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Doctoral Dissertation

CHARACTERIZATION OF DURUM WHEAT GENETIC RESOURCES AND EVALUATION UNDER CYPRUS CONDITIONS

Angelos Kyratzis

Limassol, October 2017

CYPRUS UNIVERSITY OF TECHNOLOGY

FACULTY OF GEOTECHNICAL SCIENCES AND ENVIRONMENTAL MANAGEMENT

DEPARTMENT OF AGRICULTURAL SCIENCES, BIOTECHNOLOGY AND FOOD SCIENCE

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Approval Form

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ABSTRACT

Breeders need to intensify their efforts to meet the challenges arising from the growing population and from climate change. Genotypic characterization, phenotypic evaluation and exploitation of high throughput technologies will be crucial in order to achieve their goals. The main objectives of this doctoral dissertation were (1) to characterize a set of 55 durum wheat accessions, including modern varieties from different breeding programs and landraces originated from Cyprus and other Mediterranean areas using microsatellites, (2) to evaluate a subset of twenty modern varieties for grain yield and other important agronomic traits under diverse climatic conditions, and (3) to evaluate the usefulness of high throughput, remote sensing techniques for plant phenotyping under a water limited and heat stressed environment.

High genetic diversity was detected. Landraces formed distinct groups and depicted higher genetic variability than modern varieties reinforcing their potential use to plant breeding. The highest genetic variability was observed in Cypriot landraces suggesting the existence of unique genetic diversity within this genepool. High genetic diversity was also detected in Cypriot landrace "Kyperounda". The genetic diversity was higher in accessions conserved as sublines. The genetic differentiation between "Kyprerounda" accessions and the different clustering of landrace accessions conserved under the same name signifies that passport data is not a reliable method to eliminate duplicates in genebanks.

Phenotypic evaluation revealed the existence of genetic diversity for all the traits, however, for most of them the highest percentage of variance was explained by the environmental effect. In most cases, number of seeds per m^2 was significantly correlated with grain yield and the correlations were stronger in the low yielding environments. Number of seeds per spike was the trait consistently related with number of seeds per m^2. Trait offs between yield components were recorded. Varieties had different yield formation strategies. The two highest yielding varieties had the highest number of fertile tillers per m^2 combined with high number of seeds per spike or high kernel weight. The differences between varieties for plasticity were significant for yellow pigment content, protein content, gluten index, growing degree days to heading, thousand kernel weight and plant height. In some cases, significant correlations were found between plasticities and between plasticities and the traits *per se*.

Statistically significant differences between varieties were observed for Spectral Vegetation Indices derived by Unmanned Aerial Vehicle imagery. Grain yield was significantly correlated with SVIs, when recorded at early reproductive stages. Both positive and negative correlations were observed indicating that the environmental conditions during grain filling can affect the sign of the correlations. The predictive ability of SVIs was affected by plant phenology. Correlations of grain yield with SVIs were stronger as the correlations of SVIs with heading were weaker or not significant. These findings highlight the potential use of SVIs derived by UAV imagery for durum wheat phenotyping at low yielding Mediterranean conditions.

Keywords: diversity, durum wheat, genetic resources, grain yield, Mediterranean, microsatellites, spectral vegetation indices

ΠΕΡΙΛΙΨΗ

Ο γενετικός χαρακτηρισμός γενετικών πόρων αποτελεί σημαντική προϋπόθεση για την βελτιστοποίηση των στρατηγικών διατήρησης γενετικού υλικού και την αξιοποίηση του σε προγράμματα βελτίωσης. Η φαινοτυπική αξιολόγηση κάτω από διαφορετικά περιβάλλοντα επιτρέπει την εκτίμηση της παραλλακτικότητας για σημαντικά αγρονομικά και φυσιολογικά χαρακτηριστικά και τον υπολογισμό των συσχετίσεων τους με την απόδοση. Οι τεχνολογίες υψηλής απόδοσης, όπως η τηλεπισκόπηση, έχουν αποκτήσει ιδιαίτερο ενδιαφέρον για τη φαινοτύπηση φυτών επειδή είναι γρήγορες και φτηνές μέθοδοι. Οι κύριοι στόχοι αυτής της διδακτορικής διατριβής ήταν: (1) ο γενετικός χαρακτηρισμός 55 καταχωρήσεων σκληρού σιταριού, συμπεριλαμβανομένων μοντέρνων ποικιλιών από διαφορετικά προγράμματα βελτίωσης και παραδοσιακών ποικιλιών προερχόμενων από την Κύπρο και άλλες περιοχές της Μεσογείου με μικροδορυφόρους, (2) η αξιολόγηση 20 μοντέρνων ποικιλιών για απόδοση και άλλα σημαντικά αγρονομικά χαρακτηριστικά κάτω από διαφορετικές εδαφο-κλιματικές συνθήκες, και (3) η αξιολόγηση της χρησιμότητας μεθόδων υψηλής απόδοσης τηλεπισκόπησης για μαζική φαινοτύπηση κάτω από ένα ξηρό και θερμό περιβάλλον.

Οι μικροδορυφόροι κατέδειξαν την ύπαρξη μεγάλης γενετικής παραλλακτικότητας. Οι παραδοσιακές ποικιλίες δημιούργησαν ξεχωριστές ομάδες και παρουσίασαν μεγαλύτερη γενετική παραλλακτικότητα από τις μοντέρνες ποικιλίες ενισχύοντας τη δυνατότητα αξιοποίησης τους στα προγράμματα βελτίωσης. Η μεγαλύτερη γενετική παραλλακτικότητα παρατηρήθηκε στις κυπριακές παραδοσιακές ποικιλίες υποδεικνύοντας την πιθανή ύπαρξη μοναδικής ποικιλομορφίας εντός αυτής της γενετικής δεξαμενής. Μεγάλη γενετική παραλλακτικότητα παρατηρήθηκε και εντός της κυπριακής παραδοσιακής ποικιλίας «Κυπερούντα». Η γενετική ποικιλομορφία ήταν μεγαλύτερη εντός των καταχωρήσεων που διατηρούνται ως ξεχωριστές γραμμές προερχόμενες από ένα στάχυ. Η γενετική διαφοροποίηση των καταχωρήσεων της «Κυπερούντας» και η διαφορετική κατάταξη των καταχωρήσεων με ίδιο όνομα στην ανάλυση συστάδων υποδεικνύει ότι τα δεδομένα συλλογής δεν είναι αξιόπιστος τρόπος για περιορισμό διπλών δειγμάτων.

Η φαινοτυπική αξιολόγηση κατέδειξε την ύπαρξη σημαντικής γενετικής παραλλακτικότητας για όλα τα χαρακτηριστικά που εξετάστηκαν, παρόλα αυτά, για τα περισσότερα η επίδραση του περιβάλλοντος ήταν μεγαλύτερη. Παρατηρήθηκαν στατιστικά σημαντικές συσχετίσεις μεταξύ του αριθμού σπόρων ανά τ.μ. και της απόδοσης στα περισσότερα περιβάλλοντα και οι συσχετίσεις ήταν μεγαλύτερες στα δύο περιβάλλοντα με τη χαμηλότερη απόδοση. Ο αριθμός σπόρων ανά στάχυ ήταν το χαρακτηριστικό που συστηματικά συσχετιζόταν σημαντικά με τον αριθμό σπόρων ανά τ.μ. Παρατηρήθηκαν επίσης αρνητικές συσχετίσεις μεταξύ των συστατικών απόδοσης. Οι ποικιλίες είχαν διαφορετικές στρατηγικές διαμόρφωσης της απόδοσης. Οι δύο ποικιλίες με την μεγαλύτερη παραγωγή είχαν το μεγαλύτερο αριθμό γόνιμων αδελφιών ανά τ.μ. σε συνδυασμό με ψηλό αριθμό σπόρων ανά στάχυ, ή βάρος χιλίων κόκκων, αντίστοιχα. Οι ποικιλίες διέφεραν μεταξύ τους ως προς την πλαστικότητα τους για τα περισσότερα χαρακτηριστικά. Σε ορισμένες περιπτώσεις, παρατηρήθηκαν σημαντικές συσχετίσεις μεταξύ των πλαστικοτήτων των ποικιλιών ή μεταξύ των πλαστικοτήτων και των μέσων όρων των ποικιλιών για διαφορετικά χαρακτηριστικά.

Παρατηρήθηκαν στατιστικά σημαντικές διαφορές μεταξύ των ποικιλιών για τους Δείκτες Βλάστησης υπολογιζόμενους με ανάλυση φωτογραφίας προερχόμενη από μη επανδρωμένα σκάφη. Σε ορισμένες περιπτώσεις, η απόδοση συσχετιζόταν σημαντικά με τους δείκτες βλάστησης. Οι συσχετίσεις ήταν θετικές ή αρνητικές υποδεικνύοντας ότι οι περιβαλλοντικές συνθήκες κατά τη διάρκεια του γεμίσματος του σπόρου μπορούν να επηρεάσουν το πρόσημο των συσχετίσεων. Η υπολογιστική ικανότητα της απόδοσης των Δεικτών Βλάστησης επηρεάστηκε από τη διαφορετική φαινολογία των ποικιλιών. Οι συσχετίσεις της απόδοσης με τους Δείκτες Βλάστησης ήταν μεγαλύτερες όταν οι συσχετίσεις του χρόνου ξεσταχυάσματος με τους Δείκτες Βλάστησης ήταν μικρότερες ή μη σημαντικές. Τα αποτελέσματα αναδεικνύουν την δυνατότητα χρήσης των Δεικτών Βλάστησης, υπολογιζόμενοι από φωτογραφία προερχόμενη από μη επανδρωμένα σκάφη, για φαινοτύπηση σκληρού σιταριού σε Μεσογειακά περιβάλλοντα χαμηλής απόδοσης.

