**UNIVERSITÀ DEGLI STUDI
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Ciclo XXXII

Development of a high throughput plant phenotyping system for innovating durum wheat breeding in the Mediterranean

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Abstract

The central challenge of modern genetic analysis is to understand the biological determinants of quantitative phenotypic variation. The power of whole genome sequencing as a unifying force in biology has motivated the development of diversity panels and large mapping populations in many crop species to facilitate trait dissection and gene discovery. More accurate and precise phenotyping strategies are necessary to empower high-resolution linkage mapping and genome-wide association studies and for training genomic selection models in plant breeding. Unfortunately, phenotyping under field environmental conditions remains a bottleneck for future breeding advances, limiting the power of genetic analysis and genomic prediction. Field conditions are notoriously heterogeneous and the inability to control environmental factors makes results difficult to interpret. One of the possible solutions is to employ Unmanned Aerial Vehicles (UAVs) commonly known as drones, to collect phenotypic records through different sensors, technologies and wavelengths. Compared with other aerial survey methods, drones generate more precise and more frequent data about the condition of crops. The goal of the present research program is to develop a high-throughput phenotyping platform based on the use of drones. Useful for obtaining detailed measurements of durum wheat plant communities that collectively provide reliable estimates of phenotypic traits (e.g. soil coverage%). In order to carry out the Ph.D. project, in Foggia, at the experimental farm of the CREA Research Centre for Cereal and industrial Crops (CREA-CI), two experimental designs were set up: a varietal comparison, and an agronomic trial. Variety comparison trial: for the preparation of the trial 401 genotypes of durum wheat of different origins were used. The genotypes were grown in plots of one square meter according to a randomized block design with 3 repetitions. The trial was sown with a seed drill, two applications of fertilizers were made, one at sowing and one at the tillering phase. During the growing season, post-emergence chemical treatment for weed control and fungicide treatment for disease control were performed. During the three crop seasons, the following measurements were taken: acquisition of RGB and multi spectral images; morphological assessments and qualitative analyses on grains. The analysis of RGB images, indicative of the genotypes' ability to cover the soil more or less rapidly, showed a high degree of variability and a good discriminatory ability of the used indices (covering capacity, green index, etc.) between the tested genotypes. The analysis confirms the usefulness of automated equipment for the determination of morpho-physiological characters in order to facilitate and make the breeder's evaluation more and more objective. Agronomic trial: on the basis of information obtained from the first trial a second experimental design was set up, using a randomized block design with three factors and three repetitions scheme. The factors considered were: I) two Varieties; II) two different Sowing densities and III) five nitrogen fertilization levels. During the three growing seasons, post-emergence chemical pest control was carried out and also a fungicide treatment for disease control. The fertilization theses were differentiated by administering nitrogen at four different phenological stages. During the

growing seasons, for both the trials, the following measurements were carried out: soil cover index, heading time, number of spikes per linear meter, multispectral and RGB assessments of plots by UAV system. In addition to the plot yield, the grain quality parameters were evaluated. The crop response in terms of production and grain quality was evident considering all the measured factors. The vegetation indices derived from the multispectral and RGB evaluation also showed a good correlation with morphological parameters registered manually, particularly with the soil's cover capacity.

Sintesi

La sfida centrale dell'analisi genetica moderna è comprendere i determinanti biologici della variazione fenotipica quantitativa. Sono però necessarie strategie di fenotipizzazione più accurate e precise per potenziare la descrizione della mappa genetica e gli studi di associazione genomica per allestire modelli di selezione genomica per il miglioramento delle piante. Sfortunatamente, la fenotipizzazione sta rapidamente emergendo come il principale collo di bottiglia operativo che limita il potere dell'analisi genetica e della previsione genomica. Una delle soluzioni è quella di impiegare un veicolo aereo senza pilota (UAV) comunemente noto come drone. L'obiettivo del programma di ricerca è stato quello di realizzare piattaforme di fenotipizzazione ad alto rendimento basate sull'uso di droni utili per ottenere misurazioni dettagliate delle caratteristiche delle piante di frumento duro, che forniscano collettivamente stime affidabili dei tratti fenotipici. Al fine di svolgere il progetto di dottorato di ricerca., a Foggia, presso il Centro Sperimentale di Ricerca sui Cereali e le Colture Industriali (CREA-CI), ho allestito due prove sperimentali, una sperimentazione agronomica e un confronto varietale; *Prova di comparazione varietale*: per l'allestimento della prova sono stati utilizzati 401 genotipi di grano duro di diversa origine e provenienza. I genotipi sono stati coltivati in parcelle di un metro quadrato secondo un piano a blocchi randomizzato con 3 ripetizioni. La prova è stata seminata con una seminatrice parcellare, sono state realizzate due concimazioni, una alla semina e una nella fase di accestimento. Durante le stagioni di crescita, è stato eseguito un trattamento chimico post-emergenza per il controllo delle infestanti e il trattamento fungicida per il controllo delle malattie. Durante le tre annate agrarie, sono stati eseguiti i seguenti rilievi: acquisizione di immagini RGB e multispettrali; valutazioni morfologiche e analisi qualitative sulla granella. L'analisi delle immagini RGB, indicativa della capacità dei genotipi di coprire il terreno più o meno rapidamente, mostra un elevato grado di variabilità e una buona capacità discriminatoria degli indici utilizzati (capacità di copertura, indice verde, NDVI, ecc.) tra i genotipi testati. L'analisi conferma l'utilità della raccolta di immagini da droni per la determinazione dei caratteri morfofisiologici al fine di facilitare e rendere sempre più obiettiva la valutazione del breeder. *Prova agronomica*: Sulla base dei risultati ottenuti dalla prima prova è stata allestita una seconda prova, secondo uno schema a blocchi randomizzati con tre fattori e tre ripetizioni. I fattori considerati sono stati: due varietà; due diverse densità di semina e cinque livelli di concimazione azotata. Durante le tre annate agrarie, sono stati effettuati trattamenti chimici per il controllo delle infestanti e anche trattamenti fungicidi per il controllo delle malattie. Le tesi di concimazione sono state differenziate somministrando azoto in quattro diverse

fasi fenologiche. Durante le annate agrarie, sono state eseguite le seguenti misurazioni: indice di copertura del suolo, data di spigatura, numero di spighe per metro lineare, valutazioni multispettrali e RGB con sistema UAV. Oltre alla resa, sono stati valutati alcuni parametri qualitativi della granella. La risposta del raccolto in termini di produzione e qualità del grano è evidente considerando tutti i fattori misurati. Gli indici di vegetazione derivati dalla valutazione multispettrale e RGB mostrano anche una buona correlazione con i parametri morfologici rilevati a terra, in particolare con la capacità di copertura del suolo.

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1. Introduction

Durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.)) is a cereal of considerable importance for human nutrition in different parts of the world (Bozzini, 1988). The importance that cereals took already in the first civilizations and that has been preserved over time, derives from some of their characteristics: for giving a product that is suitable for human nutrition due to its caloric value, protein content, lipid content, mineral salts and vitamins and being a dry product is easily transportable and storable, suitable to build stocks to be used in the same year of production or in the following years (Nithya et al., 2011). Currently the durum wheat cultivation covers around 17 million hectares worldwide and is prevalent mainly in the Mediterranean basin regions, Canada, USA and Mexico. Italy covers around 11% of the entire global production, with 4.2 million tons per year out of a total of 38.5 million tons of grain estimated in 2018 (source: Aretè, Durum Days 2018; Foggia). The Mediterranean basin, and in particular south Italy, is the most important cultivation area. Durum wheat is cultivated in dry farming conditions and in very diverse environments, generally characterized by scarce and very variable rainfall, where production is often threatened by extreme growth conditions and where biotic and abiotic stresses can cause losses in terms of yield and quality (Nuttall *et al.*, 2017). For these reasons, some of the objectives of genetic improvement in durum wheat focus attention on some specific aspects, in view of the cultivation in difficult areas due to climate irregularities, the uneven distribution of rainfall during the year, high temperatures and the strong potential of evapotranspiration in the maturation period (Atkinson and Urwin, 2012). Furthermore, the growing interest in sustainable and organic farming, requires that the new varieties must have characteristics that make the crop yield more sustainable for the environment (Caubel *et al.*, 2015). In other words, modern durum wheat breeding aims to increase productivity respecting the environment while meeting current food safety standards (Chakraborty and Newton, 2011). The new varieties must not require excessive agrochemicals applications and must have morphological characteristics that allow them to compete with weeds and pathogens/parasites (e.g. early vigor, plant height, tillering ability and soil coverage ability).

Obtaining DNA markers at genomic scale — once inconceivably difficult — is now easy and routine procedure, and the central challenge of modern genetic analysis is to understand the biological determinants of quantitative phenotypic variation. The power of whole genome sequencing as a unifying driver in biological research has motivated the development of diversity panels and large mapping populations in many crop species, to facilitate trait dissection and gene discovery. On the other hand, collecting plant phenotypic data with sufficient resolution (in both space and time) and accuracy represents a long standing challenge in plant science research, and it has been, and still is a major limiting factor for the effective use of genomic data for crop improvement. This is particularly true in plant breeding where collecting large-scale field-based plant phenotypes can be very labor intensive and costly. More accurate and precise phenotyping strategies are necessary to empower high-resolution linkage mapping and genome-wide association studies, as for training genomic selection models in plant improvement. Much of the research on high throughput phenotyping has focused on the measurement of single plants using automated imaging in environmentally controlled greenhouses (Campbell et al., 2015; Fahlgren et al., 2015). These greenhouse-based systems are proven useful to quantify certain plant traits such as biomass dynamics or growth rate of plants and their organs, but they also face a number of major limitations. First, plants in greenhouses are grown in artificial environments (such as pot, soil, water and nutrient distribution, closed aerial environment, and artificial lighting) that can significantly alter the normal pattern of plant growth and development, In addition, plants are grown in dense communities in the field, which affects the kinds of traits that can be measured effectively at the plot level compared to those at the single plant level. For these reasons, phenotyping under field environmental conditions remains a bottleneck for future breeding advances, limiting the power of genetic analysis and genomic prediction. Moreover, field conditions are notoriously heterogeneous and the inability to control environmental factors often makes results difficult to interpret. One of the solutions for field phenotyping of cereal (wheat) communities is to employ Unmanned aerial vehicle (UAV), commonly known as drones, to collect phenotypic records by different sensors, technologies and wavelengths. Compared with other aerial or satellite survey methods, drones can generate more precise and more frequent data about the condition of crops.

