not significant.

Experiment	Treatment	Crop plant height, cm	Sign	BW plant height, cm	Sign
CH	CH	50.4 ± 4.3 **a**	***	-	n.s.
	$CHBW_{AR}$	42.4 ± 4.8 **b**		53.6 ± 8.8	
	$CHBW_{25\%}$	47.0 ± 5.5 **a**		54.3 ± 7.7	
	$CHBW_{50\%}$	50.7 ± 4.2 **a**		56.8 ± 5.1	
	BW	-		57.4 ± 7.1	
LN	LN	39.4 ± 2.4	n.s.	-	n.s.
	$LNBW_{AR}$	40.7 ± 4.0		54.5 ± 8.4	
	$LNBW_{25\%}$	40.1 ± 2.6		53.7 ± 7.0	
	BW	-		52.0 ± 6.4	

Aboveground dry biomass measured at harvest is graphically represented in Figures 4.7-4.8. In the chickpea experiment (Figure 4.6), no difference was assessed among DW_{ABV} of both chickpea and buckwheat in different treatments. Although, Weeds DW_{ABV} was significantly higher in pure buckwheat plots. The same trend was observed in lentil. In fact, no difference was assessed among crop biomass in different treatments, however, weeds DW_{ABV} was significantly higher in pure buckwheat. About buckwheat biomass, it was higher in pure buckwheat, where also plant density was higher (see Table 4.13).

Measured values of yield are represented in Figures 4.9-4.10. In chickpea, lower legume was observed in the AR layout, with 0.44 ± 0.12ton ha^{-1}, significantly lower from the other treatments where yield reached a maximum of 1.24 ± 0.18ton ha^{-1} in pure crop. Buckwheat yield was also lower in $CHBW_{AR}$ and $CHBW_{25\%}$, however, values were not significantly different from buckwheat in monoculture. In lentil, no difference was assessed in terms of legume yield among different treatments. Measured values were generally high, with a minimum of 2.05 ± 0.18ton ha^{-1} in $LNBW_{AR}$ and a maximum of 2.40 ± 0.22ton ha^{-1} as pure strains.

Measured yield generally followed the same trend of DW_{ABV}. Hence, we did not observe any difference for any crop in terms of Harvest Index (Figures 4.11-4.12). HI values were approximatively 0.40 in chickpea and buckwheat, and 0.50 in lentil.

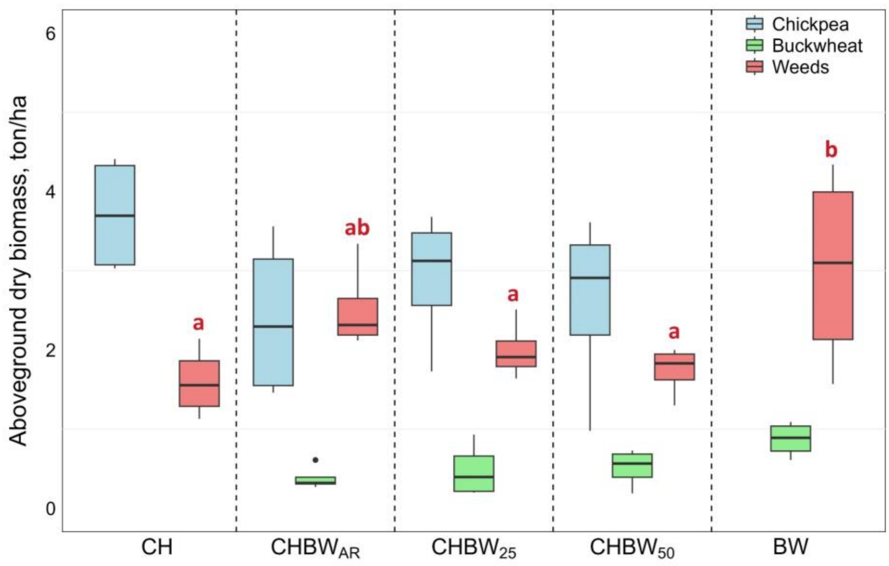

Figure 4.7. Aboveground dry biomass of chickpea (blue boxes and letters), buckwheat (green boxes and letters) and weeds (red boxes and letters), measured at harvest in treatments of the chickpea experiment. Statistical differences are denoted by different letters.

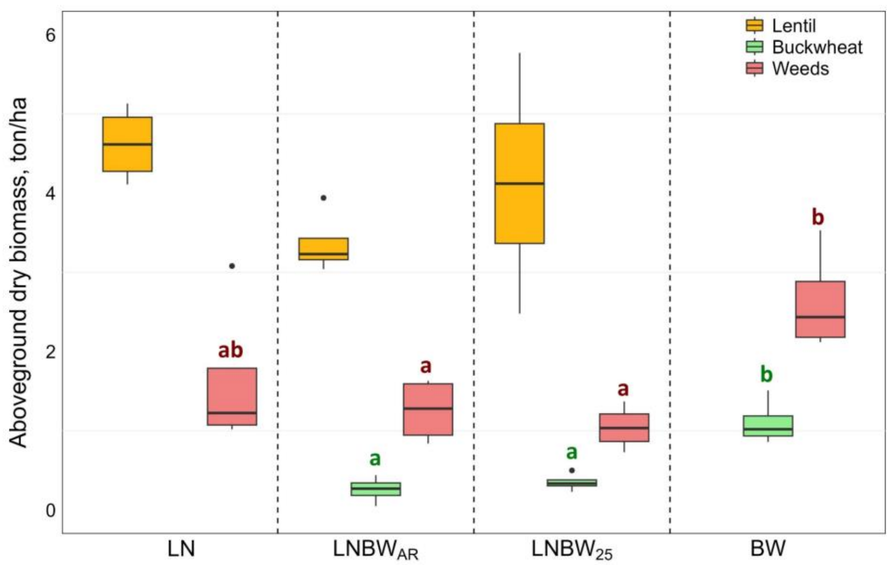

Figure 4.8. Aboveground dry biomass of lentil (yellow boxes and letters), buckwheat (green boxes and letters) and weeds (red boxes and letters), measured at harvest in treatments of the lentil experiment. Statistical differences are denoted by different letters.

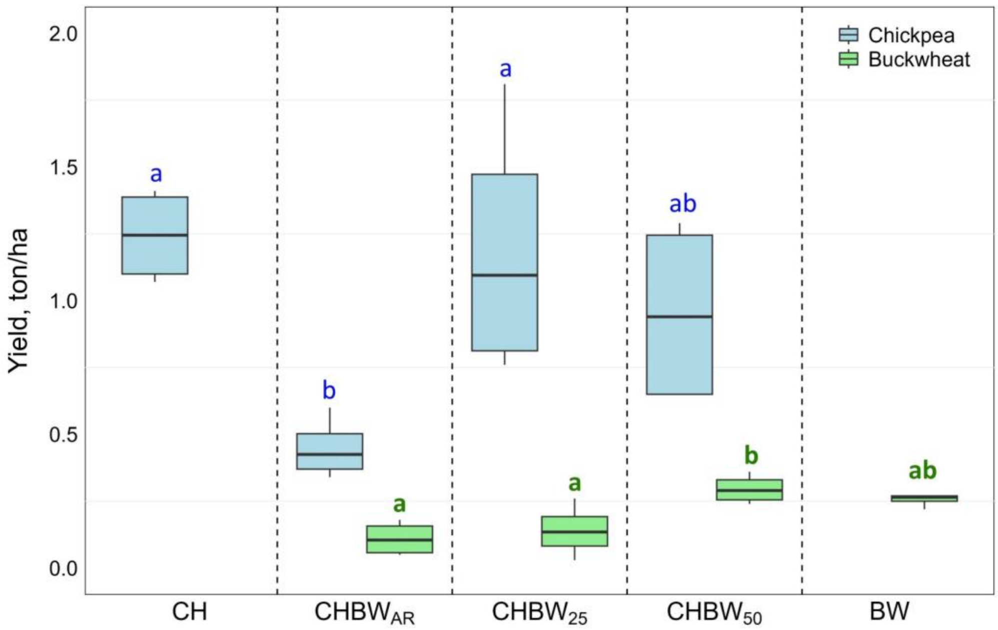

Figure 4.9. Yield of chickpea (blue boxes and letters) and buckwheat (green boxes and letters) measured at harvest in treatments of the chickpea experiment. Statistical differences are denoted by different letters.