Λέξεις κλειδιά: ποικιλομορφία, παραλλακτικότητα, σκληρό σιτάρι, γενετικοί πόροι, απόδοση σε σπόρο, Μεσόγειος, δείκτες βλάστησης.

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AFLP:	Amplified Fragment Length Polymorphism
ANOVA:	Analysis of Variance
AMOVA:	Analysis of Molecular Variance
ARI:	Agricultural Research Institute
CAR:	Yellow Pigment Content
CIMMYT:	International Maize and Wheat Improvement Program
CV:	Coefficient of Variation
DArTs	Diversity Arrays Technology Markers
DEM:	Digital Elevation Model
Dj:	Discriminating Power
GxE:	Genotype x Environment Interactions
GDD / GDDHD:	Growing Degree Days to Heading
GDDPM:	Growing Degree Days from Heading to Physiological Maturity
GI:	Gluten Index
GNDVI:	Green Normalized Difference Vegetation Index
GRYLD:	Grain Yield
HI:	Harvest Index
ICARDA:	International Center for Agricultural Research In the Dry Areas
LAI:	Leaf Area Index
LSD:	Least Significant Difference
NDVI:	Normalized Difference Vegetation Index

NIF:	Near Infrared
NSSM:	Number of Seeds per m^2
NTLSM:	Number of Fertile Tillers per m^2
PC:	Principal Component
PCA:	Principal Component Analysis
PCoA:	Principal Coordinate Analysis
PCR:	Polymerase Chain Reaction
PH:	Plant Height
PIC:	Polymorphism Information Content
PRO:	Protein Content
RGB:	Red Green Blue
RP:	Resolving Power
SPS:	Seeds per Spike
SR:	Simple Ratio
SSR:	Simple Sequence Repeats
SVI:	Spectral Vegetation Indices
TKW:	Thousand Kernel Weight
UAV:	Unmanned Aerial Vehicle
UPGMA:	Unweighted Pair Group Method with Arithmetic Mean
USDA:	United States Department of Agriculture
VW:	Volume Weight

Introduction

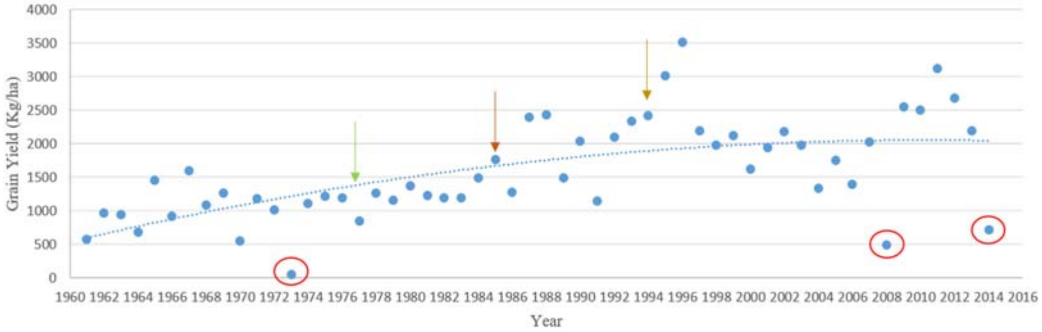
Durum wheat [*Tritigum turgidum* subsp. *durum* (Desf.) Husn.] is a major crop worldwide. The main producing area is the Mediterranean basin (Habash, Kehel and Nachit, 2009; Royo, et al., 2010) where about of 75% of the world's production is produced (Alsaleh, et al., 2016). This area is also the most significant import market and the largest consumer of durum wheat commodities (Royo, et al., 2017). European Union produced almost 25% of the total production, and the four leading countries are Spain, France, Italy and Greece. Other important durum wheat areas are Kazakhstan, North American great plains, India, Australia, Mexico and Argentina (Elias and Manthey, 2005). In Cyprus, durum wheat is the 2nd most important arable crop, after barley. The crop covers about 7.500 hectares (area under the effective control of the Republic of Cyprus) and the main producing areas are Larnaca and Paphos districts (Statistical service, 2010). The annual production and the standard output varies from 4.445 to 23.740 tonnes and from €1.147.000 to €6.315.000, respectively (Statistical service, 2014).

Durum wheat is better adapted in semi-arid Mediterranean climates, characterized by frequent drought and heat stress conditions during the critical stage of grain filling (Araus, et al., 2002; García del Moral, et al., 2003; García del Moral, et al., 2005; Habash, Kehel and Nachit, 2009). In this harsh environment, durum wheat can be more productive than bread wheat (*Triticum aestivum* L.), especially under environments favouring higher yields (Josephides, 1993; Marti and Slafer, 2014). Semolina from durum wheat is the major ingredient for pasta and the Mediterranean conditions favour the production of high quality grains desirable by the pasta industry (Subira, et al., 2014). Nevertheless, durum wheat is also widely used for the production of other commodities such as couscous, bulgur, frekeh or firik and bread (Elias and Manthey, 2005; Guzmán, et al., 2016).

After the 2nd world war, the growing population imposed the establishment of intensive plant breeding programs with the mission to increase production to meet human demands. CIMMYT and ICARDA, that are members of the Consultative Group for International Agricultural Research (CGIAR centres), have a predominant role in durum wheat breeding and national breeding programs are largely based on material received from these centres (De Vita, et al., 2007; Royo, et al., 2010; Kabbaj, et al., 2017). Systematic enhancement of durum wheat at CIMMYT started in 1965 under the leadership of the Nobel laureate Dr. Norman Borlaug. Early breading efforts focused on the introgression of dwarfing genes, and alleles for photoperiod insensitivity, the improvement of floral fertility and enhanced biotic resistance (Pfeiffer and Payne, 2005). The introgression of the dwarfing genes conferred tolerance to lodging and hence adaptation to high rates of fertilizer application, and photoperiod insensitive genes allowed durum wheat, a long-day species, to be grown under short winter days (Royo, Elias and Manthey, 2009). This pioneer work of Dr Borlaug let to the green revolution and breeding efforts thereafter sustained yield increase, as it is evident by the several retrospective studies examining yield improvement through time (De Vita, et al., 2007; Araus, et al., 2008; Subira, et al., 2015). The germplasm cultivated before green revolution, that was landraces, was abandoned by the farmers and replaced by semi-dwarf modern varieties (Royo, Elias and Manthey, 2009).

Durum wheat breeding activities in Cyprus were initiated after the 2nd world war (Parisinos, 1956; 1965), however, intensive plant breeding was commenced after the establishment of the Agricultural Research Institute in 1962 and the initiation of the national breeding program. This program aims to improve productivity, stability and quality of durum wheat. To achieve these goals, the program incorporates genetic material received from CIMMYT and ICARDA and other breeding programs (Josepides and Kyratzis, 2007). The vast majority of durum wheat area in Cyprus is cultivated with varieties released by the national breeding program.

Figure 1 shows average yield per hectare in Cyprus over the last 56 years. A clear trend for yield increase was evident until the 2000. The last 20 years, there is a great fluctuation of yield over years due to the variation of the climatic conditions. Another point of consideration is the two extreme drought events occurred over the last eight years. The fluctuation in environmental conditions and therefore to grain yield and the occurrence of extreme drought events can be attributed to climate change. It expected that in the near future, climate change will cause precipitation reduction and temperature elevation in the Mediterranean (Giorgi and Lionello, 2008), increasing the yield uncertainty of rain-fed crops.



Source (FAO statistics, 2017)

Figure1: Durum wheat yield per hectare over years. Green, red and brown arrows show the year of release of the varieties "Aronas", "Karpasia" and "Macedonia", respectively. Red circles show extremely drought years.

On the other hand, breeders need to further increase yields to meet the demand from the growing population (Araus, et al., 2008). This is a daunting challenge in drought prone environments like the Mediterranean, where the impact of climate change is more severe (Reynolds and Tuberosa, 2008; Habash, Kehel and Nachit, 2009). Landraces gained a lot of attention over the last years, since they constitute an unexplored genepool that can be effectively used to confer resistance to abiotic stress and to cope climate change (Newton, et al., 2010; Lopes, et al., 2015; Dwivedi, et al., 2016).

Breeders need to intensify their efforts to meet the arising challenges. Genotypic characterization, phenotypic evaluation and exploitation of high throughput technologies will be crucial in order to achieve their goals. Genotypic characterization allows the assessment of the genetic diversity and the genetic structure of the germplasm (Soriano, et al., 2016; Kabbaj, et al., 2017) and to design crosses in order to increase the genetic variability in segregating populations (Royo, et al., 2010; Ren, et al., 2013) and ultimately to broadening the genetic basis of the varieties that are available to farmers. In addition, genotyping facilitates the rationalisation of *ex situ* conservation of genetic resources to genebanks and their utilization to breeding programs (Dobrovolskaya, et al., 2005; Vikram, et al., 2016; Riaz, et al., 2017). Phenotypic evaluation under diverse environments allows the assessment of variability for important agronomic and physiological traits and to evaluate their correlations with yield (García del Moral, et al., 2005; Royo, et al., 2010; Del Pozo, et al., 2016; Gonzalez-Navaro, et al., 2016). Phenotypes with superior traits can be used in crossing blocks in order to incorporate desired characteristics to commercial varieties. High throughput technologies, such as remote sensing techniques, hold great promises because they are fast and cost efficient methods that can be used for plant phenotyping and screening breeding plots (Reynolds and Tuberosa, 2008; Deery, et al., 2014; Sankaran, et al., 2015).