1.1 Research Purpose

The goal of the research program is to develop and test a high-throughput phenotyping platform based on the use of drones useful for obtaining detailed measurements of plant characteristics that collectively provide reliable estimates of phenotypic traits. These characteristics allow to determine new indexes useful to select genotypes, to be introduced in new breeding programs, aimed at satisfying the new needs of modern agriculture. In particular, because of the growing interest in organic farming, we tried to find tools to select genotypes suitable for this kind of management. One of the main problems for this type of agricultural system is weed control. And, one of the solutions for that is to identify genotypes that can compete with weeds especially in terms of space, and interception of light for photosynthetic accumulation and nutrients (Baum et al. 2003). It is therefore essential that the crop covers the soil as soon as possible. This is usually not attributed to a single morpho-physiological trait, either within or between varieties. It is in fact the interaction between a series of desirable characteristics to be important in weed competition (Eisele & Köpke, 1997), and this will include strengths in some characteristics compensating for weaknesses in others. Certain key characteristics are indicated as generically desirable for organic wheat varieties to improve weed suppression: good establishment ability, high tillering ability, increased plant height (Wicks et al., 1986; Korr et al., 1996; Didon & Hansson, 2002), planophyle leaf habit, and high leaf area index (LAI) through production of larger leaves (Niemann, 1992; Hucl & Huel, 1996; Seavers & Wright, 1999), plant growth habit and leaf inclination (Eisele, 1992; Niemann, 1992; Lemerle *et al.*, 1996). All these features contribute to increasing or reducing the soil cover ability of the different genotypes in the early stages of growth.

In order to carry out the present project, in Foggia, at the experimental farm of CREA Research Centre for Cereal and industrial Crops (CREA-CI), two experimental devices were set up: a varietal comparison trial and an agronomic trial. <u>Variety comparison trial</u>: For the preparation of the trial 401 genotypes of durum wheat of different origin and provenance have been used. The genotypes were grown in plots of five square meters according to a randomized block design with 3 repetitions. During three cropping seasons, the following data were collected: acquisition of RGB and multi spectral images; morphological assessments and qualitative analyzes on grains. The analysis of RGB images, indicative of the genotypes ability to cover the soil more or less rapidly, showed a high degree of variability and a good discriminatory ability of the used indices (covering capacity, green index, NDVI, etc.) between the tested genotypes. <u>Agronomic trial:</u> From the analysis of the first trial data, two genotypes with contrasting ability of soil coverage were selected to grow in an agronomic trial, to evaluate the behavior due to different cultivation techniques (seed rate and different fertilization). The experiment was designed as a randomized blocks scheme with three factors and three repetitions. The factors considered were: two Varieties; two different Sowing densities and five nitrogen fertilization levels. During the growing season, the following measurements were carried out through the growth cycle: soil cover index, heading date, number of spikes per linear meter, multispectral and RGB assessments by UAV system. The vegetation indices derived from the multispectral and RGB evaluation showed a good correlation with morphological parameters collected at the ground level, particularly with the soil's coverage capacity.

2. Materials and Methods

2.1 Experimental site

This study was performed at the Cereal Research Centre (CREA-CI, Foggia, Italy; 41◦28_N, 15◦32_E; 75 m a.s.l.) in the 2016/17, 2017/18 and 2018/19 growing seasons, on a clay-loam soil (Typic Chromoxer-ert). Soil traits were: 36% clay, 47% sand; pH 7.8; 17.3 g kg−1totalC; 1.5 g kg−1total N. The mean long-term rainfall of the experimental site is 479 mm, and the mean air temperatures are 12.2°C in autumn, 8.2°C in winter, and 17.6°C in spring. The mean minimum and maximum annual temperatures are 9.9°C and 21.0°C, respectively.

Figure 1: CREA-CI aerial view

2.2 Experimental design and crop management

In order to carry out the project two experiments were set up, a varietal comparison and an agronomic trial:

Variety comparison trial: The panel of 401 durum wheat genotypes used in this study was characterized by different geographical origins, breeding history and year of release. The genotypes were grown in plots of one square meter according to a randomized block plan with 3 repetitions. The trial was run in a single year, the first one of the project; trial was sown with a seed drill on 15/12/2016, two fertilizations were made, one at sowing with 200 kg/ha of bi-ammonium phosphate 18-48 kg / ha and one with 150 kg/ha of urea at the tillering phase. During the growing season, one post-emergence herbicide treatment for weed control and one fungicide treatment for disease control were performed (Amistar ultra 1,25 l/ha, Traxos pronto 1 l/ha).

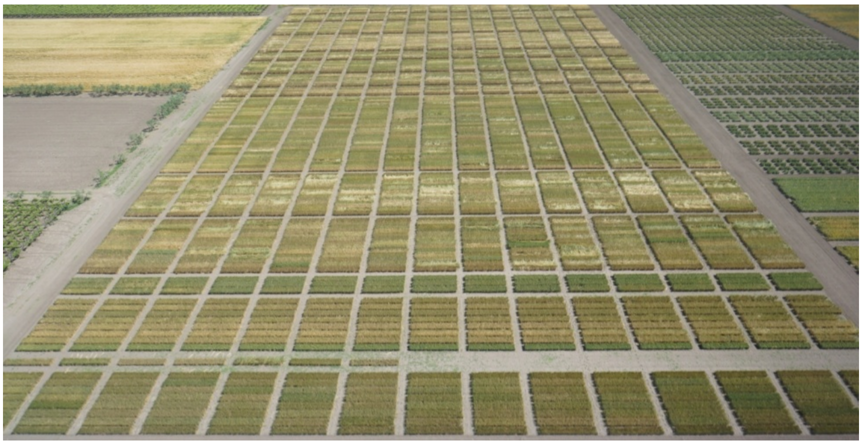

Figure 2: Variety comparison trial

Harvesting was carried out with a plot thresher machine on 29/06/2017, and in addition to the plot yield the following grain quality parameters were evaluated: protein content, yellow index and sedimentation index in SDS (NIR analysis – Foss Analytical)

Agronomic trial: The experiment was set up as a randomized block design with three factors and three repetitions, and grown in 10.2 m^2 plots. The factors considered were Variety (Natal and Nadif); Sowing density (200 and 400 germinable seeds per square meter) and five different nitrogen fertilization levels (0, 60, 120, 180 and 240 Kg N / ha.).

Figure 3: Agronomic trial

The seed needed for the trial was provided by Foggia CREA-CI, owner of the two durum wheat varieties. The trial was repeated three years, and sowing was carried out around mid-December in all the three growing seasons (13/12/2016; 11/12/2017 and 14/12/2018), using a plot seeder. The meteorological conditions and soil at the time of sowing were optimal. During the growing season, a post-emergence chemical weed control was carried out, and a fungicide treatment for disease control. The various fertilization theses were differentiated by administering nitrogen in four different phenological stages (21, 30, 45 and 59 of Zadoks's scale).

2.3 Data collected

During the vegetative and reproductive cycle of the trials phenological observations and surveys of phenotypic, agronomic and biometric characters were conducted in order to evaluate the extent of trait variation among the genotypes of the collection. In order to obtain valid agro-phenological information, a standard methodology for the detection of phenotypic data has been adopted, responding to the criteria of representativeness, objectivity and the possibility of statistical data processing. The phenotypic data that have been detected in the field (on the ground) are the following:

- Soil cover index (Zadoks GS 10-21)
- Heading data (Zadoks GS 55)
- Plant height (Zadoks GS 70-79)
- NDVI (Normalized Difference Vegetation Index) (Zadoks GS40-49; GS85)
- Grain yield
- Protein content
- Test weight

Heading Date is an important pheno-phase of the biological cycle of the durum wheat, as it is related to precocity. It is expressed as the number of days that have elapsed since April 1, up to the date on which about 50% of the plot's main culms have the spike halfway out of the flag leaf sheath.

Figure 3: Heading date GS 55

Plant height was measured during milky-waxy ripening (GS 70-79). In durum wheat this phenological phase is that in which the maximum height is reached (Zadoks et al., 1974). The survey was carried out on the main culm and at three different points of each plot in order to operate on plants representative of the genotype. The height was expressed as distance (in cm) from the crown to the base of the spike (entire culm excluding the spike).

Soil cover Index: this is an important agronomic trait that should be considered because a dense ground cover affects the interception of light for photosynthetic accumulation, the inhibition of weed growth and the reduction of water evaporation from soil (Baum et al. 2003). For this reason, it is an important trait for genotypes selection in organic management. The acquisition of this character was achieved by drone, the first step was to draw the area to be mapped on a smartphone (Pix4d Capture software) . For this operation Google Heart images were used. Once the drone has mapped the area, whit a dedicated software (Pix4d Mapper) was possible to create an orthomosaic photo of the trial (Figure 4).

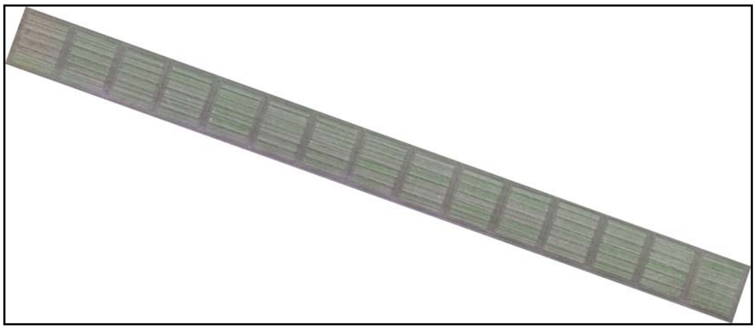

Figure 4: Agronomic trial Orthomosaic

To collect the soil coverage data, (three surveys for both trial in the first phenological stages (GS 11 to GS 32)
it is necessary to process the photo generated, with another open source software (Qgiss 3.6). With Qgiss we can finally draw a grid on the orthomosaic where each box on the grid is the equivalent of the plot in the field (Figure 5)

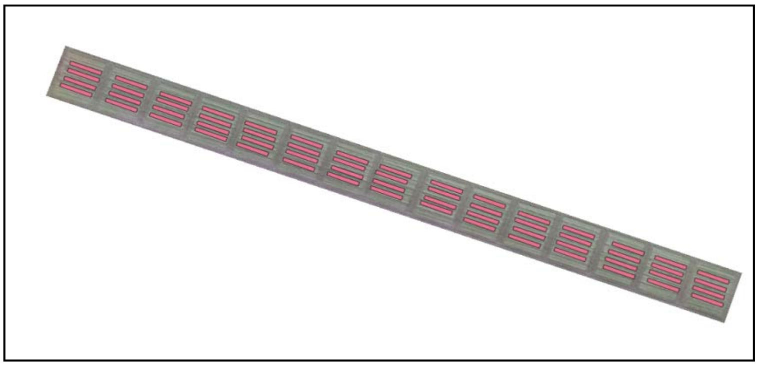

Figure 5: Trial grid drawn with Qgiss

The layer of the grid is geolocalized so the same grid was used for all data records on the same trial. Whit this grid we extracted the image of each single plot from the orthomosaic (Figure 6); then with the green color band was isolated and selected from the background, also modifying the parameters of brightness and saturation in order to create a mask that filters the vegetation elements from the others (soil, non-vegetative elements) by using the Image J free software (Figure 7). Finally, the number of pixels masked and compared to the total area was calculated, in order to obtain the numerical percentage of the vegetative elements.