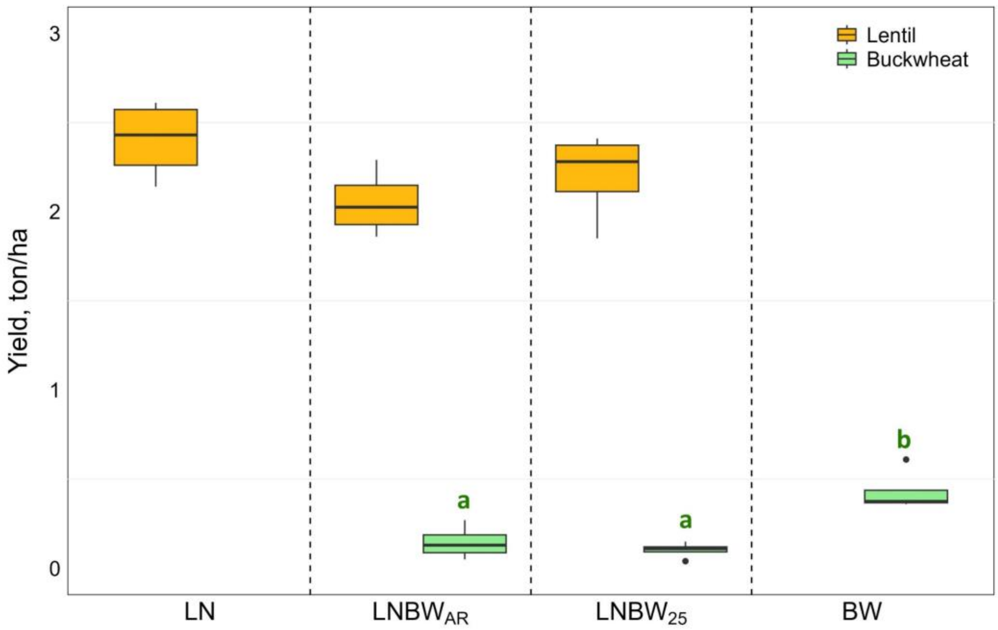

Figure 4.10. Yield of lentil (yellow boxes and letters) and buckwheat (green boxes and letters) measured at harvest in treatments of the lentil experiment. Statistical differences are denoted by different letters.

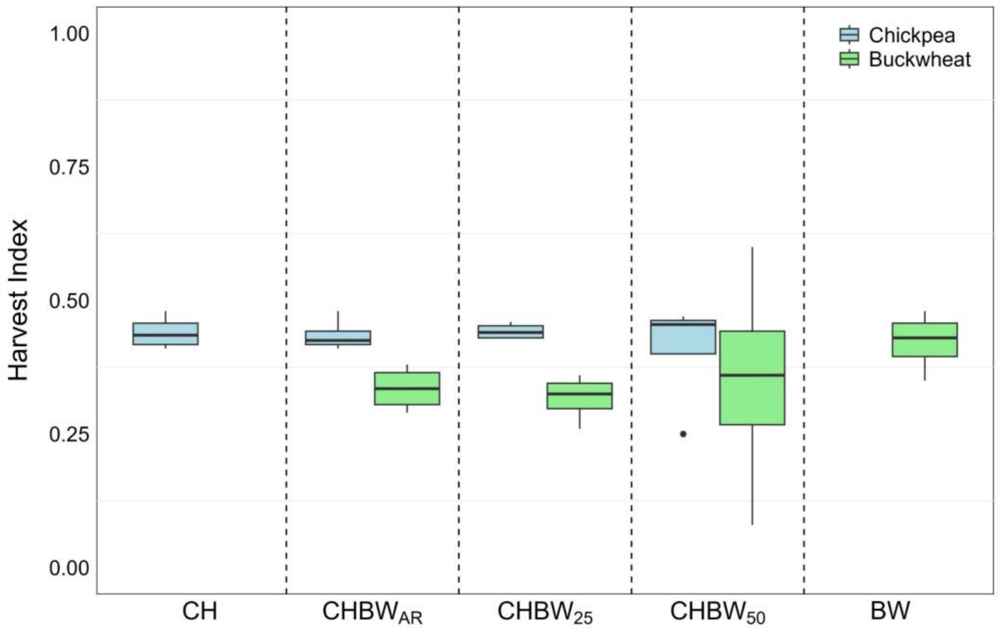

Figure 4.11. Harvest Index of chickpea (blue boxes and letters) and buckwheat (green boxes and letters) measured at harvest in treatments of the chickpea experiment. No statistical difference was assessed.

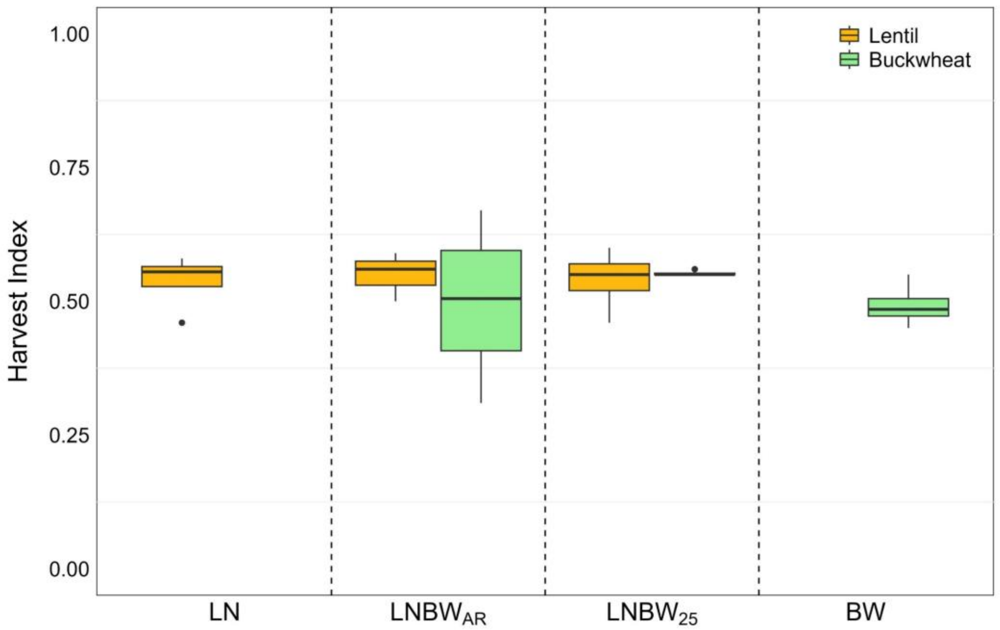

Figure 4.12. Harvest Index of lentil (yellow boxes and letters) and buckwheat (green boxes and letters) measured at harvest in treatments of the lentil experiment. No statistical difference was assessed.