The main objectives of this doctoral dissertation were: (1) to characterize durum wheat genetic resources, including modern varieties from different breeding programs and landraces originated from Cyprus and other Mediterranean areas using molecular markers **(Chapter 1)**, (2) to evaluate a subset of twenty modern varieties, originated from different breeding programs, for grain yield and other important agronomic traits under diverse climatic conditions in Cyprus **(Chapter 2)**, and (3) to evaluate the usefulness of high throughput, remote sensing techniques for plant phenotyping in the subset of the twenty varieties grown under a water limited and heat stressed environment **(Chapter 3)**.

1. Chapter 1 - Genetic Characterization of Durum Wheat Genetic Resources

1.1. Introduction

The center of wheat domestication is located at the Fertile Crescent, where its cultivation is evident since 12.000 Before Present (MacKey, 2005). From there, wheat cultivation was gradually spread to the adjacent areas. Recent archeological evidence confirmed the presence of wheat in Cyprus just after its domestication to the Fertile Crescent (Vigne, et al., 2012). Among the various tetraploid forms, durum wheat has definite agricultural significance (MacKey, 2005), and gradually replaced its ancestors. The long history of the crop in the area, in combination with the diverse soil and climatic conditions, resulted to the development of highly diverse landraces (e.g. Medini, et al., 2005; Moragues, et al., 2007; Ruiz, et al., 2012; Soriano, et al., 2016).

According to the definition of Villa, et al. (2005) "a landrace is a dynamic population(s) of a cultivated plant that has historical origin, distinct identity and lacks formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming systems". Large number of durum wheat landraces were cultivated in Cyprus until the 1980 when phenotypic variation between and within them was still present, especially within the landrace "Kyperounda". This landrace was widely cultivated because of its adaptation and good quality traits (Bennet, 1973; Hadjichristodoulou and Della, 1976). The typical "Kyperounda" head is characterized by a dense, short and slightly to intensively black colored spike and black awns. Landraces were collected in 1978 in a joint collecting mission with the International Board for Plant Genetic Resources (IBGRI), and are *ex situ* conserved in the Genebank of the Agricultural Research Institute, Cyprus (ARI Genebank). Each accession is composed of 50-150 ear lines (sublines) depending on the phenotypic variability observed in the collecting site (Della, Farias and Josephides, 1980).

Onwards the green revolution, landraces were gradually replaced by modern varieties due to their higher productivity, especially under more favourable condition (Moragues, et al., 2007; Royo, Nazco and Villegas, 2014; Soriano, et al., 2016). Modern varieties are bred by national breeding programs based, in most cases, on material received from CIMMYT and ICARDA (Maccaferri, et al., 2005; Royo, et al., 2010; Ren, et al., 2013; Kabbaj, et al., 2017). Correspondingly in the case of Cyprus, landraces were replaced by modern varieties bred by the national breeding program (Josephides and Kyratzis, 2007).

However nowadays, there is a trend of reviving the long forgotten landraces via their use in plant breeding programs. Given their past evolutionary history and adaptation to stress environments, landraces can contribute to breed varieties with improved productivity, adaptation, nutritional value (Lopes, et al., 2015; Dwivedi, et al., 2016) and quality attributes (Alsaleh, et al., 2016). It has been reported that landraces depict higher genetic variability than modern varieties (Ganeva, et al., 2010; Soriano, et al., 2016), even though other authors concluded the opposite (Maccaferi, et al., 2003; Martos, et al., 2005). However, in all cases, landraces group differently from modern varieties in cluster and structure analysis based on molecular data (e.g. Maccaferi, et al., 2005; Royo, et al., 2010; Kabbaj, et al., 2017; Riaz, et al., 2017). Thus, landraces constitute a source of unexplored alleles (Laidò, et al., 2013), and their introgression to breeding programs will undoubtedly contribute to the broadening of the genetic base and to the improvement of the next generation wheat varieties (Vikram, et al., 2016).

Molecular markers have been widely used for the assessment of the genetic diversity, because of their superiority to detect patterns of relatedness among genotypes compared to phenotypic data (Maccafferi, et al., 2003) and co-ancestry analysis (Laidò, et al., 2013). Among molecular markers, microsatellites are frequently employed because they are widely distributed to the genome, codominant, highly polymorphic, stable, reproducible and cost effective when developed (Röder, et al., 1995; Roder, et al 1998; Gupta, et al., 2002; Song, et al., 2005). In the case of durum wheat, microsatellites have been extensively used for the genetic characterization of genetic resources (e.g. Royo, et al., 2010; Ruiz, et al., 2012; Soriano, et al., 2016). Data from microsatellites were significantly correlated with results from AFLPs (Maccafferi, et al., 2007; Moragues, et al., 2007) and DArTs (Montovani, et al., 2008; Ruiz, et al., 2012; Laidò, et al., 2013).

Genetic characterization allows the development of rational strategies for *ex situ* conservation and enhance the germplasm use in breeding programs (Dreisigacker, et al., 2005; Ruiz, et al., 2012; Ren, et al., 2013; Baloch, et al., 2017). Data from molecular markers provide the basis for the construction of core collections (Zhang, et al., 2006; Vikram, et al., 2016) and the designation of genetically unique accessions that further facilitates the integration of regional and global initiatives, such as the European Genebank Integrated System (AEGIS). Most importantly, the knowledge of genetic proximity between genotypes gives clues about the heterosis that can be expected when making crosses among them in order to create polymorphic populations (Royo, et al., 2010; Ren, et al., 2013).

Despite that several studies were conducted over the last years aimed at the genetic characterization of durum wheat genetic resources, there is still large genetic variation within *in situ* and *ex situ* collections, especially in landraces, that remains undiscovered (Dreisigacker, et al., 2005; Vikram, et al., 2016, Baloch, et al., 2017). For instance, Hagenblad, Zie and Leino (2012) indicated that little is known about the diversity within landrace populations. In the case of durum wheat, only few studies were designed to investigate the genetic diversity within individual landraces (Pagnotta, et al., 2004; Ribeiro-Carvalho, et al., 2004; Zhang, et al., 2006; Colomba and Gregorini, 2011; Alsaleh, et al., 2016; Mangini, et al., 2017). Some studies in the past included a very limited number of durum wheat landraces originating from Cyprus to investigate the genetic diversity and structure of durum wheat genetic resources (e.g. Moragues, et al., 2007; Laidò, et al., 2013; Ren, et al., 2013, Soriano, et al., 2016). To the best of our knowledge, there is no robust information available regarding the genetic constituents within Cypriot durum wheat landraces and modern varieties.

The main objectives of the present study were (1) to investigate the genetic diversity and genetic structure in a set of germplasm, including 22 modern varieties from different breeding programs and 33 landraces originated from Cyprus and other Mediterranean areas, and (2) to examine the genetic / phenotypic diversity and genetic structure of the Cypriot landrace "Kyperounda".

1.2. Materials and methods

1.2.1. Genetic diversity and structure of modern varieties and landraces

1.2.1.1. Plant material

Fifty-five durum wheat (*Triticum turgidum* subsp. *durum*) accessions (22 modern varieties and 33 landraces) were included in the present study, designated to four groups **(Table 1)**. Group 1 was composed with six varieties originated from the national breeding program of Cyprus, representing the main commercial varieties cultivated in Cyprus for the last 40 years. Group 2 contained sixteen varieties improved by other breeding programs, including varieties that are widely cultivated in the Mediterranean Basin. Group 3 was composed with twenty Cypriot landraces, most of them conserved to ARI Genebank and group 4 with thirteen landraces originated from the Mediterranean (10), Iran, Armenia and Azerbaijan. Group 4 accessions were kindly provided by the ICARDA Genebank. The variety "Chinese spring" (*Triticum aestivum*) was also included as a control of admixtures and to verify size products. The landraces at the ARI Genebank are conserved as sublines. Therefore, ten seeds were randomly selected from each subline to create a bulk for each accession. All accessions were cultivated to single plots in the same field. Off-types and hexaploid plants were discarded.

1.2.1.2. DNA extraction and PCR amplification

After harvesting, 60 seeds from each plot (accession) were randomly selected and grown in a glasshouse before DNA extraction. Genomic DNA was extracted from two-week old leaves, from the germinated seedlings, using the Invisorb® Spin Plant Mini Kit (STRATEC Biomedical AG, Birkenfeld, Germany), following the manufacturer’s instructions. DNA concentration and quality was assessed by Nanodrop 1000 (Thermo Scientific, Wilmington, USA) and verified with agarose electrophoresis.

Twenty-one microsatellite markers (SSRs) were selected based on their polymorphism and chromosomal location. These markers have been previously described by Röder, et al. (1995; 1998), Gupta, et al. (2002) and Song, et al. (2005) **(Table 2)**.

Table 1: List of accessions used for the assessment of the genetic diversity.