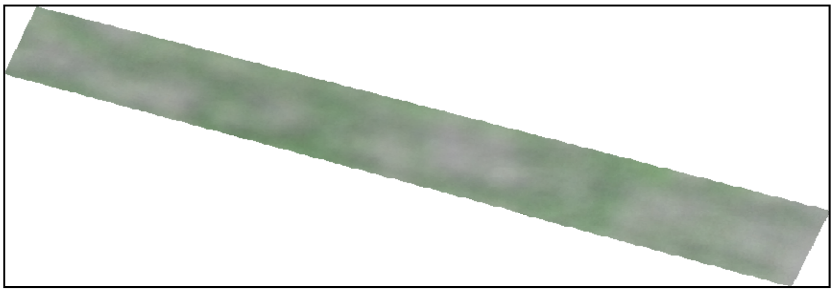

Figure 6: Plot image

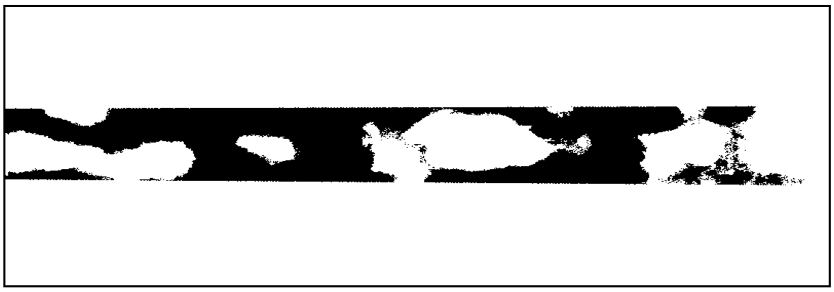

Figure 7: Mask of the green area

The NDVI (Normalized Difference Vegetation Index) is a vegetation index, correlated with the LAI (Leaf Area Index) and the green biomass, which describes the level of vegetative vigor of the crop. This index is given by the ratio between the difference and the sum of the radiations re-emitted in the near infrared and in the red, *i.e.* as (NIR-RED) / (NIR + RED) (Jackson and Huete, 1991; Hansen and Schjoerring, 2003; Araus and Cairns, 2014). The interpretation of the absolute value of the NDVI is highly informative, since it allows to immediately recognize the areas of the field that present developmental problems. This data was acquired by drone equipped with a multispectral cam, and the process is pretty the same described for the soil cover index acquisition. In this case it is possible to extract the NDVI value with Qgiss directly from the Geotiff image acquired, using the grid previously drawn. To collect the NDVI data three flights were performed at three different phenological stages (GS37, GS58 and GS80 Zadoks)

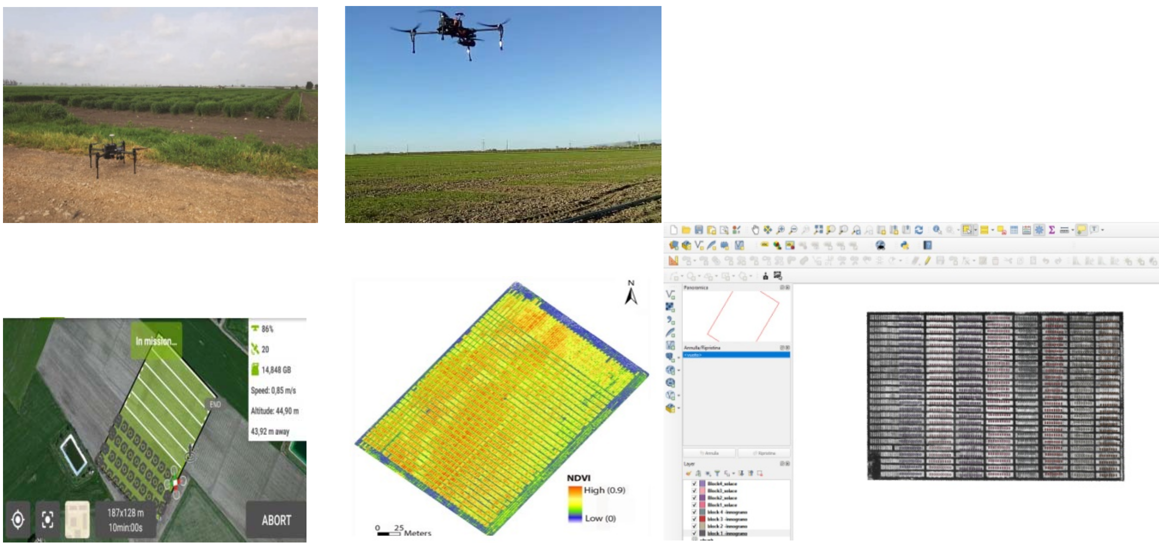

Figure 8: pre-flight check, flight monitoring by device, data detect and creation of the grid in Qgiss

UAV unmanned aerial vehicle: The drones used for the phenotypic evaluations were a Parrot bluegrass equipped whit a multispectral cam (Sequoia), able to take pictures at four different wavelenghts, for NDVI records; and a Matrice 100 drone equipped with a RGB camera to detect the soil cover index. Both drones are from DJI company.

Figure 9: Drones and cameras use

Qualitative grain analysis : The qualitative analysis on grain, *i.e.* protein content, test weight and humidity were made with a cereal analyzer Infratec Foss, based on NIT technology and NIR FOSS xds near infrared (Figure 10)

Figure 10: Grain analyzer Infratec^{TM} FOSS and NIR xds FOSS

Climate data: Climate data was provided by the meteorological service of CREA-CI. The meteorological station is located in Foggia inside the experimental farm ("Manfredini") of the research Centre, where the trials of the present project have been run (north latitude 41 ° 27 '06 ", east longitude 15 ° 33' 05" from Greenwich) (Figure 11)

Figure 81: Meteorological station at CREA-CI

3. Result and Discussion

3.1 Weather trend

The three growing seasons, in the experimentation area, were characterized by very different thermo-pluviometric trends (Figure 12 and 13). These differences had a significant effect especially on the results of the agronomic trial. The total amount of precipitation during the crop cycle 2018/19 was abundant, above the long-term average, 543 mm against 472 mm, while the 2016/17 season was characterized by a rainfall lower than long-term average, and abundant rainfall during the month of January. The growing season 2017/18 was characterized by a rainfall lower than the average, and it was higher in the last phase of the cycle.

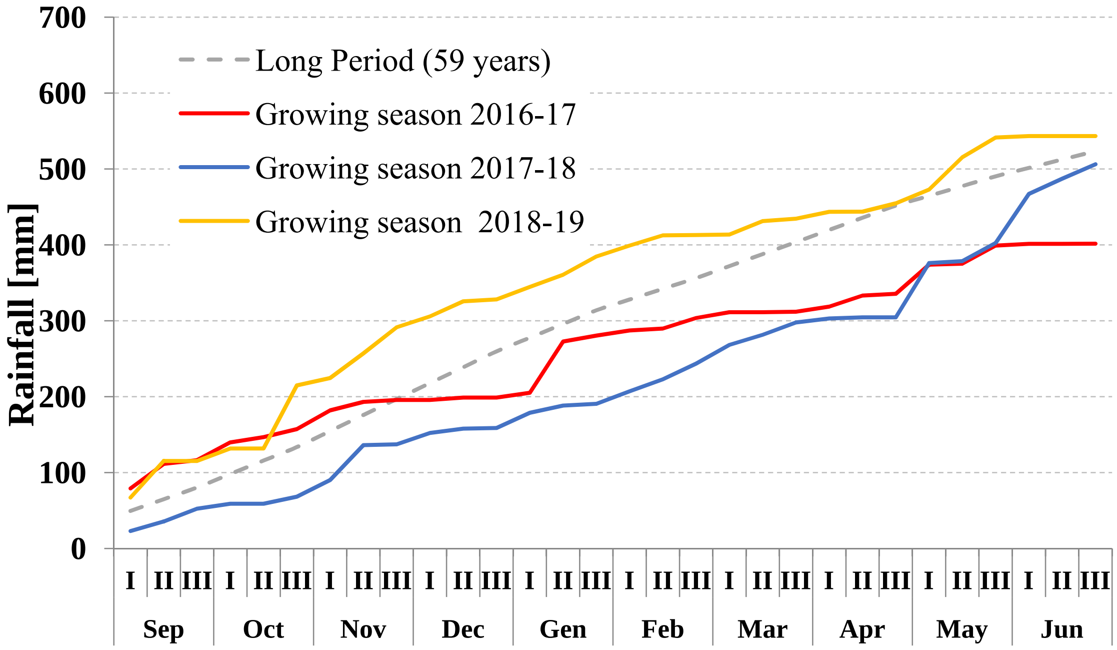

Figure 12: Thermopluviometric data detected during the three growing seasons at Foggia