3.4. Allelochemical analysis

Measured values of Rutin and Quercetin are listed in Table 4.19. Both allelochemicals were quantified in grinded buckwheat and in the water extract prepared for the in-vitro experimentation (d05), while Rutin was not detected in leached water from the greenhouse experimentation. Extraction of allelochemicals from water resulted extremely inefficient for dissolving allelochemicals into solution. However, in terms of concentration per volume of solution, Quercetin values were mostly similar, while Rutin in water was approximatively 20% compared to the quantified amount of the grinded buckwheat.

Table 4.19. Mean values with corresponding standard deviations of Rutin and Quercetin quantified by HPLC analysis. Values are reported both as a concentration in the analyzed solution (mg $^{l-}$1) and referred to the weight of buckwheat (BW) used for the extraction (mg g^{-1}).

Sample	**Rutin**		**Quercetin**	
	mg l^{-1}	mg g^{-1}	mg l^{-1}	mg g^{-1}
Water extract 1:5	6.15 ± 0.12	0.03 ± 0.001	1.35 ± 0.04	0.007 ± 0.0002
Grinded BW (extracted in CH3OH)	30.11 ± 1.56	1. 78 ± 0.02	1.45 ± 0.03	0.09 ± 0.002
Leached water	0		1.05 ± 0.07	

4 Discussion

4.1. Effect of aqueous buckwheat extract on seed germination.

In this trial, we tested the effect of buckwheat water extracts on the germination of seeds of crops, weeds, and model species. We tested water extracts at two different concentrations: i) d10 – more diluted (buckwheat and water at 1:10 weight/volume ratio); ii) d05 – more concentrated (buckwheat and water at 1:5 weight/volume ratio). A consistent source of bias in this experiment may attributed to molds, which occurred even though the petri dishes were sterile, and the seeds were sterilized before incubation. The cause of contamination is probably due to the buckwheat extract, which was not possible to sterilize. However, this problem primarily affected few species, and was notably in tobacco, where it was not possible to measure the root length.

Through HPLC analyses, we quantified the actual presence and abundance of two of the main allelochemicals in buckwheat, Quercetin and Rutin. The measured values for both compounds are extremely low, approximately 50 times lower than the values reported in the literature (Table 4.1). However, buckwheat extracts had a generally remarkable effect on all tested species, both in terms of seed germination and root elongation, resulting in significant differences in estimated germination index. Since the extract dose per Petri dish was the same for every species, we hypothesized that this effect might be due to the size of the seed, which determines its susceptibility, Although, neither Germination Index at d10 and d05 were related to the thousand seed weight (TSW) and for example *C. dactylon* (TSW of 0.68g) was the second less susceptible species, while in chickpea (TSW of 467g) at d05 no one seed germinated.

Species response was quite different between the d10 (more diluted) and d05 (more concentrated) treatments, both in terms of germination and root length. For example, among weeds, seed germination was not strongly reduced in *C. dactylon* and *E. crus-galli* both in d10 and d05, while root length was approximatively halved compared to the control. On the other hand, in *S. italica*, the Relative Seed Germination was significantly affected while in the d05 treatment the Relative Root Growth was approximatively 70%. However, in general, all species were heavily affected by buckwheat extracts. Excluding quinoa values at d10, for which root length values are anomalous, the less suppressed species was soybean with a GI of 75.9 at d10 and 40.9% at d05. At d10, the three most resistant species after soybean where weeds, *S. italica* (GI 57.0%), *E. crus-galli* (GI 55.1%) and *C. dactylon* (GI 46.7%). At d05 there was not such trend and all species resulted heavily affected by the extract.

Up to our knowledge, this is the first study investigating this methodology for buckwheat. Nevertheless, it has been demonstrated the efficacy of buckwheat in suppressing multiple species. Evidence has been widely provided for weeds (Golisz et al. 2007, Kumar et al. 2009, Falquet et al. 2015, Wirth and Gfeller 2016), however knowledge is still limited for crops (Kalinova et al. 2005, 2007), especially major crops (Cheriere et al. 2020). This study represents a first assessment of this aspect, more investigations are required, with the possibility of also including reference toxic chemicals generally used in germination tests (*e.g.* Sodium Chloride, Copper Sulfate, Potassium Dichromate) to compare buckwheat extracts toxicity with a standard reference

4.2 Intercropping with buckwheat in controlled conditions

In this trial we set up a pot experiment in a controlled environment (greenhouse) to evaluate the allelopathic effect of buckwheat, consociated with two weeds: *A. retroflexus* and *A. theophrasti* and two grain legumes: chickpea and lentil. To distinguish between competition and allelopathy, we also tested each species with a staircase device experimental design, where plants were grown in monoculture and irrigated with leached water from pots containing pure buckwheat. This methodology has been reputed one of the most solid in distinguishing the effect of allelopathy from competition (Mahé et al. 2022).

Through HPLC analysis, we determined that there was no Rutin in the leached water used for irrigating the pots in the staircase device treatment. However, the Quercetin concentration is comparable to the one measured in the water extract d05 used for the in-vitro experimentation.

A significant reduction in aboveground dry biomass was observed in chickpea and lentil under intercrop treatment, and in chickpea and *A. theophrasti* under staircase device treatment. While we cannot exclude the possibility of shading effects in chickpea irrigated with leached water from buckwheat, the lack of differences in plant height in chickpea suggests that this issue may be negligible and implies a potential inhibition effect on this crop due to allelopathy. Furthermore, based on the results of in-vitro experiments, chickpea appears to be one of the more sensitive crops to buckwheat allelochemicals. The same conclusions may not apply to the results for *A. theophrasti*. In fact, in the staircase device treatment, it exhibited a significant reduction in aboveground dry biomass, plant height and root length, a phenomenon not observed in the intercropping treatment. Therefore, we suggest that shading may have been a significant factor affecting this crop, potentially introducing bias into the measurements conducted in the staircase device treatment. The most affected species in the intercropping treatment was lentil. Compared to the control, lentil in consociation exhibited a significant reduction in both aboveground and root dry biomass, which did not occur in the staircase deivce treatment. It is likely that this crop may have suffered from competition by buckwheat.

No effect was observed in any treatment for *A. retroflexus*. This result confirms the observations of Wirth and Geller (2016), who tested *A. retroflexus* and lettuce in both field and pots experiments using soils where buckwheat had previously been grown for varying durations. By their results, there was no influence of buckwheat on germination and development of both species. In contrast, Falquet et al. (2014) provided conflicting evidence. In their pot experiment, they examined the effects of buckwheat intercropped with *A. retroflexus*, highlighting a significant inhibitory impact due to competitive shading effect and root interaction (potentially allelopathy).

It's interesting to note that according to the Competitive Balance Index, resulted being the most competitive species in every sampling (Figure 4.6). However, it is difficult to distinguish competitive traits in this species, as buckwheat plants exhibited limited canopy extension and prostrate growth tendency, which results in a relatively weak shading effect on nearby plants. Moreover, based on our results, buckwheat was the species with the shallowest roots, a trait generally related to scarce competition for resources (Violle et al. 2009). This data is further supported by the calculated growth rate values, which are generally higher for species in monoculture compared to intercropping, especially during the period between first and second sampling. This result supports the observations on lettuce, which growth was inhibited by the allelochemicals produced during the early growth stages of buckwheat (Kalinova et al. 2005, Kato-Noguchi et al. 2007). Accomplishing for the unfavorable conditions of the experiment, we suggest that it might have been particularly challenging to observe a prominent allelopathic effect of buckwheat. Nevertheless, this result represents an interesting insight that may be explored by further investigation.