A/A	Accession number	Origin / Registration	Accession name	Cat.	Group
1		Cyprus	Aronas	VAR	1
2		Cyprus	Mesaoria	VAR	1
3		Cyprus	Karpasia	VAR	1
4		Cyprus	Makedonia	VAR	1
5		Cyprus	Ourania	VAR	1
6		Cyprus	Hekabe	VAR	1
7		ICARDA	Ammor 6	VAR	2
8	IG129081	Syria		VAR	2
9		Greece	Anna	VAR	2
10		Greece	Atlas	VAR	2
11		Greece	Matt	VAR	2
12		Greece	Mexikali 81	VAR	2
13		Greece	Pisti	VAR	2
14		Italy	Simeto	VAR	2
15		Italy	Duilio	VAR	2
16		Italy	Iride	VAR	2
17		Italy	Claudio	VAR	2
18		Italy	Svevo	VAR	2
19		ICARDA	Adnan2	VAR	2
20		ICARDA	Omrabi5	VAR	2
21		ICARDA	Korifla	VAR	2
22		ICARDA	Waha	VAR	2
23	IG127457	Cyprus		LR	3
24	IG96271	Cyprus		LR	3
25	ARI00002	Cyprus	Kyperounda	LR	3
26	ARI00007	Cyprus	Psathas	LR	3
27	ARI00020	Cyprus	Maurotheri	LR	3
28	ARI00024	Cyprus	Tripolitiko	LR	3
29	ARI00027	Cyprus	Famira	LR	3
30	ARI00030	Cyprus	Kyperounda	LR	3
31	ARI00061	Cyprus	Maurotheri	LR	3
32	ARI00076	Cyprus	Famira	LR	3
33	ARI00084	Cyprus	Loizos	LR	3
34	ARI00095	Cyprus	Kokkino	LR	3
35	ARI00099	Cyprus	Maurokyperounda	LR	3
36	ARI00102	Cyprus	Kampouriko	LR	3
37	ARI00104	Cyprus	Aspris	LR	3
38	ARI00106	Cyprus	Irakinos	LR	3
39		Cyprus	Kyperounda Br*	LR	3
40		Cyprus	Famira Far**	LR	3
41	ARI00017	Cyprus	Broulias	LR	3
42	ARI00062	Cyprus	Kyperounda	LR	3
43	IG84979	Spain		LR	4
44	IG140526	Azerbaijan		LR	4
45	IG85710	Greece		LR	4
46	IG86179	Iran		LR	4
47	IG86653	Israel		LR	4
48	IG95789	Syria		LR	4
49	IG97359	Algeria		LR	4
50	IG98726	Livia		LR	4
51	IG83901	Israel		LR	4
52	IG99151	Tunisia		LR	4
53	IG126364	Armenia		LR	4
54	IG97193	Jordan		LR	4
55	IG96437	Morocco		LR	4
56			Chinese spring***		

LR- Landraces. VAR- Varieties, * Conserved by national breeding program, ** Collected from farmers in 2011, ****Triticum aestivum*

Table 2: Chromosomal location, sequence, repeat motif and annealing temperature in the set of SSR primers.

Primer	**Chrom.**	**Sequence**	**Repeat motif**	**Anneal. temper. (ºC)**
WMS752	1AS/1BL	*	GT	55
WMS268	1BL	AGG GGA TAT GTT GTC ACT CCA (F) TTA TGT GAT TGC GTA CGT ACC C (R)	$(GA)_{17}TA(GA)_{27}$	57
WMS312	2AL	ATC GCA TGA TGC ACG TAG AG (F) ACA TGC ATG CCT ACC TAA TGG (R)	$(GA)_{37}$	60
WMS148	2BL	GTG AGG CAG CAA GAG AGA AA (F) CAA AGC TTG ACT CAG ACC AAA (R)	$(CA)_{22}$	60
WMS619	2BL	*	$(CT)_{19}$	50
WMS5	3AL	GCC AGC TAC CTC GAT ACA ACT C (F) AGA AAG GGC CAG GCT AGT AGT (R)	$(TC)_{23}(T)_4(GT)_{12}(GA)_{10}$	55
WMS155	3AL	CAA TCA TTT CCC CCT CCC (F) AAT CAT TGG AAA TCC ATA TGC C (R)	$(CT)_{19}$	60
WMS299	3B	ACT ACT TAG GCC TCC CGC C (F) TGA CCC ACT TGC AAT TCA TC (R)	$(GA)_{31}(TAG)_4$	55
WMS389	3BS	ATC ATG TCG ATC TCC TTG ACG (F) TGC CAT GCA CAT TAG CAG AT (R)	$(CT)_{14}(GT)_{16}$	60
WMC161	4A	ACC TTC TTT GGG ATG GAA GTA A (F) GTA CTG AAC CAC TTG TAA CGC A (R)	$(GT)_{25}$	61
WMC89	4B/4A/4D	ATG TCC ACG TGC TAG GGA GGT A (F) TTG CCT CCC AAG ACG AAA TAA C (R)	$(CA)_{19}(CT)_8$	51
WMS304	5AS/2A	AGG AAA CAG AAA TAT CGC GG (F) AGG ACT GTG GGG AAT GAA TG (R)	$(CT)_{22}$	57
BARC74	5B	GCG CTT GCC CCT TCA GGC GAG (F) CGC GGG AGA ACC ACC AGT GAC AGA GC (R)	$(GA)_{13}(GATA)_7(GA)_9$	60
WMS540	5BS	TCT CGC TGT GAA ATC CTA TTT C (F) AGG CAT GGA TAG AGG GGC (R)	$(CT)_3(CC)(CT)_{16}$	55
WMS169	6AL	ACC ACT GCA GAG AAC ACA TAC G (F) GTG CTC TGC TCT AAG TGT GGG (R)	$(GA)_{23}$	55
WMC104	6BS	TCT CCC TCA TTA GAG TTG TCC A (F) ATG CAA GTT TAG AGC AAC ACC A (R)	$(GT)_{31}$	60
WMC83	7A	TGG AGG AAA CAC AAT GGA TGC C (F) GAG TAT CGC CGA CGA AAG GGA A (R)	$(GT)_{28}$	61
WMS260	7A	GCC CCC TTG CAC AAA TC (F) CGC AGC TAC AGG AGG CC (R)	$(GA)_{20}$	57
WMS46	7B	GCA CGT GAA TGG ATT GGA C (F) TGA CCC AAT AGT GGT GGT CA (R)	$(GA)_2(GC)(GA)_{33}$	60

* Primers with patent

It has been established that durum wheat fields grown with landraces are frequently contaminated with hexaploid wheats (Bennet, 1973; Zeven and Waninge, 1989; Zhang, et al. 2006; Figliuolo, Mazzeo and Greco, 2007). In the present study, alongside to the removal of hexaploid plants from the multiplication plots, primers WMS52 and WMC233 were employed as a further step to verify the absence of hexaploid admixtures. These primers are exclusively located in the D genome (Röder, et al., 1998; Gupta, et al., 2002). The hexaploid varieties "Gavdos" and "Chinese Spring" were included as positive controls.

Amplification reactions were set up in a 25µl volume of a mixture containing 50ng of genomic DNA, 1x Type-it® Multiplex PCR master mix (Type-it® Microsatellite PCR kit, Qiagen, Venlo, Netherlands) and 0.2µM of each primer (the forward primers were 5΄-end labeled with FAM (5-carboxy-fluorescent). Five µl of 1x Q solution were added to the master mix for primers WMC104, WMS155 and WMS260 to improve the quality of the amplified products. PCR amplification was performed in a PTC-200 thermocycler (Bio-Rad, Hercules, USA) under the following temperature profile: 5min at 95°C, followed by 30 cycles (40 cycles were used for primer WMC161), each one included 30s at 95°C, 1.30 min at annealing temperature depending of the primer pair **(Table 2)**, 30sec at 72°C and a final extension for 30 min at 60°C. A negative PCR control was included in each set of PCR amplification to verify the absence of contamination during PCR. A subset of accessions (10%) was amplified twice to check the reproducibility of the markers. Two primers, WMS6 and WMS459, showed inconsistent amplification products within accessions and they were disregarded from the analysis.

Amplified PCR products were run on an ABI3130 genetic analyzer (Applied Biosystems, Foster City, CA, USA). Size standard GeneScan^{TM} 500LIZ$^{®}$ (Applied Biosystems) was added to each sample to delineate allele sizes. Data were analyzed using GeneMapper Software version 4.1 (Applied Biosystems, Foster City, CA, USA).

1.2.1.3. Data analysis

Allele fragments were converted into a binary data matrix table. The discriminating capacity, level of polymorphism and informativeness were described by the indexes of number of alleles, number of rare alleles, allele range, number of private alleles, Discriminating Power (Dj, Tessier, et al., 1999) and Resolving Power (RP, Prevost and Wilkinson, 1999). Dj represents the probability that two randomly chosen accessions are distinguishable from each other and it is considered an extension of

PIC, while RP represents the ability of a primer to distinguish between accessions. Average number of alleles per locus and accession was estimated as an indicator of the heterogeneity within accessions. Analysis of Molecular Variance (AMOVA) was performed to assess the variance between and within groups using GenAlEx 6.4 (Peakall and Smouse, 2006). The significance of the resulting variance components and the inter-population genetic distances were tested using 999 random permutations. Putative population structure was analyzed using Structure 2.3.4 (Pritchard, Stephens and Donnelly, 2000). The structure algorithm was run using the admixture model, with 10 independent replicate runs per K value (number of clusters) ranging from 1 to 10. Each run involved a burning period of 500,000 iterations and a post burning simulation length of 500,000. Validation of the most likely number of clusters K was performed with the Structure Harvester (http://taylor0.biology.ucla.edu/structureHarvester). An accession was considered to belong to a cluster if its membership coefficient was ≥ 0.8 (Ren, et al., 2013; Soriano, et al., 2016). Genetic similarities were calculated based on Nei and Li (Dice) index and a bootstrapped dendrogram was constructed with FreeTree software (Pavlicek, Hrda and Flegr, 1999) using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). The dendrogram was visualized with the implementation of TreeView (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html).