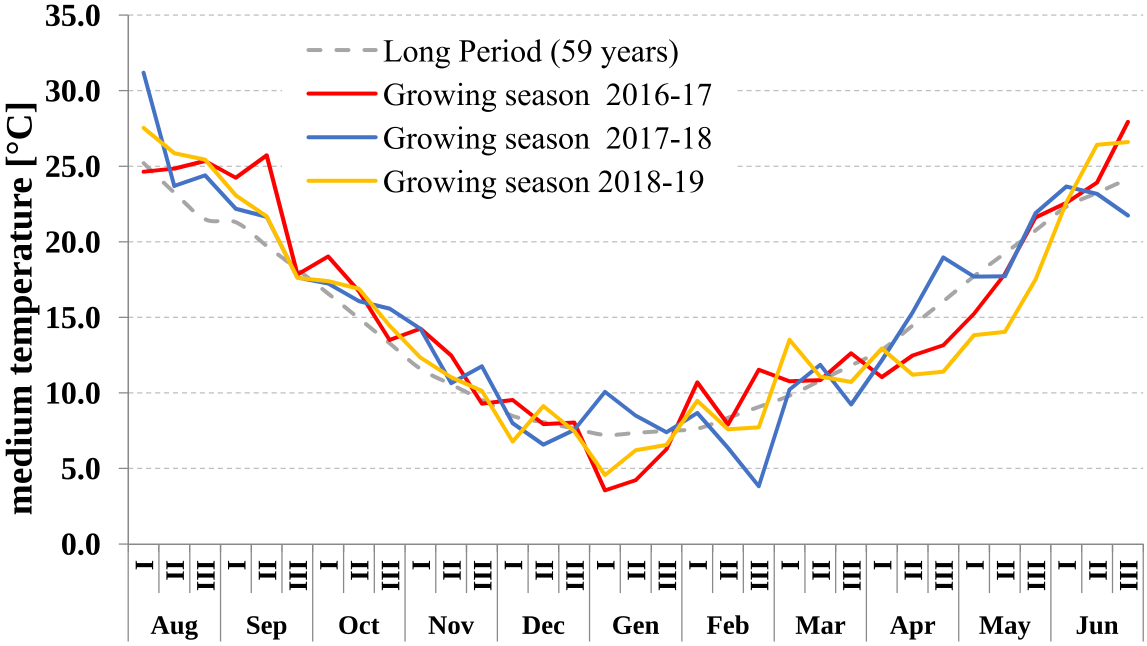

Figure 13: Medium temperature measured during the three growing season at Foggia

3.2 Variety Comparison trial results

Encouraging results have been achieved from the analysis of RGB images, that indicated the genotypes' ability to cover the soil in the early phase of growth. The results showed a high degree of variability and a good discriminatory ability among genotypes in term of soil covering ability (Figure 14). *Nadif* was the genotype with the highest coverage capacity (163,1%), ideal to be used as a genotype for organic farming. While *Don Pedro* turned out to be the variety with the lowest covering power (92,6%). *Ghibli* was instead the most productive with a good covering capacity (149,6%) and a medium plant height (74,5 cm) (Table 1). *Chelta* on the other hand turned out to be the least suitable genotype for Mediterranean area, with the lowest production (4.6 t/ha), a very long life cycle (Heading date 6 of

May) and a height too high (97,5 cm) therefore subject to the lodging risk. Observing only the first soil cover survey (flight of 09/03/2017), we noted that the variety *Olimpo* was the one with the higher percentage of coverage at that time, although in subsequent surveys this trait remained almost unvaried for this cultivar.

From the statistical analysis (Table 2), it appears that the genotype (G) had a significant effect on all the traits analyzed, including soil cover surveys, demonstrating that also this trait can be used as a discriminant in the selection of genotypes.

Based on the results of soil coverage, two varieties (*Natal* and *Nadif*), with opposite behavior, were selected to be used in the agronomic trial, in order to evaluate the discriminating effectiveness of the UAV system in different agronomic conditions (sowing density and fertilization).

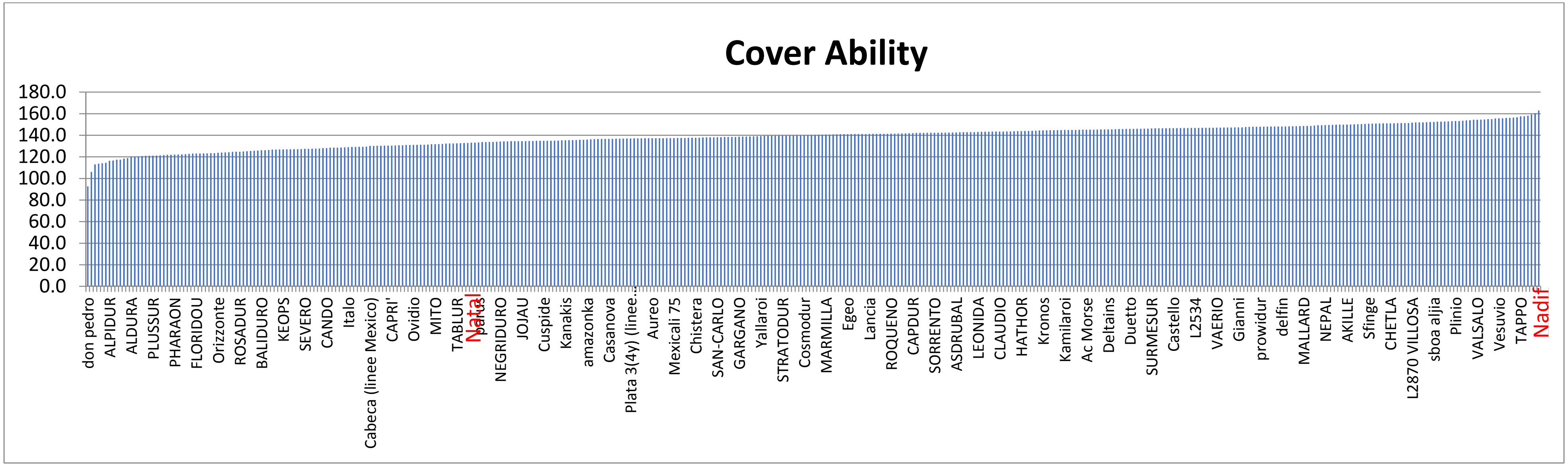

Figure 14: Different cover ability of 401 durum wheat genotype

		Soil Coverege% 09/03/17		Soil Coverege% 23/03/17		Soil Coverege% 10/04/17		Grain yield (t/ha)		Heading date (from 1th of April)		Plant Height (cm)	
	DF	MS	*p*	MS	*p*	MS	*p*	MS	p	MS	*p*	MS	*p*
Genotype	400	72	0.0000	59	0.0000	39	0.0000	7.5	0.0000	125.4	0.0000	198	0.0000
Error	805	47		36		18		3.1		6.9		20	
Total	1205												

Table 1: Anova analysis

Genotype	Soil Coverege% 09/03/17	Soil Coverege% 23/03/17	Soil Coverege% 10/04/17	Cover ability	Grain yield (t/ha)	Heading date (from 1th of April)	Plant Height (cm)
Ac Avolnea	45.4	52.7	56.8	154.9	9.4	32	90.0
Ac Morse	34.3	50.6	60.3	145.2	8.8	30	96.5
Ac Navigator	34.5	44.4	47.3	126.2	9.4	38	86.5
Achille	35.2	46.9	52.5	134.5	11.0	29	84.5
Ac-Melita	43.9	48.0	55.3	147.1	9.0	31	101.5
ACONCHI-89	39.1	52.8	52.9	144.8	7.0	34	68.5
ACTISUR	33.5	49.2	54.6	137.3	10.1	30	72.5
Adone	39.4	51.7	53.5	144.5	9.9	29	84.5
Agathe	41.6	49.2	53.3	144.1	9.1	33	76.0
Ajaia (linee Me	40.6	46.1	53.2	139.9	13.2	22	73.5
AKENATON	34.7	41.2	46.2	122.2	10.4	33	71.5
AKILLE	43.2	50.8	56.0	150.0	12.0	26	92.5
aksinit	26.8	37.0	53.6	117.4	7.6	25	68.0
ALCAO	42.4	50.5	53.8	146.8	11.3	28	63.5
ALDURA	31.0	42.0	47.0	120.0	9.7	22	59.0
Alemanno	43.3	50.7	57.9	151.9	10.6	18	70.5
alena	33.6	52.0	55.1	140.6	8.4	37	85.5
ALLUR	35.6	42.8	47.3	125.7	8.7	33	60.0
ALPIDUR	25.0	39.1	52.2	116.3	8.2	38	77.5
ALTAR-84	26.7	51.0	58.2	135.9	10.2	21	83.5
Altin	36.4	46.4	51.2	134.0	8.2	36	70.5
amazonka	36.6	48.4	50.9	136.0	9.0	37	73.5
Ambral	37.7	45.9	51.3	134.9	9.5	32	80.5
Anco Marzio	38.2	51.2	50.4	139.7	9.6	22	83.0
ankara 98	31.1	42.9	47.8	121.7	7.5	37	81.5
Antalis	35.0	46.1	49.9	131.0	12.7	25	76.0
Antas	41.1	56.7	55.0	152.8	11.2	25	72.5
Arcangelo	37.9	45.4	52.1	135.4	9.8	25	69.5
Arcobaleno	34.9	43.5	44.7	123.1	10.9	25	78.0
Ardente	40.6	50.2	53.1	143.9	11.7	25	80.0
argonavt	35.3	51.6	54.4	141.3	9.3	37	75.0
ARHIPELAG	37.3	57.2	58.2	152.6	10.9	30	80.0
Ariosto	39.8	48.4	52.9	141.1	11.5	31	73.0
ARISTAN	42.8	45.6	53.4	141.8	11.7	34	68.0
Arnacoris	38.5	50.3	56.5	145.3	13.5	20	71.5
ARTEMIDE	36.1	51.1	58.2	145.4	13.6	19	69.0
ASDRUBAL	39.5	49.2	53.9	142.6	11.7	19	85.5
Asterix	33.4	51.7	55.9	140.9	11.4	19	73.0
Athena	40.3	50.9	49.6	140.8	9.7	35	82.5
ATHORIS	35.6	51.2	53.1	140.0	11.0	19	71.0
auradur	29.2	38.0	47.0	114.1	6.2	25	83.5
Aureo	33.7	48.7	54.9	137.3	9.2	23	69.5
AURIS	41.3	52.0	51.9	145.2	10.8	34	73.5
AVENTUR	27.3	39.6	46.8	113.7	9.8	38	72.5
AVISPA	23.9	39.8	42.5	106.2	8.3	20	66.0
BABYLONE	34.7	47.6	48.9	131.2	8.4	36	68.5
BAIO	40.7	47.5	53.0	141.1	8.9	26	78.0
BAKARDI	32.5	45.7	47.0	125.2	8.9	31	80.5
BALIDURO	29.2	41.8	55.2	126.2	10.0	25	71.0
BALSAMO	37.8	47.2	53.4	138.5	10.2	23	79.5