4.3. Intercropping with buckwheat for weed management in field.

We set up a field trial to test the allelopathic properties of buckwheat when intercropped with grain legumes, with the aim of mitigating the competitive effect exerted by weeds. The hypothesis of the experiment was that the allelochemicals produced by buckwheat are sufficient to replace weeding, which represents a major issue in organic agriculture. Weeding by hoeing was conducted 25 days after crops emergence. Is efficacy was evaluated one month later, at legumes flowering (58 days after crops emergence) and at harvest.

In terms of weeds diversity, there was no difference in any treatment, whether hoeing was performed or not. As confirmed by calculated Shannon Diversity Index, the weed community had a limited diversity, with few dominant species. Both at flowering and harvest, weeds biomass was mostly affected by hoeing. At flowering and harvest, aboveground dry biomass of weeds was significantly higher in the alternate-row treatment and in pure buckwheat within chickpea treatments, while within lentil, only in pure buckwheat. This result may be due partially attributed to the not-executed hoeing in those treatments and partially to variations in soil cover. In fact, the soil cover in buckwheat was notably lower than in plots with legumes, hence favoring weeds growth. These results refute our hypothesis and support that there was not any significant effect in weeds inhibition, neither for specific species. Our findings are in line with the observations of Wirth and Geller (2016), who did not observe any inhibition in growth of *A. retroflexus* and lettuce immediately sown in a field where buckwheat was cropped for varying durations. However, in literature, several studies support the inhibitory effect of buckwheat on several weeds (Tominaga and Uezu 1995, Hayashi 1999, Kalinova et al. 2005, Kumar et al. 2009, Falquet et al. 2015). According to our results, *C. album* was the dominant weed, occurring in 100% of plots. This result is in contrast with previous research, suggesting that *C. album*, together with *E. crus-galli* and *Portulaca oleracea* should be strongly inhibited (Tominaga and Uezu 1995, Hayashi 1999).

In terms of crops productivity, intercropping with buckwheat did not significantly affect crops aboveground dry biomass and yield but in the alternate-row layout for chickpea. This result is likely due to the absence of weeding in that treatment, hence may be caused by a higher weed competition. However, even if biomass of weeds was higher in the chickpea-buckwheat alternate-row layout, difference with hoed treatments was quite low and not significantly different. Since results of the previously discussed experiments, chickpea has performed as a possible susceptible species by buckwheat allelochemicals. A further result supporting this hypothesis consists in the measured plant density, which was lower in both crops when intercropped with buckwheat, even if the sown amount of seeds was the same. It is known that buckwheat might be able to produce and release several allelochemicals already during germination and early development (Lee et al. 2004, Iqbal et al. 2003, Kalinova et al. 2005, Falquet et al. 2014). Accomplishing for this evidence, we can rule out a possible inhibition of crops by allelopathy from interaction with buckwheat, however, further investigations are required to better evaluate this aspect.

5 Conclusions

Water extracts of buckwheat inhibited the germination of seeds in all tested species. Germination inhibition occurred in terms of both reduced germination rate and root elongation. The occurrence of molds due to contamination from the buckwheat extract itself was a main source of bias. Nevertheless, results of this experiment are promising, and potential applications of these extracts may involve the production of bioherbicides. Further investigation may consider including treatments with a standard toxin or conventional herbicide to better assess the scale of the inhibitory effect exerted by the extract.

The pot trial conducted in the greenhouse did not provide significant evidence of the allelopathic activity of buckwheat in association with weeds or crops. The experiment was constrained by the availability of only low-quality soil and greenhouse infrastructure issues, which led to shading in one treatment and overheating. A possible allelopathic effect may have occurred in chickpea and *A. theophrasti*. Nevertheless, buckwheat resulted as the most competitive species, reducing the growth of all species when in association. Given the limited canopy extension of buckwheat, which exhibited a prostrate growth tendency and shallow roots, we hypothesize that the competitive effect exerted by this species should have been quite limited.

Field trials did not support the hypothesis that consociation with buckwheat is an effective strategy to mitigate weed competition. According to vegetation sampling results, there was no noticeable effect on containing weed infestation, and the most prevalent species was *C. album*, which, according to literature, should be particularly sensitive to buckwheat allelochemicals. Overall, weed management through hoeing proved to be a more efficient strategy. In treatments without weeding, buckwheat did not inhibit weed growth, and crop yield was significantly reduced. Further investigation is still needed, and future perspectives may involve testing the allelopathic effect of buckwheat by applying relay intercropping, where buckwheat is sown before the companion legume.

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Chapter 5. Random Forest Classification of Protein Crops with UAS Multispectral Data

Keywords: grain legume, protein grain, vegetation index, NDVI, UAV

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1 Introduction

Thanks to the increase in accessibility of remote sensing technology, thematic maps have gained significant appeal among researchers (Ok et al. 2012). While the use of such tools has gained high value for comprehending the land use and cover attributes of land surface, interesting applications involve the classification of agricultural landscapes, determination of land use areas and the detection of crop residues in agricultural fields (Daughtry et al. 2006, Huang and Fipps 2006, Stehman and Miliken 2007, Duro et al. 2012). Such applications may play a fundamental role in assessing many important aspects related to land use like soil erosion (Ganasri et al. 2016), weed management (Huang et al. 2018) crops stress (Sun et al. 2019) yield (Bu et al. 2017), and also quantifying important ecological services like Carbon stock (Guo et al. 2021).

For this purpose, image classification algorithms have been developed, in order to face diverse applications and classification challenges. These algorithms are in various categories according to their mechanism of classification. The simplest ones are pixel-based algorithms, like Maximum Likelihood Classification (MLC), which assign a class to each pixel in an image based on its spectral characteristics (Horning 2010). More complex are object-based algorithms, which use different object features (*e.g.* spectral reflectance, shape and texture), and the relationship among different objects to assign a specific label for grouped pixels (Gitas et al., 2004). Classification models are applied across a broad spectrum of applications, including precision agriculture, crop management decision-making, monitoring and modeling food production and security (Bad and Kayaalp 2021. In precision agriculture, they play a crucial role in aiding informed decisions related to crop management (Mishra et al. 2016). Additionally, they find applications in land use planning and ecosystem services assessment, aiding in environmental protection efforts by monitoring land use changes and assessing the impact of agriculture on natural resources (Liakos et al. 2018).

Recently, a wide ensemble of machine learning-based algorithms has been developed, as an alternative to conventional pixel-based and object-based methods, resulting more accurate and reliable models for image classification purpose (Ok et al. 2012). Conversely to the usual approaches, machine learning methods do not rely on parametric statistics, as they do not require data distribution and independence assumptions. These methods are data driven and are structured to “learn” the relationship between data predictor and response (Breiman 2001, Horning 2010).