1.2.2. Genetic diversity and structure of the "Kyperounda" landrace

1.2.2.1. Plant material

For the assessment of the intra-genetic diversity and structure of the "Kyperounda" landrace, five accessions were selected **(Table 3)**. Three accessions were selected from the genetic material conserved by the ARI Genebank (representing three distinct regions with diverse environmental conditions) and two accessions from entries conserved and kindly provided by USDA-ARS (National Small Grains Collection). The latter accessions were collected at least 20 years before the collection of the genetic material conserved in ARI genebank. One hundred and sixty sublines from the three ARI accessions were grown in single plots to eliminate off-types and hexaploids. Forty seeds were sown from each subline to rows 1m long. Heading dates and plant heights were recorded in each plot. Ears from each row were hand harvested and further examined to the laboratory for three distinctive morphological characteristics i.e. length of the beak, ear color and glume hairiness. The ears from each subline were threshed and 5 seeds were randomly picked from the bulk for DNA extraction. Genomic DNA was extracted from one seedling per subline. The two accessions from USDA are conserved as bulks. Seeds from these accessions were sown in single plots to ensure that off-types adventitious present in the accessions were eliminated. Leaves from individual plants were collected for DNA extraction.

Table 3: List of populations (accessions) of the landrace "Kyperounda" and meteorological data from the collecting sites.

Population	Accession No.	Collecting site	Registration	Collection form	No of sublines / plants phenotyped	No of sublines / plants genotyped
Pop 1	ARI00002	Athienou	1978	Sublines	51	40
Pop 2	ARI00030	Neo Chorio Pafou	1978	Sublines	54	52
Pop 3	ARI00062	Pareklisia	1978	Sublines	55	53
Pop 4	PI210951	unknown	1953	Bulk	46	46
Pop 5	PI210960	unknown	1953	Bulk	26	26
	Average maximum temperature (Nov – Feb)	**Average maximum temperature (Mar – May)**	**Average minimum temperature (Nov – Feb)**	**Average minimum temperature (Mar– May)**	**Average precipitation (Oct – Feb)**	**Average precipitation (Mar – May)**
Pop 1	17.63	24.30	7.10	10.67	229.10	74.40
Pop 2	18.19	21.95	9.25	11.40	350.10	76.80
Pop 3	20.25	24.97	8.43	11.57	351.80	79.00

1.2.2.2. DNA extraction and PCR amplification

The DNA extraction procedure was as described above (1.2.1.2). Five primer pairs (BARC 74, WMC 104, WMS 268, WMS 5 and WMC 89) were selected from the complete set of the 19 microsatellite markers, based on their informativeness in the "Kyperounda" bulk accessions. Amplification reactions were set up in a 10μl volume of a mixture containing 25ng of genomic DNA, 0.5U Kapa Taq (Kapa Biosystems, Wilmington, MA, USA), 1x Kapa buffer A, 0.2μM of each primer and 0.2mM dNTPs. PCR amplification was performed in a PTC-200 thermocycler (Bio-Rad, Hercules, USA) under the following temperature profile: 5min at 95°C, followed by 35 cycles, each one included 30s at 95°C, 30s at 57°C, 30sec at 72°C and a final extension for 15min at 60°C. Analysis of the amplification products was conducted as previously described (1.2.1.2).

1.2.2.3. Data analysis

The five "Kyperounda" accessions were considered as five populations. Descriptive statistics for the phenotypic data and analysis of variance between populations was carried out using SPSS version 22 (IBM). Genetic similarities between populations were calculated based on Rogers index (Rogers, 1972) which takes into account alleles frequencies within populations. A bootstrapped dendrogram was constructed with FreeTree software using the UPGMA method. The dendrogram was depicted with the implementation of TreeView. Principal Coordinate Analysis (PCoA) was performed to determine the associations between sublines / plants. PCoA and the calculation of genetic variation indices within each population (Number of different alleles, Number of effective alleles, Number of different alleles with a frequency ≥5%, Number of alleles unique to a single population, Shannon's Information Index, Observed heterozygosity, Expected heterozygosity, Unbiased expected heterozygosity, Fixation index) were performed using GenAlEx 6.4 (Peakall and Smouse, 2006). Number of alleles, number of rare alleles, allele range, discriminating power, resolving power per locus, AMOVA and STRUCTURE analysis were performed as described above. However, for the STRUCTURE analysis each run involved a burning period of 100,000 iterations and a post burning simulation length of 100,000. Validation of the most likely number of clusters K was performed with the Structure Harvester (http://taylor0.biology.ucla.edu/structureHarvester).

1.3. Results

1.3.1. Genetic diversity and structure of modern varieties and landraces

All microsatellite primers used in the present study were polymorphic (**Table 4**). The average discriminating power (Dj) was 0.84, 0.89, and 0.55 for the whole set of accessions, landraces, and modern varieties, respectively; similarly, the Resolving Power (RP) was 3.11, 3.61 and 1.67, respectively. Dj and RP for each primer were higher in landraces, except for primer WMS260. Primer WMS540 was polymorphic in the group of landraces and monomorphic in the group of modern cultivars. Three microsatellites consistently produced at least two alleles per accession (WMS752, WMS304 and WMC89), while null alleles were observed only for WMS540.

Microsatellite markers revealed a high level of polymorphism. In total, 224 alleles were detected with an average of 11.79 alleles per locus. Forty-nine alleles were classified as rare, with an average 4.56 of rare alleles per locus, since they appeared with a frequency lower than 0.05. Landraces had a higher number of private alleles compared to modern varieties. Private alleles were observed on both landraces originated from Cyprus and landraces originated from other areas. Modern varieties accessions were homogeneous in comparison to landrace accessions **(Figure 2)**. The average number of alleles per locus and accession was 1.99 and 1.25 for landraces and modern cultivars, respectively. The landraces conserved in the ARI Genebank exhibited higher heterogeneity compared to the landraces from ICARDA. "Kyperounda" accessions had the highest average number of alleles per locus and accession.

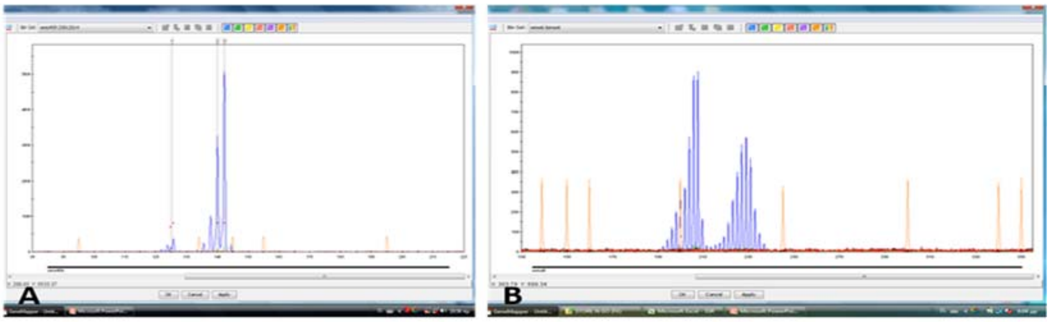


Figure 2: Electropherograms of homogeneous (A) and heterogeneous accessions (B).

Table 4: Levels of polymorphism detected by SSRs for the accessions studied.

	All accessions (56 accessions)					**Landraces (33 accessions)**					**Modern (22 accessions)**				
Primer	**No of alleles**	**Nr**	**Range (bp)**	**Dj**	**RP**	**No of alleles**	**No of private alleles***	**Range (bp)**	**Dj**	**RP**	**No of alleles**	**No of private alleles****	**Range (bp)**	**Dj**	**RP**
WMS752	14	4	105-161	0.93	4.43	13	7(2.2)	105-159	0.94	4.91	7	1(0.1)	105-161	0.73	2.64
WMS268	35	14	182-276	0.92	6.75	33	26(7.5)	182-276	0.99	9.27	9	2(1.0)	197-256	0.48	1.82
WMS312	15	8	184-246	0.82	3.18	14	13(6.4)	184-246	0.87	3.27	2	1	184-223	0.25	0.55
WMS148	9	2	139-167	0.89	2.89	8	4(0.2)	139-167	0.93	3.09	5	1	141-167	0.64	2.55
WMS619	12	5	135-164	0.88	2.93	10	9(1.3)	135-164	0.92	3.39	2	1	145-153	0.50	1.09
WMS5	8	-	158-176	0.93	3.54	7	3(0.1)	162-176	0.95	4.48	5	1(1.0)	158-170	0.80	2.00
WMS155	8	1	124-142	0.70	2.07	8	6(1.2)	124-142	0.89	2.85	2	0	124-128	0.09	0.18
WMS299	11	4	188-221	0.82	2.71	9	7(2.2)	188-221	0.89	3.21	3	1	192-215	0.64	1.91
WMS389	10	3	115-134	0.87	2.50	8	5(0.0)	115-134	0.92	2.85	4	1(1.0)	115-128	0.54	1.45
WMC161	16	9	137-185	0.80	3.25	14	9(4.4)	137-185	0.88	3.58	5	0	153-179	0.59	2.36
WMC89	9	3	124-178	0.89	3.86	6	2(1.0)	124-145	0.92	4.12	5	1	126-176	0.71	2.09
WMS304	9	1	196-216	0.87	2.93	8	2(0.1)	196-210	0.87	3.03	6	0	196-208	0.79	2.36
BARC74	13	6	146-187	0.88	3.04	10	6(0.5)	157-187	0.93	3.70	5	1(0.1)	167-177	0.64	1.82
WMS540	6	3	112-127	0.64	1.64	6	5(2.0)	112-127	0.76	2.26	1	0	114	0	0
WMS169	8	3	185-205	0.82	2.32	7	5(2.0)	185-197	0.85	2.55	3	1(1.0)	189-205	0.56	1.82
WMC104	14	5	120-188	0.90	4.11	14	10(3.1)	120-188	0.95	4.55	4	0	146-184	0.62	2.73
WMC83	8	2	95-167	0.71	1.75	7	5(0.2)	95-163	0.82	2.24	2	0	95-163	0.37	0.91
WMS260	6	2	143-166	0.78	2.25	4	1(1.0)	143-149	0.63	1.39	4	1(1.0)	145-166	0.71	2.00
WMS46	13	7	157-187	0.89	3.00	12	6(2.2)	157-185	0.95	3.94	6	0	171-183	0.59	1.36
Mean	11.79	4.56		0.84	3.11	10.42	6.89		0.89	3.61	4.21	0.68		0.55	1.67
Sum	224	49				198	131				80	13			

*first number: private alleles in landraces originated from other areas; second number: private alleles in landraces originated from Cyprus. ** first number: private alleles in varieties originated from other breeding programs; second number: private alleles in varieties originated from Cypriot breeding program. Dj: Discriminating power. Rp: Resolving power Nr=No of alleles with a frequency ≥5%.