BANCO	36.9	46.9	55.9	139.7	11.4	33	81.0
BELDUR	36.1	49.4	46.4	131.9	11.0	20	77.0
Belfuggito	28.8	44.8	49.5	123.1	6.7	19	93.0
Berillo	41.2	48.1	57.6	146.8	7.6	29	79.0
BISKRI	41.1	54.9	56.6	152.6	5.1	36	101.0
BRADANO	49.2	53.8	48.5	151.4	9.8	26	71.0
BRAVO	34.1	44.2	49.7	128.1	10.7	32	72.5
BRENNUR	38.4	53.0	53.5	144.9	9.7	29	69.5
Bronte	39.4	55.3	57.4	152.1	12.0	20	76.0
burshtin	30.4	40.2	47.8	118.4	10.7	34	87.0
Cabeca (linee M	34.8	47.2	48.2	130.2	10.1	27	69.0
CALCAS	42.5	46.1	53.9	142.5	12.4	22	67.0
CALO'	35.7	51.8	50.6	138.1	10.3	19	75.5
CAMPIONE	36.0	43.2	49.3	128.5	8.5	38	65.0
Campodoro	38.7	52.4	54.8	145.9	6.8	34	80.0
CANDO	26.3	48.8	53.1	128.1	10.4	35	80.5
Cannizzo	34.7	55.8	59.4	149.9	7.3	35	82.5
Canyon	40.1	47.5	55.4	143.0	12.9	22	75.0
CAPDUR	40.1	48.8	53.1	142.0	6.4	35	68.5
CAPRI'	35.5	44.9	50.0	130.4	6.0	35	74.5
CARIOCA	43.7	46.4	52.1	142.2	10.3	20	71.5
CARPIO	35.3	44.5	50.4	130.2	10.6	20	78.5
Casanova	35.8	47.1	53.8	136.7	8.6	25	66.0
Castello	38.8	55.5	52.3	146.6	9.2	28	66.5
Catasta	36.8	52.4	57.5	146.7	11.0	22	78.5
CATERVO	37.0	57.3	55.0	149.3	11.2	26	75.0
Ceedur	34.9	45.2	53.1	133.1	10.7	30	75.0
CELESTINO	40.4	52.2	57.9	150.4	7.1	19	71.0
CELSO	27.4	38.9	55.1	121.3	9.7	16	52.5
Cer904	36.3	42.1	46.6	125.0	10.8	21	76.5
Cesare	43.9	47.2	53.6	144.6	12.3	29	73.5
CHAM3	43.7	50.6	55.8	150.1	10.0	19	74.0
CHANDUR	37.7	51.2	54.7	143.5	11.4	21	70.5
CHETLA	39.7	52.7	58.7	151.1	4.6	36	97.5
Chiara	39.8	50.1	53.1	142.9	10.5	20	74.0
CHILI	44.0	53.1	61.1	158.2	6.9	37	104.0
Chistera	32.4	49.6	55.8	137.8	10.7	33	86.0
Ciclope	41.6	53.4	56.0	151.0	12.4	24	64.5
CINCINNATO	30.4	52.7	53.9	137.0	10.0	30	68.0
Cirillo	49.6	51.9	51.3	152.8	10.1	31	79.5
CLAUDIO	36.7	50.5	56.2	143.5	12.6	23	76.5
cliodur	32.9	42.0	47.4	122.2	5.5	37	84.5
CLODIS	48.4	48.1	53.9	150.5	12.6	24	79.5
Colombo	32.6	44.4	50.6	127.6	9.3	31	74.5
COLORADO	32.4	50.3	51.8	134.5	8.8	20	66.0
Colosseo	43.0	54.6	58.3	156.0	8.6	28	77.5
CONCADORO	48.4	52.4	53.1	154.0	6.6	22	74.0
CONDURUM	35.7	41.9	48.2	125.8	7.0	38	68.5
Core	29.7	49.4	56.0	135.1	11.9	21	74.5
CORPUR	40.6	52.7	51.4	144.7	9.2	36	70.5
Cortez	41.4	54.5	51.1	146.9	11.8	22	80.5
Cosmodur	37.2	48.1	54.8	140.0	11.0	19	66.5
COUSSUR	30.0	51.7	52.6	134.3	10.2	31	75.5
Credit	37.1	53.0	52.8	142.9	11.6	20	75.0
Creso	28.1	44.9	56.1	129.2	10.3	30	77.0

CRIVU	39.4	55.1	52.4	147.0	10.6	16	69.5
CURZIO	35.7	55.7	56.0	147.4	10.0	25	71.0
Cuspide	34.1	47.9	53.0	135.0	11.1	26	81.0
Dario	37.6	50.2	51.9	139.6	9.0	28	70.5
DAUNIA	41.2	53.7	54.1	149.0	11.9	20	74.5
DEDALO	33.2	57.6	58.9	149.7	10.9	18	73.0
delfin	43.8	52.9	51.4	148.2	7.1	24	77.5
delta	40.5	51.5	58.8	150.9	7.8	35	75.5
Deltains	36.3	52.5	56.6	145.5	11.4	26	80.0
Desert King (or	36.8	46.0	51.7	134.5	11.0	22	71.3
don pedro	22.8	27.8	42.1	92.6	9.2	33	73.5
DON RICCARDO	42.6	50.9	56.3	149.9	11.1	22	74.0
Doral	43.1	51.4	57.4	151.9	9.8	22	87.0
Dorato	33.3	51.3	56.8	141.4	12.5	25	75.5
Drysdale	39.5	58.5	55.4	153.4	11.2	19	84.5
Duetto	46.9	46.7	52.2	145.9	12.5	34	79.0
Duilio	40.8	53.2	58.3	152.3	10.5	17	76.0
Dupri	37.5	42.9	49.0	129.3	9.8	29	69.5
DURABON	34.6	48.7	57.1	140.5	9.1	36	92.5
duramar	30.5	44.4	46.3	121.2	9.0	37	85.0
Durango	41.3	50.8	54.0	146.1	10.9	33	73.5
Durfort	34.2	52.7	56.5	143.5	10.7	26	68.5
durobonus	32.6	53.2	52.3	138.2	10.0	33	75.5
Dylan	38.6	54.4	58.1	151.1	12.5	28	79.5
Egeo	38.9	50.9	51.2	141.1	11.7	24	83.5
ELIOS	32.1	44.3	49.8	126.2	11.6	24	75.5
Elsadur	32.9	44.6	49.3	126.8	10.8	34	87.0
Emilio Lepido	35.5	48.2	52.8	136.5	12.2	21	63.5
Enduro	35.9	55.2	59.2	150.2	8.8	16	75.0
ERCOLE	40.8	49.7	54.7	145.2	10.8	30	71.5
ERMOCOLLE	27.8	51.7	53.4	132.9	11.2	21	61.5
Esperto	35.8	43.9	45.9	125.5	12.0	30	66.0
Ettore	42.0	48.2	48.0	138.2	12.9	24	75.0
Exeldur	39.2	36.8	42.9	118.9	8.4	29	62.5
FABULIS	42.1	51.5	53.5	147.1	8.7	33	72.0
Fauno	41.6	47.9	52.7	142.2	10.3	18	71.0
FENICE	39.5	50.2	52.7	142.3	9.8	30	69.5
Fenix	32.8	49.8	49.3	131.9	6.3	35	74.5
Fiore	33.5	47.4	55.7	136.6	10.1	22	70.5
FLAMINIO	37.1	50.2	56.1	143.3	9.7	25	72.5
FLAVIO	34.9	47.5	52.2	134.5	8.4	24	75.5
floradur	33.1	51.0	53.4	137.6	9.0	36	72.5
FLORIDOU	29.6	46.2	47.3	123.1	11.5	37	71.5
Fortore	35.9	49.1	52.0	137.0	10.6	18	66.0
GAN	32.8	54.7	58.7	146.2	12.2	23	73.0
gardemarin	32.8	37.1	46.7	116.6	10.4	37	75.5
Gardena	41.2	52.9	56.1	150.2	9.4	19	64.0
GARGANO	40.5	45.8	52.4	138.7	9.2	18	69.0
GATTUSO	38.1	47.4	56.1	141.7	7.5	21	65.5
gelios	38.9	55.8	58.5	153.1	9.1	26	116.5
GENNARO	49.8	48.2	53.3	151.3	12.6	25	76.0
GHIBLI	45.6	48.5	55.5	149.6	13.8	24	74.5
Gianni	41.6	51.8	53.9	147.3	10.3	18	67.0
Gibraltar	34.2	48.9	50.0	133.1	9.5	25	81.0
GIBUS	49.6	51.5	53.4	154.4	9.4	33	78.5

GIOVE	33.3	46.2	50.9	130.4	9.3	21	60.5
GIUSTO	40.7	51.7	55.7	148.1	12.5	25	79.5
gk betadur	32.2	40.3	44.9	117.4	8.6	36	58.0
gk julidur	37.6	49.0	47.3	133.9	11.1	36	84.0
gk novodur	36.9	48.1	45.5	130.6	7.6	35	64.0
gk pannondur	30.9	44.2	46.8	122.0	9.6	38	69.5
gk selyemur	38.7	45.8	47.9	132.4	7.3	37	75.5
gk tiszadur	37.3	51.5	50.7	139.6	8.4	37	75.0
gk-aga	35.8	51.1	50.4	137.4	9.6	37	85.0
gk-minaret	34.8	47.3	48.2	130.3	8.5	33	81.0
GLOCODUR	37.0	53.5	50.8	141.3	11.6	21	80.0
gordeiforme 114	34.6	44.7	50.0	129.4	9.7	31	70.0
gordeiforme 6	45.8	46.8	48.8	141.5	9.0	37	99.0
Granizo	33.1	37.9	49.1	120.1	8.3	37	76.0
Grazia	35.5	45.3	50.6	131.3	11.7	29	69.0
HATHOR	41.7	50.4	51.7	143.9	10.4	26	75.5
HISPASANO	36.3	44.2	44.1	124.6	11.1	23	70.0
Homer	34.5	53.9	59.8	148.3	9.6	25	85.5
ICARO	38.1	50.0	59.1	147.3	10.8	21	63.0
IONIO	37.7	52.7	53.9	144.2	12.0	24	79.5
IRIDE	36.0	49.7	51.8	137.5	12.4	23	65.0
is pentadur	37.8	49.4	51.7	139.0	8.2	38	81.0
Isidur	31.9	43.0	49.1	124.0	10.8	33	56.5
ISILDUR	34.7	44.2	48.8	127.6	11.7	36	63.0
ISMUR	39.0	51.6	51.7	142.4	12.1	27	75.5
Italo	29.2	49.0	50.8	129.0	8.6	20	75.0
IXOS	30.1	48.9	48.2	127.2	9.4	29	71.0
JANEIRO	38.1	52.0	50.3	140.3	10.6	35	70.5
jaschma	36.2	48.8	47.8	132.8	8.1	32	76.5
JOJAU	41.5	37.2	55.9	134.6	9.7	33	79.0
JORDAN	40.9	55.5	58.7	155.1	5.5	29	75.0
JUSTIDUR	29.7	51.6	50.4	131.8	11.7	31	67.0
K26	39.4	53.9	54.7	148.0	10.2	29	73.5
Kamilaroi	42.9	49.5	52.5	144.9	11.4	17	70.0
Kanakis	35.7	46.6	53.1	135.4	12.4	22	70.0
Karel	39.3	51.1	56.6	147.0	12.6	21	79.5
KARIM	34.0	52.2	57.2	143.4	9.5	19	65.5
KEOPS	35.9	43.4	47.6	126.9	11.4	23	62.5
kermen	34.7	43.5	44.9	123.2	8.9	37	85.5
kharkovskaya-32	36.0	48.3	54.8	139.1	7.0	38	82.0
Khiar	31.0	40.6	50.3	121.9	10.7	22	77.0
KIKO'	38.2	49.1	49.8	137.1	10.6	22	71.0
KIKONICK	41.3	49.5	55.9	146.7	10.9	19	73.3
kiradur	40.9	47.5	51.8	140.1	9.0	34	80.0
kiziltan 91	33.2	50.4	50.9	134.6	9.2	37	72.5
Kofa	35.7	54.4	55.1	145.2	9.8	18	70.0
KOMBO	35.8	48.8	52.2	136.8	10.3	30	60.0
koralodesskij-1	37.0	54.5	51.9	143.4	9.0	37	90.5
Kronos	41.1	51.0	52.5	144.6	11.7	15	68.5
krupinka	33.5	52.2	59.2	145.0	10.1	21	80.0
kurant	26.5	38.2	49.8	114.5	9.3	28	67.0
L1864	38.6	43.1	54.1	135.8	9.3	21	70.5
L2081	27.3	44.5	55.2	127.0	11.0	17	84.5
L2284=P22D84	39.5	45.7	47.1	132.3	11.7	26	77.5
L2300=NATAL	30.6	49.2	48.7	128.5	10.8	28	80.1