The most widely used machine learning-based algorithms are Random Forest models. Their classification algorithm is considered being particularly effective as it is poorly sensitive to noise and tends to not overfit (Belgiu and Drăguţ 2016). These models have been successfully used to classify many features like crops (Tatsumi et al. 2015), invasive species (Cutler et al. 2007, Jay et al. 2009) and crop residues (Barnes et al. 2021). Random Forest models are typically applied to satellite data, utilizing spectral bands and derived variables. These variables are often obtained through calculations of multispectral indices or tasseled cap transformation (*e.g.* Long et al. 2013, Hao et al. 2015, Tatsumi et al. 2015,2016). As an example, Hao et al. (2015) utilized MODIS satellite time series data to develop a Random Forest (RF) model for classifying 9 different crop types in the agricultural landscape in the Central Great Plains of Kansas. The model was fed with multispectral indices (NDVI and NDWI) as well as phenological metrics derived from spectral data. By their results, the NDVI contributed as the most significant features for crop mapping, which concurs with the most part of literature on the subject (Huang et al. 2021).

Despite the widespread use of these models, to our knowledge, there is no evidence of their use on crops investigated in the various trials of this thesis. Hence, we conducted a study aimed to classify chickpea (*Cicer arietinum),* faba bean *(Vicia faba)*, lentil (*Lens culinaris*) and quinoa (*Chenopodium* quinoa) by applying Random Forest modelling techniques with multispectral and photogrammetric data acquired by unmanned aerial system (UAS) in Italy and Netherlands. The hypothesis of this study is that it is possible to implement a Random Forest classification model capable of automatically distinguishing these crops. To achieve this goal, we aim to evaluate the models' performance by combining both spectral and photogrammetric information.

2 Materials and Methods

2.1 Study areas

The study took place in 2021 by exploiting two different varietal studies conducted in Udine (IT) and Lelystad (NL). Crops investigated were chickpea, faba bean, lentil and quinoa (Figure 5.1).

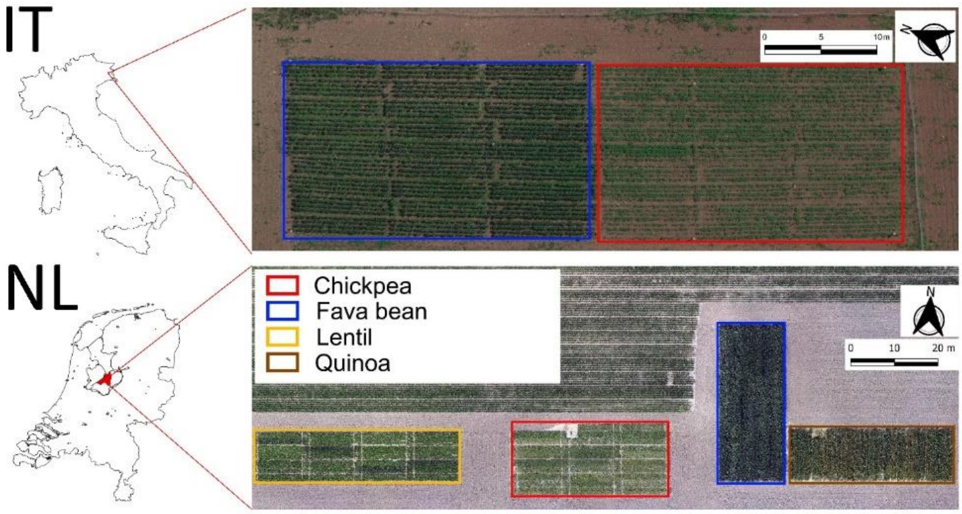

Figure 5.1. Study areas in the pilot farms in Italy (IT) and Netherlands (NL), where Chickpea, Fava bean, Lentil and Quinoa were tested in 2021.

2.1.1 Italy

In Italy the study was conducted in Udine (46°02'12''N – 13°13'23''E). The climate is Continental, with warm summers (mean temperature higher than 20°C) and relatively cold winters. Average cumulated precipitation exceeds 1400mm, regularly distributed along the year and occurring abundantly in autumn (data from ARPA-OSMER, reference period 2000-2022).

The experimental trial took place in a field situated at the experimental farm "Antonio Servadei" of the University of Udine on loam soil (36%sand. 35% silt 22% clay, 7% gravel), having 1.9% of Soil Organic Carbon, 6.8 pH and 9 C/N. The soil has a low fungi/bacteria ratio of 0.5 and microbial activity is low. The SOC balance indicates a steady state supply of C equal to 1.6 tC ha^{-1} $year^{-1}$ assuming an annual mineralization rate of 2.9% of the actual SOC. Deficiencies of Mn are foreseen for legumes; Fe, Zn, P and K plant available are evaluated rather low. The soil Water Holding capacity (at 0.25m depth) is 52mm.

Crops under investigation were: chickpea, faba bean and lentil. Treatments were sown following a randomized block design, replicated in three blocks, in plots of 12.6m^2. A detailed summary of the experimental design is reported in Table 5.1 and for more information refer to chapter 1.

2.1.2 Netherlands

In Netherlands the experimentation was conducted in Lelystad (52°32'51''N - 5°34'39''E). The climate is Oceanic, with fresh summers (mean temperature generally lower than 18°C) and cold winters. Average cumulated precipitation is approximatively 650mm, regularly distributed along the year (data from Royal Netherlands Meteorological Institute https://dataplatform.knmi.nl, reference period 2014-2021).

The experimental trials took place in a field located at the experimental farm “Waiboerhoeve” of Delphy B.V., on loam soil (46% of sand, 32% of silt and 15% of clay) with neutral acidity (pH 7.5) and medium-low fertility (total Nitrogen stock 2.6x10^{3}kg ha^{-1}, C/N ratio 11, N-supplying capability 45kg ha^{-1}, available Phosphorus for plants 1.9kg ha^{-1}).

Crops tested were chickpea, faba bean, lentil and quinoa. Treatments were sown following a randomized block design, where each treatment was replicated in four plots, one per block. Plot dimensions were 18m^2 (6 rows with row-distance of 0.25m at a length of 12m). A detailed summary of the experimental design is reported in Table 5.1.

Table 5.1. Experimental conditions for field trials in Italy and Netherlands

Study site	Italy – Udine	Netherlands
Sowing date	Chickoea: 30/03/2021 Faba bean: 30/03/2021 Lentil: 30/03/2021	Chickoea: 08/06/2021 Faba bean: 26/04/2021 Lentil: 08/06/2021 Quinoa: 26/04/2021
Soil type	Loam	Loam
Sowing density	Chickpea: 45 plant m^{-2} Faba bean: 35 plants m^{-2} Lentil: 120 plants m^{-2}	Chickpea: 40 plants m^{-2} Faba bean: 80 plants m^{-2} Lentil: 135 plants m^{-2} Quinoa: 100 plants m^{-2}
Plot size	12.6m^2	18m^2
Cultivars / Accessions / Treatments	Chickpea: *Eq.3279, Eq.3282, Eq.3283, Eq.3284, Sultano, Sultano + Eq.3282, Sultano + Eq.3284* Faba bean: *Alexia, Fanfare, Fuego, GL Emilia, Lynx, Taifun, Tiffany* Lentil: *Anicia, Flora, Itaca*	Chickpea: *Eq.3279, Eq.3280, Eq.3282, Eq.3283, Eq.3284, Eq.1396, Sultano* Faba bean: Alexia, Fanfare, Fuego, GL Emilia, Lynx, Tiffany Lentil: *Anicia, Flora, Itaca, Paula* Quinoa: *Eq.1002, Eq.1002 fertilized, Eq.1010, Puno, Titicaca, Titicaca fertilized, Zeno*
Emergence (>80%)	Chickpea: 14/04/2021 Faba bean: 14/04/2021 Lentil: 14/04/2021	Chickpea: 17/06/2021 Faba bean: 09/05/2021 Lentil: 17/06/2021 Quinoa: 09/05/2021
Flowering (>80%)	Chickpea: 01-07/06/2021 Faba bean: 01-07/06/2021 Lentil: 01-07/06/2021	Chickpea: 13/07/2021 Faba bean: 09/06/2021 Lentil: 13/07/2021 Quinoa: 07/06/2021
Harvest Maturity	Chickpea: 29/07/2021 Faba bean: 16/07/2021 Lentil: not reached	Chickpea: 24/09/2021 Faba bean: 28/08/2021 Lentil: 10/10/2021 Quinoa: 16/08/2021
Weeding	Periodically, hand hoeing	Periodically, hand hoeing