Analysis of molecular variance (AMOVA) revealed that 81% of the total variation was attributed within group, while the remaining 19% was attributed among groups. The highest variability was recorded for landraces originated from Cyprus (*SS=491*), followed by landraces from other Mediterranean areas (*SS=269*), modern varieties from other breeding programs (*SS=174*) and modern varieties from Cyprus (*SS=43*). The genetic distance between clusters was significant (*Fst=0.186; p=0.001*), suggesting the presence of deviation between groups. The more diverged groups were the varieties originating from other breeding programs and the landraces originating from Cyprus (*Fst=0.266; p=0.001*), while genetic affinity was observed between the two landrace groups (*Fst=0.052; p=0.006*).

Figure 3 depicts the UPGMA unrooted dendrogram based on Dice genetic similarities. "Chinese spring", the only *Triticum aestivum* accession, was clustered separately from the core of durum wheat accessions entries. With the exception of "Omrabi5", "SYRIA IG129081" and "Simeto", all other modern varieties were clustered together. The four varieties released from the national breeding program of Cyprus ("Ourania", "Hekabe", "Makedonia" and "Karpasia") were closely related. Landraces did not follow a specific pattern according to their origin. However, "Kyperounda" accessions were clustered together alongside to "Cyprus-IG96271", "LIVIA-IG98726" and "MAROCCO-IG96437". "Famira" accessions were also grouped together with the "LOIZOS-ARI00084" and "TUNISIA-IG99151".

A Bayesian based approach was used to examine the population structure and the allocation of the genetic diversity. The range from K=1 to K=10 was investigated and the posterior probability for each K value was calculated using the estimated log likelihood of K. The optimum for the ad hoc quantity based on the second order rate of change of the likelihood function with respect to ΔK was observed for K=4 (**Figure 4**).

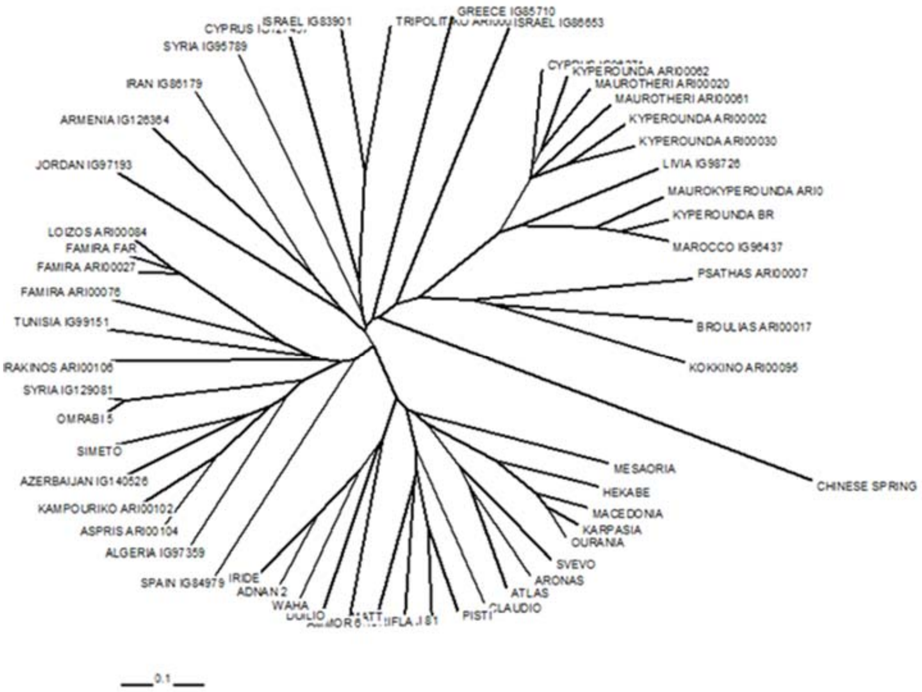


Figure 3: Unrooted UPGMA dendrogram of 56 durum wheat accessions based on Dice genetic similarity.

Structure analysis is comparable with the results of the cluster analysis. Forty-three out of the 55 accessions had a proportion of membership higher than 0.8. Modern varieties were assigned to Cluster 1 with an average proportion of membership 0.91. In general, the proportion of membership was higher than 0.8, with the exceptions of "Simeto", "Omrabi 5" and "IG129081". "IG129081" and "Omrabi5" had admixed genotypes having affinity to Cluster 1 while "Simeto" was affiliated to Cluster 2.

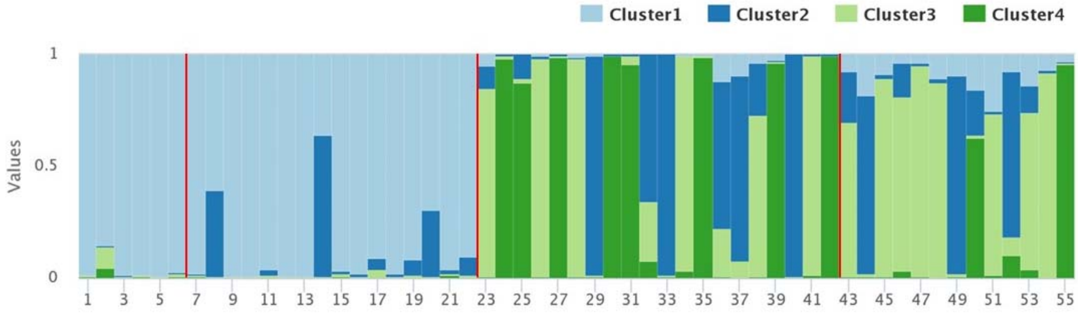


Figure 4: Population structure analysis of modern varieties and landraces with optimum cluster K = 4.

Landraces were genetically assigned to Clusters 2, 3 and 4. No specific pattern related to their origin was observed. "Famira" accessions were assigned to Cluster 2 along to "Aspris" and "Loizos" and two landraces originating from Algeria ("IG97359") and Azerbaijan ("IG140526"). A significant proportion of Cluster 2 membership, was also recorded from a landrace originating from Tunisia ("IG99151"). Cluster 3 contained landraces from Cyprus, Jordan ("IG97193"), Syria ("IG95789"), Israel ("IG86653") and Greece ("IG85710"). Lower percentage of membership, though higher than 0.7, was recorded for three landraces originating from Israel ("IG83901"), Armenia ("IG126364"), Cyprus ("Irakinos") and Iran ("IG86179"). "Kyperounda" type accessions were assigned to Cluster 4 with landraces "IG96271" (originating from Cyprus) and "IG96437" (originating from Morocco). A high proportion of membership to Cluster 4, was also recorded for a landrace originating from Libya ("IG98726").

1.3.2. Genetic diversity and structure of the "Kyperounda" landrace

From the 160 "Kyperounda" sublines of the three populations (accessions) conserved at ARI genebank examined in the field plots, 15 sublines were discarded from further analysis as off-types or hexaploid wheat **(Figure 5)**.

Figure 5: Off-types sublines in "Kyperounda" accessions.

In general, variation within sublines for heading date and plant height was recorded **(Figure 6)**. Population 1 differed significantly in heading date from the other two populations (*p<0,0001*). Most sublines had dense and short spikes, without hairiness and short beak. However, sublines with hairs on the glumes, long beak and intense black ear color or slightly colored were observed. These sublines existed in all populations, even though the variation within populations 2 and 3 was greater **(Figure 7)**.

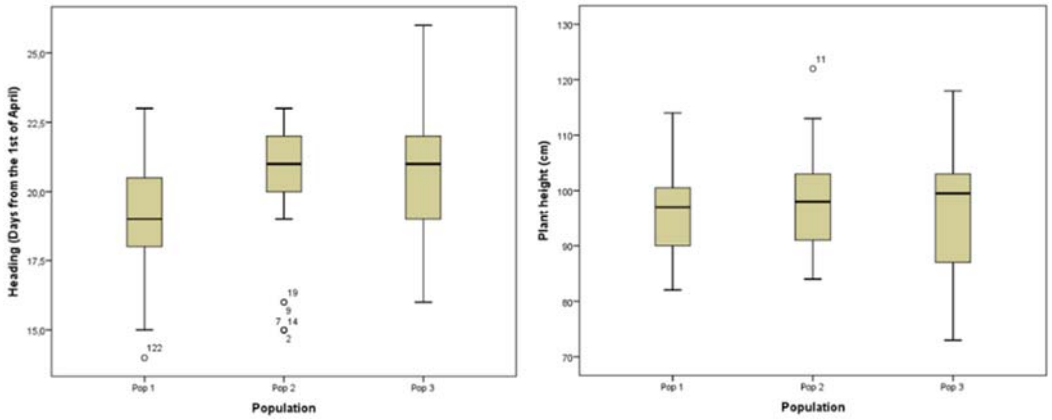


Figure 6: Variation between and within "Kyperounda" populations for heading date and plant height.