L2443=NADIF	46.8	56.8	59.5	163.1	11.5	24	74.0
L2445	32.3	52.3	52.9	137.5	10.8	33	73.5
L2518 VILLOSA	40.0	50.7	57.2	147.9	10.2	19	84.0
L2534	43.2	49.8	53.9	146.8	9.5	18	76.0
L2542	34.5	47.9	51.4	133.8	9.3	22	74.5
L2554	41.6	51.2	55.3	148.1	10.5	22	75.5
L2574	39.6	50.8	54.5	145.0	11.6	24	84.5
L2578 GLABRA NN	31.4	40.8	48.8	121.0	8.8	27	72.0
L2842	40.5	46.3	48.7	135.5	11.8	24	75.5
L2870 VILLOSA	41.9	56.7	53.0	151.6	12.1	30	75.5
Lancia	42.7	49.0	49.6	141.2	11.0	30	78.5
Latino	36.4	47.6	54.5	138.5	11.7	21	79.5
LEONIDA	42.4	49.6	51.0	143.0	9.7	21	66.5
Lesina	40.6	52.1	53.3	146.1	9.5	19	62.0
leukurum 21	40.6	52.9	52.5	146.0	9.7	38	79.5
leukurum 479	30.4	52.5	47.9	130.8	7.4	38	76.5
Lira	46.3	48.6	53.5	148.3	11.7	27	75.0
logidur	30.9	42.6	49.7	123.2	9.4	27	73.0
LUMINUR	39.7	47.7	52.4	139.8	6.7	16	69.5
lunadur	33.9	43.0	51.5	128.5	8.0	37	61.5
lupidur	29.8	43.3	51.0	124.2	7.7	38	66.0
M.TRZABEY	36.0	47.2	49.5	132.7	8.7	37	83.5
Maestrale	36.1	54.2	58.4	148.7	10.9	20	80.5
MAKIT	36.6	46.5	54.1	137.2	10.9	31	69.0
MALLARD	42.7	50.9	55.0	148.6	10.6	22	74.0
MARCO	44.8	53.9	55.9	154.7	10.8	28	73.5
MARMILLA	40.0	48.2	52.5	140.7	6.7	24	64.0
MATT	41.9	53.1	53.6	148.5	11.3	21	70.5
Mexicali 75	37.8	47.6	52.0	137.4	10.8	19	76.5
MG54	35.0	50.3	52.0	137.3	10.8	30	79.5
Mida	35.0	54.1	53.4	142.5	9.9	31	72.5
MIDYNU	38.8	50.4	52.3	141.4	13.6	19	80.0
Mimmo	42.1	54.8	54.4	151.3	10.8	23	81.5
Minosse	40.3	48.6	52.9	141.9	10.3	25	71.0
miradova	35.2	45.0	50.2	130.4	10.1	34	72.0
Mirandur	22.5	54.0	51.2	127.7	9.8	34	79.5
MITO	34.5	46.1	51.2	131.8	9.7	19	62.5
Mohawk	40.9	52.7	52.0	145.6	11.4	19	71.0
Monastir	30.9	57.1	55.5	143.4	11.4	28	75.0
MONGIBELLO	35.8	50.7	51.1	137.6	10.4	20	65.5
montferrier	30.7	49.3	52.5	132.5	9.0	38	76.5
MURANO	36.5	51.2	53.4	141.1	9.0	34	77.0
mv vitadur = pe	34.4	46.4	46.4	127.2	9.0	37	68.5
NADIR	38.8	48.5	55.1	142.4	9.5	25	71.0
NAUTILUR	45.0	56.6	57.7	159.2	10.6	29	64.0
Nefer	35.4	50.8	55.0	141.1	9.4	28	75.0
NEGRIDURO	37.7	46.5	50.0	134.2	11.0	21	72.5
NEMESIS	38.5	51.0	58.6	148.1	10.2	33	68.0
Neodur	32.7	42.6	45.0	120.3	9.1	35	72.0
NEPAL	40.7	55.7	53.0	149.5	9.0	33	63.5
Nerone	42.4	51.8	50.3	144.5	10.5	35	82.5
NORA	29.6	42.6	51.3	123.5	8.9	19	81.5
Norba	35.8	51.5	57.8	145.1	7.9	19	74.5
Normanno	33.1	50.3	50.4	133.8	9.3	29	66.5
NUDURA	40.5	51.8	59.0	151.3	10.4	18	64.0

nursith	38.5	44.6	43.7	126.8	6.6	34	71.0
Obelix	42.4	51.4	51.6	145.4	11.7	33	85.0
OCOTILLO	39.4	50.7	55.7	145.8	9.9	16	75.0
odessa #65	35.5	44.4	43.6	123.5	10.0	38	90.5
Odisseo	36.3	44.6	53.0	133.9	10.4	28	74.5
Olimpo	51.7	52.1	53.9	155.7	11.5	17	64.5
OLINTO	42.2	48.7	50.3	141.2	9.2	35	74.5
OLIVER	40.3	47.0	54.2	141.6	10.0	27	75.5
Opera	44.6	45.9	53.7	144.1	10.8	25	68.0
Orizzonte	33.7	43.5	46.6	123.9	9.6	18	62.0
Ovidio	35.3	44.1	51.7	131.1	11.8	26	72.0
Pablo	35.5	45.7	49.3	130.4	8.7	30	74.5
Paleotto	39.1	51.1	56.2	146.5	12.1	28	82.5
pandur	34.4	44.1	49.1	127.5	9.0	35	78.0
PAPADAKIS	36.8	47.7	52.1	136.6	11.4	19	78.0
PAPRIKA	37.6	53.0	54.4	145.0	10.8	19	71.0
Parsifal	41.1	50.4	55.8	147.3	10.7	19	77.5
parus	38.9	44.7	49.8	133.4	8.1	37	77.0
PASTIFLUR	28.1	46.1	52.5	126.7	9.9	28	74.0
PELEO	33.4	49.2	54.8	137.3	11.1	23	69.0
PERES	35.6	52.3	52.0	140.0	9.2	28	70.0
perlyna odeska	36.2	49.6	48.8	134.6	9.2	35	75.5
Perseo	43.3	50.6	54.3	148.2	11.6	20	71.0
PESCADOU	35.9	48.4	50.6	135.0	9.5	32	77.5
Phaethon (line	37.3	52.5	56.8	146.6	9.3	23	77.5
PHARAON	32.3	42.4	47.4	122.2	9.5	34	67.0
PICENO	38.7	54.1	58.3	151.1	9.9	26	76.0
PICODUR	44.3	47.4	52.0	143.8	8.2	38	66.5
PICTUR	32.5	46.3	56.7	135.5	11.4	33	72.5
Pietrafitta	35.6	54.7	59.1	149.3	9.6	22	67.0
Pigreco	26.4	48.7	53.5	128.6	11.0	21	81.5
PITAGORA	29.7	54.3	54.9	138.9	9.2	26	69.5
Plata 3(4y) (li	35.6	51.4	50.1	137.1	8.9	20	69.0
Plinio	43.2	55.0	54.9	153.2	10.0	29	72.0
PLUSSUR	32.9	42.8	45.4	121.1	9.3	34	69.5
Poggio	40.5	53.3	54.3	148.1	9.9	31	79.5
PORTORICO	40.3	52.7	53.7	146.8	11.1	26	78.5
PR 22 D 66	37.8	49.5	52.2	139.5	10.0	31	65.0
PR-22-D-78	39.0	50.0	51.8	140.9	9.3	20	61.5
PR-22-D-89	36.2	49.0	51.5	136.7	11.2	26	77.5
PRINCIPE	40.6	45.3	51.4	137.3	8.6	21	66.5
PROCACE	36.7	52.5	57.3	146.5	9.9	31	73.5
Produra	36.2	49.5	50.6	136.3	8.8	17	66.0
PROMETEO	36.3	47.9	50.6	134.7	8.3	23	69.5
prowidur	41.2	53.2	53.6	148.0	9.2	38	68.5
Quadrato	35.8	49.1	52.9	137.8	11.7	20	69.0
Quadruro	44.4	55.6	56.2	156.2	10.4	27	78.0
QUALIDUR	35.3	54.1	51.0	140.3	11.0	33	71.0
RADIOSO	42.0	51.8	51.0	144.8	9.6	16	64.5
Ramirez	35.1	53.1	55.8	144.0	11.0	30	78.0
REAMUR	30.3	36.2	46.5	113.1	8.7	33	75.5
Rodur	34.9	51.2	51.0	137.2	7.4	35	70.0
ROMANNI	38.0	53.1	55.5	146.7	7.5	35	91.5
ROQUENO	36.7	51.1	53.7	141.5	8.7	29	81.5
ROSADUR	31.9	46.1	46.8	124.8	10.1	33	88.0