2.2 Remote sensing data acquisition

Altum and multispectral (MSP) data were acquired during the growing season on four dates in Italy: 21/05/2021, 04/06/2021, 23/06/2021, 05/07/2021, and on five dates in Netherlands: 16/06/2021, 09/07/2021, 24/04/2021, 05/07/2021, 18/08/2021. Data were acquired with a MicaSense RedEdge MX camera (MicaSense Inc., Seattle, WA, USA) equipped on a DJI Matrice 210 v2 UAV (SZ DJI Technology Co. Ltd., Shenzenm, China). In the Netherlands, same data were acquired with a MicaSense Altum camera equipped on a DJI Zenmuse P1 UAV. Both MSP cameras acquired reflected radiation in: Blue (450±16nm), Green (560±16nm), Red (650±16nm), Red-Edge (RE, 730±16nm) and Near-Infrared (NIR, 840±26nm).

Images were acquired with a multicamera system at 1 Hz frequency with minimum overlap of 80%. Pictures of the MicaSense calibrated reflectance panel were also acquired before and after each flight, in order to correct the acquired images on the day's lighting conditions and compare the results of different flights.

Spectral data were mapped with spatial resolution of 2cm using Agisoft Metashape v.1.5.2 software (Agisoft LLC, 2019). Georeferenced maps of the plots were created with R software by cropping the central area of each plot with a 500x500m mask. In order to prevent possible biases due to inhomogeneous or incomplete soil cover between rows, pixels with NDVI*height (cm) value lower than 0.1 were excluded.

Multispectral data were used to calculate an ensemble of the most used multispectral (MSP) and RGB Vegetation Indices (VIs) in literature. All VIs used are listed in Table 5.2.

In addition, Digital Elevation Models (DEMs, Figure 5.2) and Digital Terrain Models (DTMs) were generated in order to calculate the photogrammetric canopy volume (V_C, m^3m^{-2}) as:

$$V_C = \frac{\sum(H_{px} - H_g) * A_{px}}{A_m}$$

where H_{px} is the pixel height (m) referenced to the Digital Elevation Model, H_g is the ground level (m) referenced to the Digital Terrain Model, A_{px} is the pixel area (m^2) and A_m is the area of the mask, equal to $16.32m^2$ (1.6 width x 6m length).

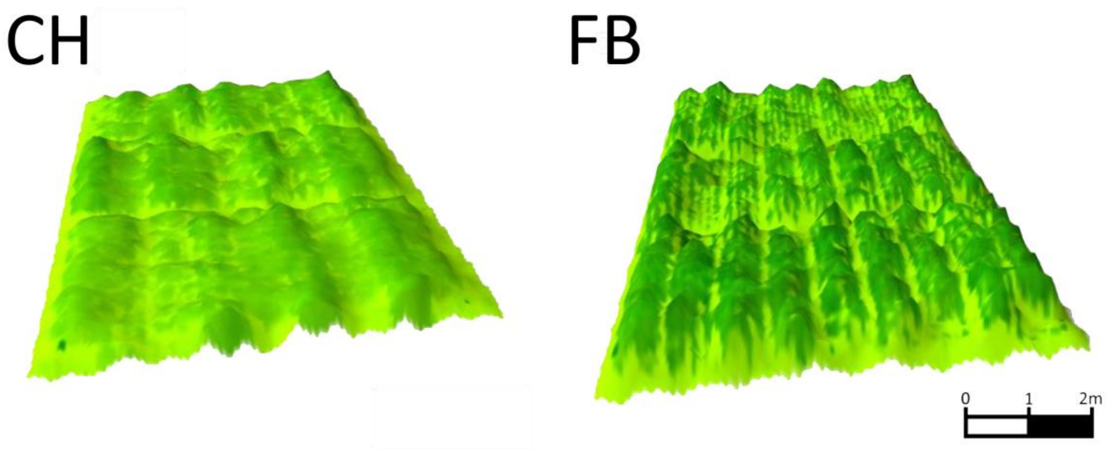

Figure 5.2. Examples of digital elevation models of the experimental fields of chickpea (CH) and faba bean (FB) in Udine, generated from the data acquired on 04/06/2023.

Table 5.2. List of spectral bands and vegetation indices tested, with corresponding abbreviations, equations and references.

Classification	Parameter	Abbreviation	Equation	Reference
Single bands	Blue	B		
	Green	G		
	Red	R		
	Red Edge	RE		
	Near-Infrared	NIR		
Simple ratio	Simple Ratio Vegetation Index	RVI	RVI = R / NIR	Pearson and Miller 1972
MSP indices	Normalized Difference Vegetation Index	NDVI	NDVI= (NIR – R) / (NIR + R)	Rouse *et al*. 1974
	Green NDVI	GNDVI	GNDVI = (NIR – G) / (NIR + G)	Gitelson *et al.* 1996
	Renormalized Difference Vegetation Index	RDVI	RDVI = (NIR – R) / $(NIR + R)^{0.5}$	Roujean and Breon 1996
	Nonlinear Vegetation Index	NLI	NLI = $(NIR^2$-R) / $(NIR^2$+R)	Goel and Quin 1994
	Normalized Difference Chlorophyll Index	NDI	NDI = (NIR – RE) / (NIR + RE)	Gitelson and Merzlyak, 1994
	Soil-Adjusted Vegetation Index	SAVI	SAVI = ((1 + L) * (NIR – R)) / (NIR + R + L)	Huete *et al*., 1988
	Green SAVI	GSAVI	GSAVI = ((1 + L) * (NIR – G)) / (NIR + G + L)	Sripada 1995
	Oprimized SAVI	OSAVI	OSAVI = (NIR – R) / (NIR + R + 0.16)	Rondeaux et al. 1996
	Infrared Percentage Vegetation Index	IPVI	IPVI = (NIR) / (R + NIR)	Crippen 1990
	Modified Triangular Vegetation Index	MTVI1	MTVI1 = 1.2 * (1.2*(NIR – G) – 2.5*(R – G))	Haboudane et al. 2004
	Modified Triangular Vegetation Index	MTVI2	MTVI2 = MTVI1 / $(((2*NIR+1)^2$ – (6*NIR- $(5*R^{0.5}))^{0.5}$ – 0.5)	Eitel et al. 2007
	Modified Red Edge Simple Ratio	MRESR	MRESR = (NIR – B) / (RE – B)	Sims and Gamon 2002
	Red MRESR	rMRESR	rMRESR = (NIR – R) / (RE – R)	/
RGB indices	Excess Blue Vegetation Index	ExB	ExB = (1.4*B-G)/(R+G+B)	Mao et al. 2003
	Excess Green Vegetation Index	ExG	ExG = (2*G)-R-B	Woebbecke *et al., 1995*
	Excess Red Vegetation Index	ExR	ExR = (1.4*R-G)/(R+G+B)	Meyer and Neto, 2008
	Excess Green minus Red Vegetation Index	ExGR	ExGR = ExG – ExR	Neto, 2004
	Green Leaf Index	GLI	GLI = (2*G-R-B)/(-R-B)	Louhaichi et al. 2001
	Green-Red Vegetation Index	GRVI	GRVI = (G-R)/(G+R)	Tucker, 1979
	Modified GRVI	MGRVI	MGRVI = $(G^2 – R^2)/(G^2 + R^2)$	Bendig *et al.*, 2015

2.3 Crops Classification

All the analyses and elaborations were conducted with the R v2.0.1 software (R Core Team, 2021) with the "*randomForest"* package (Liaw and Weiner 2002).