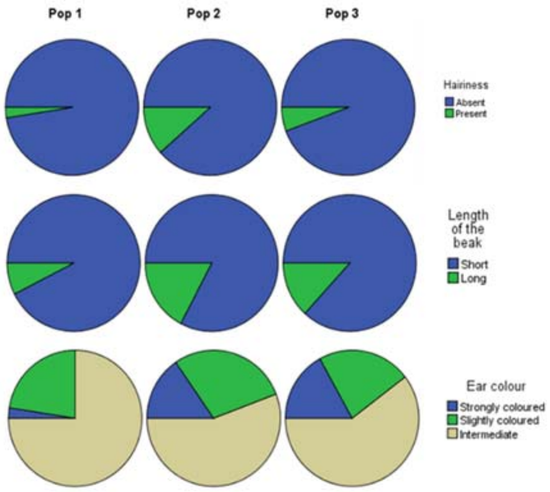


Figure 7: Variation between and within "Kyperounda" populations for ear characteristics.

The five primers were highly polymorphic **(Table 5)**. Primer WMC89 consistently produced two alleles per subline/plant. Fifty-six alleles were recorded with an average of 11.2 alleles per locus. Thirty alleles, or 53.57% of the recorded alleles, were rare. In total, 50 discrete genotypes were detected, with 35 of them having a frequency less than 0.05. On the contrary, the most common genotype had a frequency of 0.281. Principal Coordinate Analysis revealed that 59.68 (%) of the total diversity was explained by the first and the second axes **(Figure 8)**. No definite grouping of the populations was observed.

Table 5: List of the informative primers selected and the degree of polymorphism of the landrace "Kyperounda".

Primer	No of alleles	Nr	Range (bp)	Dj	Rp
BARC 74	12	5	156-187	0.71	2.19
WMC104	10	6	119-182	0.60	2.32
WMS268	17	12	182-265	0.50	2.20
WMS5	10	5	95-178	0.58	1.52
WMC89	7	2	121-145	0.55	1.48

Nr=No of alleles with a frequency ≥5%, Dj: Discriminating power. Rp: Resolving power.

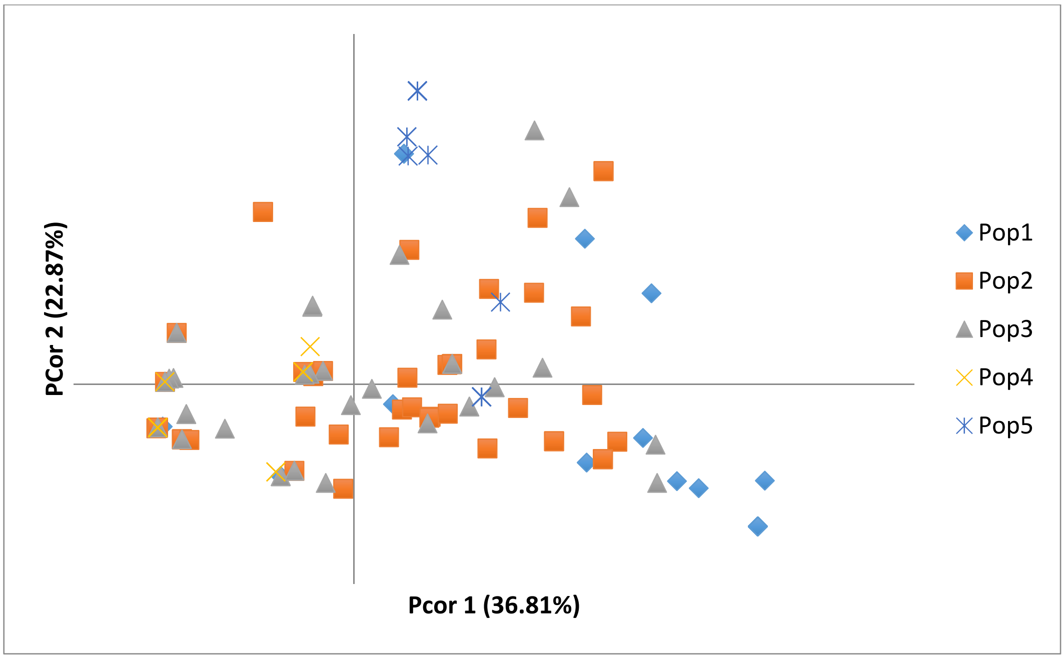


Figure 8: Principal Coordinate Analysis of "Kyperounda" populations.

AMOVA showed that 75% of the total variation was due to the within population variation and the remaining 25% due to the variation among populations. Population 2 showed the highest variability (*SS=229*), followed by Population 3 (*SS=179*), Population 1 (*SS=165*), Population 5 (*SS=68*) and Population 4 (*SS=39*).

The high genetic variation within Populations 1, 2 and 3 is further evident by the genetic diversity of the polymorphism indices depicted in **Table 6**.

Table 6: Diversity indices within "Kyperounda" populations.

	Na	Ne	Nr	Np	I	Ho	He	UHe	F
Pop 1	7	2.926	3.8	1.2	1.323	0.485	0.657	0.665	0.273
Pop 2	8.20	3.775	4.8	1.2	1.593	0.581	0.731	0.738	0.205
Pop 3	7.80	2.877	4	1	1.362	0.611	0.643	0.649	0.084
Pop 4	2.60	1.728	1.8	0.4	0.577	0.600	0.384	0.388	-0.184
Pop 5	2.80	2.229	2.4	0	0.786	0.415	0.469	0.478	0.203
Average	5.68	2.707			1.128	0.538	0.577	0.584	0.113

Na=No. of different alleles, Ne=No. of effective alleles, Nr=No. of different alleles with a frequency ≥5%, Np=No. of alleles unique to a single population, I=Shannon's Information Index, Ho=Observed heterozygosity, He=Expected heterozygosity, UHe=Unbiased expected heterozygosity, F=Fixation index

The higher genetic diversity portrayed by Population 5, compared to Population 4, was in line with field observations. Two phenotypes were observed in Population 5, while Population 4 appeared uniform **(Figure 9)**.

Figure 9: "Kyperounda" typical spikes in accession PI210951 with short beak and intermediate coloured spike (A), and in accession PI210960 with short beak and intesively coloured spike (B) and with long beak and intermediate coloured spike (C).

The genetic distance between clusters was significant (*Fst=0.253; p=0.001*) suggesting the presence of divergence among populations. The most divergent populations were Populations 4 and 5 (*Fst=0.621; p=0.001*), followed by

Populations 1 and 4 (*Fst=0.434; p=0.001*), while the closer genetic proximity was observed between Populations 2 and 3 (*Fst=0.017; p=0.054*), followed by Populations 3 and 4 (*Fst=0.124; p=0.001*). **Figure 10** depicts the UPGMA dendrogram based on Rogers genetic distances and the genetic relationships among the "Kyperounda" populations. A higher genetic affinity among Populations 2 and 3 and Populations 1 and 5 is evident, while Population 4 was allocated in between.

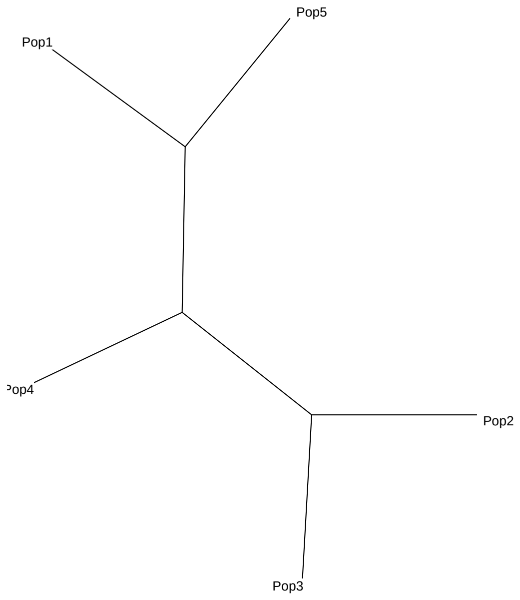


Figure 10: Unrooted UPGMA dendrogram of the five "Kyperounda" populations based on Rogers genetic distances.

A Bayesian based approach was further used to examine the population structure and the allocation of the genetic diversity. The range from K=1 to K=10 was investigated and the posterior probability for each K value was calculated using the estimated log likelihood of K. The optimum for the ad hoc quantity based on the second order rate of change of the likelihood function with respect to ΔK was observed for K=4 (**Figure 11**). Two hundred and three individuals out of 217 had a proportion of membership higher than 0.8. The most uniform population was Population 4; with almost all individuals belonging to cluster 3. The highest percentage of sublines from Population 3 were also grouped to cluster 3. Population 1 had the highest percentage of sublines grouped to cluster 1 while the highest percentage of individuals of Population 5 grouped to cluster 4. The majority of Population 2 sublines were grouped to cluster 2 and 3. Populations 2 and 3 had the highest percentage of sublines with a membership proportion lower than 0.8 (admixtures).