Rusticano	35.1	42.4	45.0	122.6	11.7	22	67.5
S. MARCO	38.3	59.8	58.3	156.4	11.8	28	75.5
SABIL 1	41.2	60.6	52.0	153.8	11.0	18	84.5
SACHEM	32.1	45.5	45.2	122.7	10.5	35	61.5
SALAPIA	40.9	55.0	60.8	156.7	11.6	22	76.0
SAN-CARLO	36.5	51.2	50.5	138.2	12.0	23	76.0
SANT-AGATA	27.8	54.4	55.7	138.0	11.0	20	81.0
SARAGOLLA	37.7	51.0	50.2	138.8	11.3	19	72.5
sboa aljia	44.6	51.6	56.1	152.4	7.4	36	116.5
Sculptur	40.8	47.7	50.1	138.5	10.8	19	77.0
selcuklu-97	38.7	45.3	52.6	136.6	10.9	38	84.5
SERAFONIK	38.2	49.1	55.3	142.6	11.7	27	75.0
SEVERO	33.2	45.2	49.1	127.5	12.8	24	74.0
Sfinge	43.4	54.1	53.2	150.7	11.7	19	67.0
Sharm 5	32.1	53.9	59.8	145.8	6.4	21	82.5
SIJAH KILAKLI	45.7	52.8	52.2	150.8	7.9	30	89.0
SIMETO	32.8	48.6	53.6	135.0	10.8	22	70.0
snowglenn	34.5	50.9	48.9	134.3	7.8	41	77.5
SOLEX	36.4	45.6	47.3	129.4	9.9	31	71.0
SOLITARIO	36.4	53.1	56.4	145.9	10.7	30	75.5
SORRENTO	38.1	50.8	53.5	142.4	11.5	23	87.0
Sorriso	29.3	40.6	51.2	121.1	10.3	23	67.0
Spartaco	40.3	47.2	53.6	141.1	9.9	20	73.5
STRATODUR	33.9	54.1	51.8	139.7	10.6	34	78.0
Strongfield	41.4	52.0	54.3	147.7	8.7	37	100.5
Suraka	35.6	40.6	48.1	124.2	12.1	25	75.5
SURMESUR	44.8	50.3	51.3	146.3	11.6	35	87.5
SVEVO	34.5	51.8	56.3	142.5	6.6	16	75.5
SY CARMA	41.4	51.2	50.9	143.5	11.0	28	66.0
SY CYSCO	39.4	43.1	48.6	131.0	9.9	29	72.0
SY-EXPERTO	38.2	44.0	46.6	128.8	11.4	27	63.0
TABLUR	36.8	46.0	49.7	132.5	10.3	35	64.0
TANIT	42.0	53.7	55.4	151.0	11.8	19	71.5
TAPPO	45.1	57.3	55.2	157.6	10.2	36	70.5
TAVOLIERE	34.8	50.4	56.0	141.1	10.0	21	65.5
Tito	42.2	46.5	48.1	136.8	7.3	37	80.5
Tiziana	48.0	51.0	56.5	155.6	12.4	27	79.5
TOPDUR	36.5	51.6	47.6	135.6	9.3	35	75.0
TORRESE	28.4	47.3	51.3	127.0	10.1	26	78.0
TRIONFO	39.6	51.9	56.9	148.4	10.6	27	74.0
TRIPUDIO	37.6	50.3	55.1	143.0	12.1	20	74.5
troubadur	31.6	47.4	52.4	131.4	9.9	36	72.0
TURCHESE	35.5	45.3	48.8	129.5	9.3	20	77.0
ULISSE	34.4	49.2	54.8	138.4	10.9	29	86.5
VAERIO	42.0	49.5	55.5	147.0	9.3	20	65.0
Valbelice	50.9	48.8	54.8	154.5	10.1	25	76.0
VALERIO	29.6	42.2	49.0	120.8	10.3	22	61.5
Valforte	40.6	54.3	52.2	147.2	10.1	27	72.5
Valgerardo	38.5	53.5	53.7	145.6	9.5	27	72.0
VALIRA	38.7	50.8	52.8	142.2	10.7	21	75.0
VALITALICO	39.2	55.8	54.6	149.5	9.6	29	75.0
VALLELUNA	35.1	50.5	57.2	142.8	5.0	26	87.0
VALRICCARDO	41.3	52.1	54.4	147.8	10.0	26	73.0
VALSALO	43.2	53.7	57.5	154.5	11.6	28	76.5
VALSELVA	45.3	56.4	55.9	157.6	8.8	28	71.5

Varano	36.1	49.3	54.1	139.5	9.9	23	72.5
Vendetta	37.9	47.4	52.5	137.8	11.2	21	75.5
VENTO	42.0	54.5	55.3	151.9	10.4	30	65.5
VESPRO	38.5	51.2	52.0	141.7	10.5	22	72.0
Vespucci	42.8	50.9	49.6	143.3	10.7	29	73.5
Vesuvio	43.2	55.0	57.5	155.6	10.7	24	77.5
VETRODUR	50.3	53.6	56.4	160.4	11.4	21	79.0
VEZIO	40.6	50.9	55.3	146.9	10.2	24	81.5
VILLEMUR	38.4	47.9	48.7	135.0	11.0	31	79.0
VITRON	38.2	53.1	55.1	146.5	12.0	21	65.5
VOLTURNO	39.9	52.5	56.2	148.6	11.0	18	72.0
wallaroi	33.6	48.4	57.0	139.0	12.5	18	59.5
windur	34.9	48.2	51.9	135.0	7.0	34	79.5
Wintergold	28.4	45.8	52.2	126.4	10.3	23	68.5
Yallaroi	37.1	49.1	53.1	139.2	8.7	20	57.5
YAVAROS	39.1	48.2	49.9	137.3	9.8	22	70.5
YELLODUR	44.1	56.9	52.2	153.2	11.5	19	77.5
yilmaz98	29.8	44.2	50.7	124.7	8.3	23	74.0
YUKON	31.7	45.2	50.2	127.1	10.8	33	69.5
ZETAE	40.6	52.9	54.4	147.9	10.1	17	63.0
zolote runo	31.8	52.6	52.9	137.3	10.4	36	71.0
lsd 0.05	10.98	9.63	6.74	4.53	2.80	4.21	7.07
Average	37.25	49.16	52.62	139.04	10.03	27	74.6

Table 2: Average of the acquired data of 401 durum wheat genotype

3.2 Agronomic trial results

All phenotypic and qualitative data collected during the experimental test were statistically processed using the General Linear Model using SAS / STAT 9.2 software (SAS Institute Inc., Cary, NC, USA). Table 3 show the data averages of all the characters acquired during the three years of experiments.

Year	**Grain yield (t/ha)**	**Heading date (from 1th of April)**	**Protein content %**	**Soil coverege % 1**	**Soil coverege % 2**	**Soil coverege % 3**
2017	4,86	24,13	14,14	8,95	19,58	49,82
2018	5,59	28,93	16,86	5,77	17,23	52,98
2019	5,72	28,88	14,21	8,04	23,46	58,22
P<0.05	***	***	***	***	***	***
Genotype	**Grain yield (t/ha)**	**Heading date (from 1th of April)**	**Protein content %**	**Soil coverege % 1**	**Soil coverege % 2**	**Soil coverege % 3**
NADIF	5,55	28,49	14,92	9,14	23,51	58,73
NATAL	5,23	26,14	15,21	6,04	16,67	48,61
P<0.05	***	***	***	***	***	***
Seed Rate	**Grain yield (t/ha)**	**Heading date (from 1th of April)**	**Protein content %**	**Soil coverege % 1**	**Soil coverege % 2**	**Soil coverege % 3**
200	5,23	27,91	15,21	4,54	16,00	47,20
400	5,56	26,72	14,93	10,64	24,18	60,15
P<0.05	***	***	***	***	***	***
Nitrogen	**Grain yield (t/ha)**	**Heading date (from 1th of April)**	**Protein content %**	**Soil coverege % 1**	**Soil coverege %**	**Soil coverege % 3**
0	5,28	26,81	13,67	7,20	20,86	50,74
60	5,34	27,00	14,96	8,41	20,46	53,65
120	5,42	27,50	15,11	7,48	18,96	53,53
180	5,48	27,44	15,62	7,68	19,71	55,05
240	5,45	27,83	15,98	7,17	20,46	55,39
P<0.05	*ns*	***	***	*ns*	*ns*	***

Table 3: data averages of all characters acquired during the three growing season

The analysis of the data showed that the year (Y) was significant for all the characters analyzed, the effect being likely mainly due to the different amount of rainfall during the three growing seasons. Sowing density also had a significant effect on the traits analyzed, in particular in the percentage of soil cover ability in the early stages of growth. Higher seeding rate is one approach that helps to increase crop competitiveness against weeds (Chauhan and Johnson, 2011). Higher seeding rates facilitate quick canopy closure, which helps suppress weeds more effectively. At low seeding rates, crop plants take more time to close their canopy, which encourages weed growth (Guillermo ate al., 2009). High seeding rates improve the ability of crops to suppress weeds and can reduce yield loss under partially weedy conditions. The data also showed the different ability of the two genotypes in terms of soil cover capacity in the early stages of growth (Figure 14)

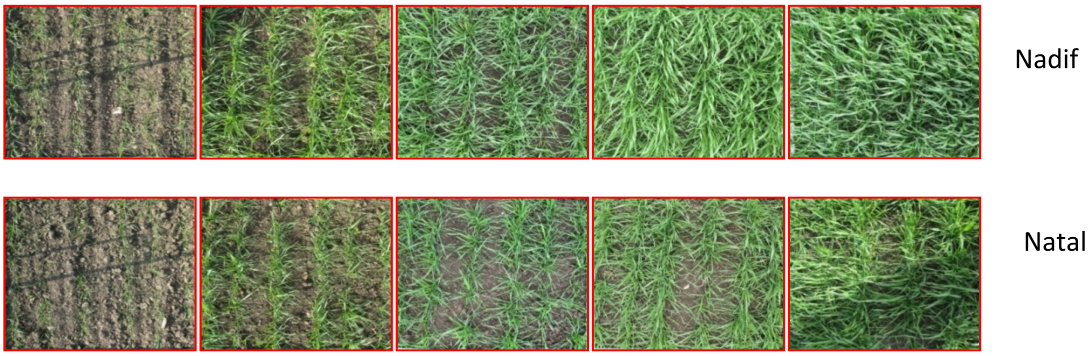

Figure 14: Different genotypes cover ability

The different levels of fertilization, on the other hand, seem to have no effect on soil cover capacity, but only, as we expected, on the protein content and on the heading date. This because fertilization has been carried out at different intervals throughout the growth cycle of the crop, so in the early stages there were no big differences between the various theses. Analysis of variance showed that there was a clear interaction between genotype and seed rate for the first and third scoring of soil coverage (p=0,000; p=0,019) (Table 4).