Remote sensing data at plot level was used to implement multiple random forest classification models. A base model (RF_{base}) was run, implementing as predictive features the acquired five spectral bands and V_C. To evaluate whether MSP bands elaboration as VIs may provide new information for classification purposes, a comprehensive model (RF_{all}) was run including all the calculated VIs as predictive features. A minimum model (RF_{min}) was then elaborated by implementing only the most significant features, selected by lack of correlation (Pearson's $R < 0.65$) and performance in terms of local importance in the RF_{all} model.

To improve models' performance, we tested multiple combinations of number of trees to grow (N): from 300 to 1500; and variables randomly sampled at each split (m): from 3 to X-1 (where X is the total amount of input variables). Each model was implemented by randomly splitting the dataset into two subsets: i) training: 70% of observations; ii) test: 30% of observations. Best models were selected by assessing global performance by reiteration of the whole process 1000 times, for each combination of model *x* N *x* m.

3 Results

Oprimal models' performances are summarized in Table 5.3. All models' performance were notable, with low values of out-of-bag error (≤0.10) and high values of accuracy (≥0.90), kappa ((≥0.86) and sensitivity ((≥0.89). Despite differences between model types are low, there is a general improvement along the three-steps procedure. The Implementation of spectral bands elaboration through VIs increased models' performance, but the best results were obtained with *RFmin* by using a restricted selection of features.

Table 5.3. Performance of Random Forest models for Crops' Classification, according to the assessed optimal number of trees to grow (*N*) and variables randomly sampled at each split (*m*).

Model	*N° of features*	*N*	*m*	*OOB error*	*Accuracy*	*Kappa*	*Sensitivity*	*Computational time (s)*
RFbase	6	400	3	0.10	0.90	0.86	0.89	0.056
RFall	28	700	12	0.09	0.91	0.87	0.90	0.249
RFmin	6	600	2	0.08	0..93	0.90	0.92	0.085

Local importances of the five best predictors for each model type, expressed as mean model accuracy decrease (%), are represented in Figure 5.2. This result is quite interesting and strongly supports the hypothesis of the study. Among spectral bands, the RE is the most recurring, one, being in the second position in RF_{base} and third in the remaining ones. About VIs, rMRESR index performed as the best. This result is quite interesting as this index does not saturate and contains both information of NIR and RE referred to R. Furthermore, the ExB index also performed well. Being an RGB index, this result suggests the suitability of RGB techniques for this purpose, however it has to be noticed that most important information used has been provided by the infrared region.

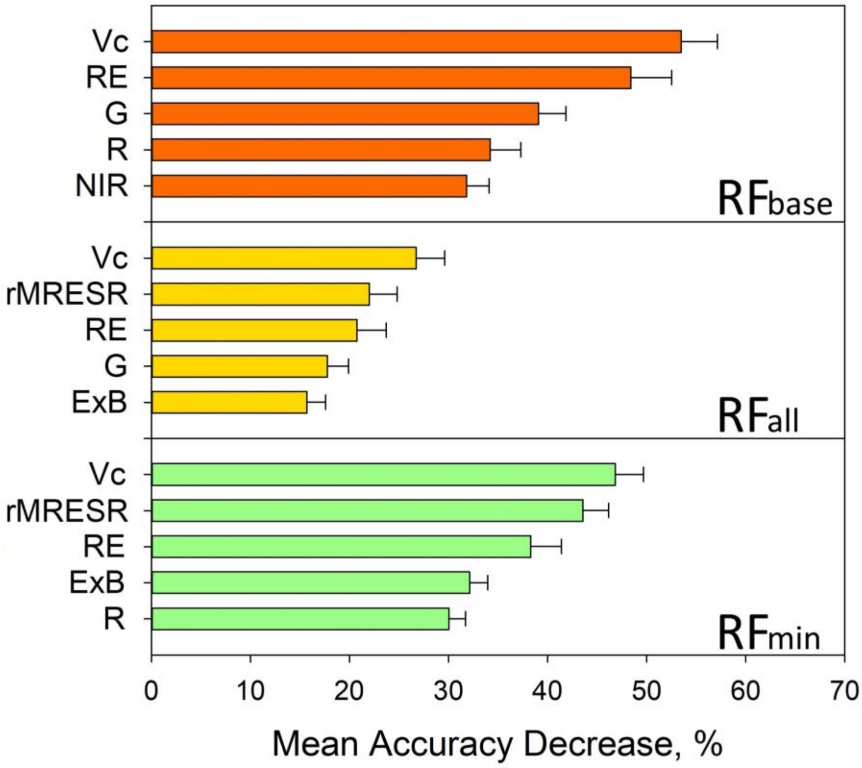

Figure 5.2. Ordered local importance of the five best predictors for each Random Forest model. Abbreviations: RF – Random Forest; V_C – Canopy volume; RE – Red Edge; NIR – Near infrared; - G – Green; R – Red; rMRESR – red Modified Red Edge Simple Ratio; - ExB – Excess Blue vegetation index.

4 Discussion

Crop classification models are highly useful for a variety of applications in agriculture, environmental monitoring, and food security. These models leverage machine learning and remote sensing technologies to identify and classify different types of crops in a given area (Ok et al. 2012). Among various classification algorithms, Random Forest (RF) has been assessed as one of the most performing and suitable for agricultural landscape (Ok et al. 2012).

In our study, we tested an ensemble of RF models that incorporated both spectral features and a structural feature, the canopy volume (V_C). All of our models performed notably, with high accuracy (>0.90) and low error (<0.10). Surprisingly among the best predictors, the NDVI never occurred and was actually discarded from the variables included in the RF_{min} model, which was the most performing one. However, there is ample literature supporting the use of alternative vegetation indices over NDVI due to its documented issues with saturation, sensitivity to soil background, and variability among different phenological phase (Huang et al. 2021). Long et al. (2013) used multispectral data from Landsat satellite to implement various RF models to classify the agricultural landscape of northeast Montana. From their results, NDVI never performed as a significant variable for this purpose. In another study conducted with Landsat 7 multispectral data to feed RF classification models for agricultural types in south Peru, it resulted that the mode and sum of the Enhanced Vegetation Index outperformed NDVI in importance for crop class separability (Tatsumi et al. 2015).

Interestingly, canopy volume resulted being the most important variable in all models. Up to our knowledge there is no evidence in literature on the use of this parameter in automatic classification, however there is some evidence where structural acquired by synthetic aperture radar (SAR) significantly improve models accuracy. Sonobe et al. (2014) classified various crop types in the western Tokachi plain (Hokkaido, Japan) with RF models implemented with radiometric data acquired from TerraSAR-X satellite. Their results demonstrate that incorporation of such variable increased classification accuracy to more than 0.91, supporting the importance of structural (*i.e.* dual-polarimetric) data for this purpose.

Since the requirement of a high amount of data for automatic classification models, such techniques are generally conducted by using sensed data from satellite rather than UAS (Ma et al. 2017). Despite the use of unmanned vehicles and the acquisition of this data in agriculture has exponentially increased in the last decade (Tsouros et al. 2019), classification models with UAS-acquired features generally rely on hyperspectral data (Wang et al. 2022) or are not applied to agricultural landscape (Feng et al. 2015, Guo et al. 2022). In this study, we have purposed a novel methodology for the automated classification of crops using commonly used precision agriculture data. Despite this research was limited in data availability, the used technique under the studied conditions shows to be effective. Further research and the integration of new data are still required and may lead to interesting results, also investigating new crop types.

5 Conclusions

We set up a study aimed to automatically classify chickpea, lentil, faba bean and quinoa by implementing a Random Forest classification model. We achieved this foal by implementing the models both with spectral and photogrammetric remote sensing data, collected by unmanned aerial system. Models were improved in a multi-step workflow which allowed us to remove redundant and noisy information, in order to achieve the minimum and most performing one.

The presented study supports the hypothesis that, despite being the investigated species all annual crops, mostly similar from a spectral point of view, the generated Random Forest models are a useful tool, able to classify the investigated protein crops. It allows to effectively combine data of crops in different sites and phenology stages. The best predictor in all models was the photogrammetric canopy volume. The integration of Vegetation Indices, particularly rMRESR, significantly improved crops classification, however, an accurate selection should be conducted to avoid information redundancy and limit the required computational power.

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General Conclusions

In this thesis we report a series of experimental trials aimed to validate the practices to optimize the protein crops production in organic agriculture, in the Friuli-Venezia Giulia region (NE Italy).

In Chapter 1 we reported a study aimed to evaluate the adaptability of chickpea, faba bean and lentil to Friuli region. Our results showed that chickpea could be a suitable species for this region, and among the varieties screened, *Sultano* was the best in terms of both yield and protein production. In faba bean, all cultivars underperformed, probably due to occurrence of biotic stress (i.e. botrytis and rust), however the protein content of seeds was high. Lentil was not harvested due to lack of grains production, which may be due to stress-induced abortion of flowers.

In Chapter 2, we present a field trial conducted on chickpea and lentil. The aim of the trial was to assess the adaptability of these crops to drought, which was particularly intense during the 2022 growing season. To this end, we tested the response of both crops to an irrigation gradient applied during the critical grain filling phase. Despite the conditions of extended drought combined with high temperatures, both chickpeas and lentils proved fairly drought resistant. Both crops had good average yields and protein production. Irrigation did not have significant effects on grain production, but it extended the flowering phase. This had a negative effect on seed quality. The plants continued to flower during the irrigation period, which resulted in an uneven ripening of the crop at harvest time. Remote sensing data has been used effectively to monitor crops' water stress and development. For this end, NDVI index and canopy temperature measure via thermal imager were efficient in detecting differences among irrigation treatments in term of stress and senescence.

In Chapter 3 we investigated the relationship between crop's measured traits (i.e., Leaf Area Index and aboveground biomass) and multispectral data acquired during the 2022 summer season. The aim of the study was to evaluate possible correlations between these data, also elaborating cumulative vegetation indices, calculated as the integral of the time dependent spline function of each spectral index. Results highlighted that for different parameters of chickpea and lentil, there is a certain discordance about the best index. The Normalized Difference Chlorophyll Index resulted being the best proxy of crop biomass while reflectance in the near infrared was associated with leaf area index. Moreover, the use of cumulative vegetation indices significantly improved correlations with crop biomass and allowed a good yield's estimate throughout the growing season.

In Chapter 4, we present a study to evaluate the allelopathic effect of intercropped buckwheat on chickpeas and lentils, in order to validate this new technique of weed control in these crops. We investigated several allelopathic effects of buckwheat on weeds and crops. The germination of seeds was studied in Petri dishes, the initial growth of plants in pots (greenhouse) and the overall response of the system up to the harvest of the crop was studied in a field plot experiment.

Results of Petri dishes' study showed that water extracts of buckwheat inhibited the seed germination in all tested species, being of particular interest for potential applications in the production of bioherbicides. No significant allelopathic activity of buckwheat on initial weed or crop growth was observed in greenhouse pots. Despite its low competitive potential, buckwheat reduced the initial growth of all species when mixed. The field trials did not support the hypothesis that intercropping with buckwheat is an effective technique for reducing weed competition; in fact, weed control by hoeing was found to be the best practice. However, this research had to face limitations and further investigation is still needed.

In Chapter 5, we present a side project that has been carried out in 2021 in Udine, Italy, and Lelystad, the Netherlands, to implement a Random Forest classification model for chickpeas, lentils, faba beans, and quinoa, based on multispectral and photogrammetry data.

The results are in support of the fact that this technique is a useful tool capable of classification with high accuracy at the species level.

Of particular interest was the unprecedented use of photogrammetric canopy volume among the input variables of the classification model. This variable was the most relevant in all the models tested. In addition, the integration of vegetation indices significantly improved crop classification. The careful selection of the input variables avoids the redundancy of the information and reduces the computational power required.

The European Union's Common Agricultural Policy (CAP) aims to ensure a fair standard of living for farmers, stabilise agricultural markets, provide consumers with a safe and affordable food supply and promote sustainable agriculture. Within this framework, organic farming is recognised as a key strategy for promoting environmental sustainability, preserving biodiversity and meeting increasing consumer demand for healthy products. The CAP has evolved over the years to meet changing economic circumstances and the needs and demands of citizens. To this end, the Farm to Fork Strategy and the Biodiversity Strategy are cornerstones of the European Green Deal. One of the key principles of this framework is that farmers should work in a sustainable and environmentally friendly way, preserving our soils and biodiversity, while being economically viable.

With this in mind, a key objective of the Smart Protein HO2020 project and our research in general has been to promote organic farming, which is recognised as a key practice for improving environmental sustainability and food safety.

Organic farming is based on a systemic agroecological approach.

Organic methods are not as effective as conventional methods in preventing yield losses due to pests, weed diseases and nutrients. More research is required to effectively address this problem, which limits the uptake of organic farming by farmers.

Research presented in this thesis supports the hypothesis of the investigated species, particularly chickpea and lentil, being suitable crops for the NE Italia region. In this Region, and in EU countries, the economic competitiveness of cereals and oil rich crops is very strong and largely explains farmers' lack of interest in other crops like protein-rich crops ones.

Both chickpea and lentil particularly performed in terms of adaptability to water and heat stress and yielded particularly well both in terms of seed and protein production. The conducted activity of cultivar screening highlighted the need to continue breeding and cultivar selection for diverse pedo-climatic region.

Within the European framework for agricultural and environmental sustainability, The Sustainable Use of Pesticides Directive (Directive 2009/128/EC) establishes a framework for community action to achieve the sustainable use of pesticides. The directive indicates the Integrated Pest Management as a key approach to minimize the impact of pests on agriculture, human health, and the environment. Integrated Pest Management involves the coordinated use of various methods and strategies to reduce the risks associated with pesticide use, protect human health and the environment, and promote alternative, non-chemical methods of pest and weed control. This is obtained through an ensemble of cultural practises including mechanical and physical control, biological control and use of cover crops and smother crops.

In our research, we performed an implementation strategy of intercropping with a smother crop (buckwheat) for weed management. Our trials did not support the hypothesis of consociation with buckwheat being an efficient practice to control weed competition. However, the presented research is still limited to the first year of trials and the experiments were affected by various methodological and technical biases which will be taken into account in future experiments.