There was also a three-way interaction, for the same record, between year, genotype and seed rate ($p=0,000$; $p=0,017$). While there were no interactions between year, seed rate and nitrogen, as also for genotype, seed rate and nitrogen ($p>0,500$). The analysis of the last year experimentation data, showed the same trend for the three growing seasons (Table 5), genotype and seed rate had significant effect on all traits acquired. A good correlation was found between all the soil coverage measurements and grain yield, as well as for the NDVI measurement, in particular for the 2nd record ($p=0,6137$), close to the flowering date (Zadoks GS 55) (Table 6); thus confirming the possible exploitation of NDVI index in this growth stage as an yield predictor (Muhammad Adeel Hassan et al., 2019).

		Grain yield (t/ha)		Heading date (from 1th of April)		Protein content %		Soil coverege % 1		Soil coverege % 2		Soil coverege % 3	
	DF	MS	p	MS	p	MS	p	MS	p	MS	p	MS	p
Year	2	12,85	**0,000**	456,10	**0,000**	144,26	**0,000**	161,65	**0,000**	594,28	**0,000**	1081,00	**0,000**
Genotype	1	4,80	**0,000**	247,30	**0,000**	3,79	**0,000**	432,73	**0,000**	2107,59	**0,000**	4605,70	**0,000**
Seed Rate	1	4,97	**0,000**	63,60	**0,000**	3,39	**0,000**	1672,17	**0,000**	3011,15	**0,000**	7548,90	**0,000**
Nitrogen	4	0,23	0,187	6,10	**0,000**	28,11	**0,000**	9,13	0,230	20,63	0,791	121,40	**0,072**
Year*Genotype	2	4,51	**0,000**	30,80	**0,000**	1,17	**0,009**	80,60	**0,000**	110,00	0,109	9,40	0,843
Year*Seed Rate	2	1,09	**0,001**	1,30	0,130	1,37	**0,004**	3,04	0,624	69,35	0,244	0,60	0,989
Genotype*Seed Rate	1	0,02	0,720	0,10	0,781	0,10	0,516	128,00	**0,000**	111,12	0,133	311,00	**0,019**
Year*Nitrogen	8	0,35	**0,021**	0,50	0,567	2,10	**0,000**	4,36	0,709	26,91	0,814	18,30	0,952
Genotype*Nitrogen	4	0,16	0,378	0,30	0,780	0,46	0,112	2,42	0,825	93,26	0,112	199,30	**0,008**
Seed Rate*Nitrogen	4	0,38	**0,041**	0,80	0,315	0,05	0,930	3,05	0,753	74,62	0,197	137,90	**0,046**
Year*Genotype*Seed Rate	2	0,43	0,060	1,60	0,095	0,13	0,595	89,44	**0,000**	105,42	0,119	231,20	**0,017**
Year*Genotype*Nitrogen	8	0,25	0,116	0,50	0,664	0,12	0,866	4,97	0,625	27,65	0,802	32,10	0,790
Year*Seed Rate*Nitrogen	8	0,14	0,492	0,80	0,302	0,16	0,740	4,86	0,641	50,38	0,413	29,60	0,826
Genotype*Seed Rate*Nitrogen	4	0,10	0,594	1,10	0,151	0,27	0,346	5,22	0,519	49,12	0,405	73,00	0,264
Year*Genotype*Seed Rate*Nitrogen	8	0,34	0,026	0,50	0,679	0,27	0,352	6,81	0,394	56,07	0,334	0,80	1,000
Error	120	0,15		0,60		0,24		6,41		48,63		55,10	
Total	179												

Table 4: Anova analysis

FACTOR	DF	Grain yield (t/ha)		Heading date (from 1th of April)		Protein content %		Soil coverege % 1		Soil coverege % 2		Soil coverege % 3		NDVI_1		NDVI_2		NDVI_3	
		MS	*p*	MS	*p*	MS	*p*	MS	*p*	MS	*p*	MS	*p*	MS	*p*	MS	*p*	MS	*p*
Genotype	1	11,864	0,0000	33,75	0,0000	3,85	0,0016	500,721	0,0000	1491,20	0,0002	1270,0	0,0001	0,01435	0,0001	0,37796	0,0000	0,470322	0,0000
Seed Rate	1	5,116	0,0000	16,02	0,0002	0,60	0,1890	656,400	0,0000	486,89	0,0245	2608,6	0,0000	0,01129	0,0003	0,00002	0,9520	0,010824	0,2027
Nitrogen	4	0,129	0,3750	4,28	0,0039	5,92	0,0000	11,187	0,2639	53,81	0,6623	53,6	0,5319	0,00151	0,0923	0,00855	0,1027	0,003957	0,6555
Genotype x Seed Rate	1	0,106	0,3501	0,42	0,5079	0,01	0,8595	304,561	0,0000	321,89	0,0647	769,4	0,0016	0,01608	0,0000	0,00010	0,8747	0,000302	0,8297
Genotype x Nitrogen	4	0,152	0,2914	0,54	0,6786	0,19	0,6842	7,192	0,4867	65,12	0,5766	41,8	0,6473	0,00016	0,9255	0,00006	0,9996	0,000672	0,9804
Seed Rate x Nitrogen	4	0,233	0,1177	0,81	0,4926	0,14	0,7867	3,939	0,7502	146,51	0,1824	103,1	0,2092	0,00020	0,8834	0,00263	0,6393	0,003261	0,7320
Genotype x Seed Rate x Nitrogen	4	0,152	0,2926	1,04	0,3625	0,24	0,5874	5,368	0,6274	113,70	0,2958	31,6	0,7563	0,00063	0,4772	0,00156	0,8233	0,001050	0,9560
Error	40	0,118		0,93		0,34		8,206		89,17		66,9		0,00070		0,00413		0,006452	
Total	59																		

Table 5: Data analysis 2019

	GY	HD	PC	SC_1	SC_2	SC_3	NDVI_1	NDVI_2	NDVI_3
GY	1								
HD	**0,2676**	1							
	p=,039								
PC	-0,2226	0,2437	1						
	p=,087	p=,061							
SC_1	**0,6699**	0,139	-0,1789	1					
	p=,000	p=,289	p=,171						
SC_2	**0,4461**	0,0728	**-0,3326**	**0,5046**	1				
	p=,000	p=,580	***p=,009***	***p=,000***					
SC_3	**0,5879**	0,0294	-0,103	**0,6798**	**0,6498**	1			
	p=,000	p=,823	p=,434	***p=,000***	***p=,000***				
NDVI_1	-0,0202	**-0,267**	0,206	-0,1869	-0,172	-0,0766	1		
	p=,878	***p=,039***	p=,114	p=,153	p=,189	p=,561			
NDVI_2	**0,6137**	**0,4939**	-0,0594	**0,392**	0,1603	0,183	-0,0606	1	
	p=,000	***p=,000***	p=,652	***p=,002***	p=,221	p=,162	p=,646		
NDVI_3	**0,48**	**0,5292**	-0,0971	**0,2548**	0,1261	0,1192	-0,1833	**0,8716**	1
	p=,000	***p=,000***	p=,460	***p=,049***	p=,337	p=,364	p=,161	***p=0,00***	

Marked correlations are significant at p < ,05000

Table 6: Correlation between traits detected

3.4 Discussion

The main challenge in the present study, was to estabilish an image based high throughput phenotyping platform able to operate in field conditions, under varying management conditions., and to automate the digital image analysis. In addition, another challenge was the identification of new traits to be practically used as a selection tool in the breeding activity. The RGB image analysis procedure used was based on the discrimination of plant and background by thresholding the excess green colour index (Meyer et al., 1998), and resulted in a reliable automated assessments. The contribution to the generation of a standard procedure was that we automated the determination of the grey-level threshold, which sets the breakpoint between vegetation and non-vegetation, and standardizing data acquisition using a georeferenced grid, in order to analyze for every score always the same plot surface. Compared with the visual assessments, which may be influenced by different operator (Rasmussen et al., 1997), our digital image analysis procedures represent a huge improvement in precision.

Regarding NDVI data, image reflectance results were estimated according to previous literature available on application of the UAV system for high throughput phenotyping (Haghighattalab et al, 2016). Good correlations of UAV-NDVI with grain yield illustrated the efficiency of platform that had made this system useful for practical breeding. Previously, several reports were published on the benefits of hand-held and vehicle-based ground sensors to evaluate canopy traits in wheat, cotton and fruit crops (Schirrmann et al, 2016). Auto-vehicle based remote sensing only decreased labor costs, but provided no improvement in time and operational costs. Field design may also confer limitations in regard to on-going field conditions such as rainfall, timing of irrigation, and space. UAV does not only reduce the labor costs, but it also provides a means of phenotyping that overcomes limitations of large field trials. Low altitude flights with large image overlaps as demonstrated in this work permit high-resolution orthomosaic generation, allowing deep spectral extraction for important secondary traits. *Sequoia* sensor had the advantage of comprising sensors with high bandwidth which gave deeper information than the *Greenseeker* one. UAV allows more data coverage across the growing season for the dynamic monitoring of NDVI and other VIs to screen large numbers of plots in a cost-effective way (Torres-Sanchez et al, 2015). Highly significant correlations between prediction of yield at the flowering stage and actual yield provided a strong evidence that UAV-NDVI can precisely explain yield variations among the genotypes (Duan et al, 2017).

The study also demonstrated the effectiveness of soil cover ability as a useful trait for selecting lines and varieties for organic farming (Baum et al. 2003), the same index is also influenced by agronomic practices, density and method of sowing, as well as weather conditions (De Vita et al, 2017), although the genetic component seems to be predominant.

4. Conclusion

The UAV field phenotyping platform is not only a rapid data acquisition system, but also reduces labor costs and problems associated with adverse weather condition. Therefore, high throughput phenotyping of important traits such as Cover Ability and NDVI appears to be a promising approach for in-season selection and yield prediction of breeding lines in advanced stages, when seed availability allows comparison between plots. As the HTP phenotyping becomes more accessible and operational in the rapid estimation of secondary traits that explain yield it will be more reliably used in breeding. Bioinformatic development is expected in the coming years by Machine learning (ML) approaches, that could make HTP more efficient by use of automated identification, classification, quantification and prediction for critical decision regarding selection of desired phenotypes in field conditions. Further improvement is also required and expected in sensor resolution and data analysis for acquisition of precise HTP data.

Finally, the acquired phenotypic information can also be valuably used in future GWAS studies to understand the genetic basis of the characters analyzed, in particular for the soil coverage percentage.

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