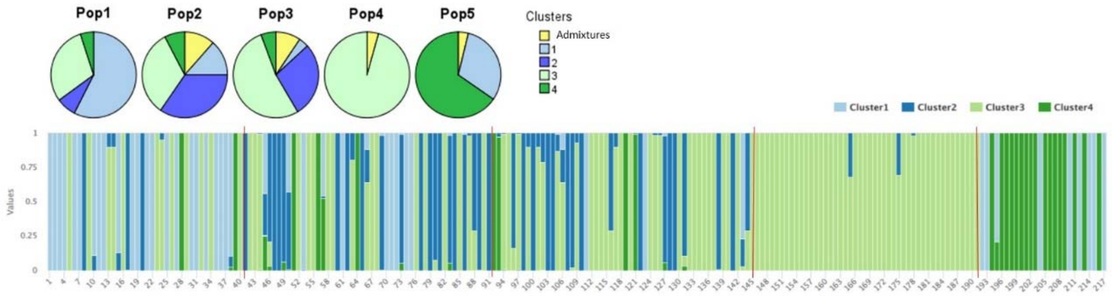


Figure 11: Population structure analysis of "Kyperounda" populations with optimum cluster K = 4.

1.4. Discussion

Genetic characterization of genetic resources enhances the development of rational conservation strategies and the utilization of germplasm to plant breeding programs. Microsatellites were employed in the present study to examine the genetic diversity and structure of a set of 55 durum wheat accessions comprised of 22 modern varieties from different breeding programs and 33 landraces originated from Cyprus and other Mediterranean areas. The genetic diversity and structure of the Cypriot landrace "Kyperounda" was further investigated.

1.4.1. Genetic diversity and structure of modern varieties and landraces

The 19 microsatellites employed were highly polymorphic revealing the existence of high genetic diversity. In total, 224 alleles were detected with an average of 11.79 alleles per locus. The average discriminating power (Dj) was 0.84. The level of polymorphism was higher within the set of landraces **(Table 4)**. The extent of variability revealed in this work is in agreement with previous studies employing durum wheat landraces (Teklu, et al., 2006; Khanjari, et al., 2007; Moragues, et al., 2007; Ruiz, et al., 2012). For example, by using 44 SSRs, Soriano, et al. (2016) detected 448 alleles, with an average of 10 alleles per locus. in a set of 192 accessions, originated from the Mediterranean Basin. Average expected heterozygosity was 0.71. Medini, et al. (2005) found 156 alleles, with an average of 10.4 alleles per locus, in 40 accessions using 15 SSRs. Average PIC value was 0.72. In the present study, the degree of polymorphism detected was higher compared to the informativeness indices in in the latter studies because all microsatellites depicted high values. The markers used in the current study detected less polymorphism within the set of modern varieties (the average number of alleles per locus was 4.21 and the average Dj was 0.55). This is in accordance with previous studies employing modern durum (Maccaferri, et al., 2005; Maccaferri, et al., 2007) and bread wheat varieties (Salem, Röder and Börner, 2015).

The higher levels of genetic diversity in landraces than in modern varieties revealed in this and in previous works (Medini, et al., 2005; Achtar, et al., 2010; Ganeva, et al., 2010; Soriano, et al., 2016; Riaz, et al., 2017) reinforce the potential use of landrace to the widening of the genetic base in modern varieties. The number of landrace accessions was higher than the number of modern varieties accessions. Imbalanced sample sizes can be a point of consideration when the genetic diversity between groups is investigated (Teklu, et al., 2006; Ren, et al., 2013). However, the fact that the set of modern varieties in this study included some of the most widely grown varieties in the Mediterranean, the narrower genetic diversity observed should be a point of consideration. The higher genetic variability was observed in Cypriot landraces and 36 alleles were detected only in this genepool. These results suggest the existence of unique genetic diversity within Cypriot landrace genepool.

Primer WMS268 generated substantially high number of alleles and the informativeness of this primer was much higher in landraces than in modern varieties **(Table 4)**. This primer also yielded a high number of alleles in other studies employing wheat landraces (Medini, et al., 2005; Teklu, et al., 2006; Khanjari, et al., 2007). Only one primer generated null allele. The presence of null allele is frequently reported (Khanjari, et al., 2007; Moragues, et al., 2007; Ganeva, et al., 2010; Oliveira, et al., 2012) and it can be related to point mutation, insertions, deletions and inversions to the flanking region of the primers (Leigh, et al., 2003; Teklu, et al., 2006).

The presence of multiple bands is frequently observed in microsatellites studies (e.g. Salem, Röder and Börner, 2015) and it can be attributed to heterogeneity within an accession, segregation at the respective SSR marker (Dreisigacker, et al., 2005) or to mutlilocus markers (Leigh, et al., 2003). Fifty seedlings were used for DNA extraction and modern varieties produced one allele per marker, with the exception of three markers that consistently produced two alleles. These primers have been mapped to more than one locus (Grain genes database, https://wheat.pw.usda.gov/GG3/), thus modern varieties were homogeneous. The higher number of alleles per locus within landrace entries can be attributed to heterogeneity between individuals within an accession. Landraces are characterized by high heterogeneity, since the variability within populations serves as important adaptive trait in stressful environments and under low inputs (Zhang, et al., 2006; Dwivedi, et al., 2016). Heterogeneity within landrace accessions is frequently reported in genetic studies (Dreisigacker, et al., 2005; Figliuolo, Mazzeo and Greco, 2007; Khanjari, et al., 2007). The heterogeneity within accessions was higher in the landraces conserved by ARI genebank. Despite that off-types were eliminated at the field plots, it is likely that plants with different genotype could have exhibited the same phenotype. These results underlines that collecting individual spike representative of the phenotype may not be adequate to collect certain genes and gene combinations within the phenotype (Alamerew, et al., 2004; Skovmand, et al., 2005) Furthermore, and in agreement with previous findings, accessions conserved with the same name were differentiated in cluster analysis ("Kyperounda" and "Famira" type accessions) (Achtar, et al., 2010). Individual wheat landrace accessions cannot always be seen as homogeneous groups of individual plants, as landraces are not static entries and episodes of introduction of new diversity may occur throughout their history (Oliveira, et al., 2012). Thus, passport data alone is not a reliable method to detect duplications in genebanks (Dobrovolskaya, et al., 2005; Zhang, et al., 2006).

Structure analysis showed a clear differentiation between modern varieties and landraces (Maccaferri, et al., 2005; Ganeva, et al., 2010; Soriano, et al., 2016; Kabbaj, et al., 2017; Riaz, et al., 2017). Modern varieties had a high proportion of membership to Cluster 1, except from three varieties. These varieties derived from crosses with landraces. "Simeto" derived from the africanum type population "Jennah Khetifa" (Maccaferri, et al., 2005) while Omrabi-5 derived from a cross of "Haurani" landrace and "JoriC69" (Kabbaj, et al., 2017). The genetic structure of modern durum wheat varieties can be related to pedigree, the targeting environment of the breeding program and the breeding history (Maccaferri, et al., 2003; Maccaferri, et al., 2005; Royo, et al., 2010; Laidò, et al., 2013; Ren, et al., 2013; Kabbaj, et al., 2017). Cluster analysis further deviated the varieties to four groups. "Mexacali 81" was grouped with "Anna", "Korifla", "Pisti" and "Matt". "Mexicali 81" and "Anna" were derived from "Mexicali 75", and "Matt" is a desert type variety originated from US. "Desert durums" from US were found to be genetically related with "Mexicali 75" in previous studies (Maccafferi, et al., 2005) and the "Mexicali 75" was found to be related with "Korifla" (Montovani, et al., 2008; Kabbaj, et al., 2017). Cypriot modern varieties formed a separate group. Selection for specific agronomic and quality traits and the share of common parental lines in their pedigrees (Josephides and Kyratzis, 2007) most likely narrowed the genetic diversity of Cypriot modern varieties. US varieties were found to form a different genetic group in previous studies (Maccaferri, et al., 2003; Montovani, et al., 2008; Kabbaj, et al., 2017). The introgression of genetic material from the North Dacota (USA) to improve technological characteristics might also contribute to the genetic differentiation, nevertheless the close genetic proximity of Cypriot varieties stresses the need for widening the genetic base of the national breeding program.

Kabbaj, et al. (2017) reported that landraces from Jordan, Syria and Iraq constitute a different genepool than landraces from Italy, Greece, Tunisia, Algeria and Spain. Since Cyprus is located to the middle of these geographical areas, it can be anticipated that the endemic genetic diversity of durum wheat landraces was modulated from both neighboring areas. Indeed, landraces form Cyprus were grouped in clusters containing landraces from the East and West Mediterranean, and from the East Balkan and Turkey (Soriano, et al., 2016). Genetic proximity between landraces from Cyprus and Turkey were also detected by Moragues, et al. (2007). In the present study, the landraces were grouped in three Clusters. Cluster 2 contained "Famira" type landraces from Cyprus and landraces from North Africa, Cluster 3 contained landraces from Cyprus and the Middle East, and Cluster 4 contained "Kyperounda" type landraces and landraces from North Africa.

Genetic proximity between landraces from Cyprus and countries from North Africa can be expected, since these areas share similar climatic conditions (Royo, Nazco and Villegas, 2014). On the other hand, the exchange of genetic material between these areas is documented since early breeding activities. "Kyperounda" was introduced to countries in North Africa and was used in breeding programs while "Hamira" is a landrace cultivated in Tunisia (Medini, et al., 2005). A landrace named "Hamira 436" was tested in yield trials in the 1930s in Cyprus as an introduced genetic material (Della, Farias and Josephides, 1980). An accession from Azerbaijan grouped in Cluster 2 appeared as an outlier. Interestingly, Kabbaj, et al. (2017) found a landrace accession originated from Azerbaijan under the same cluster of landraces originated from the Mediterranean indicating that the reported associations might not be casual. Genetic relatedness between landraces from Cyprus and the Middle East can be foreseen because of the close proximity between these areas. "Tripolitiko" and "Psathas" were among the Cypriot landraces grouped in Cluster 3. "Tripolitiko" could be an introduction to Cyprus from neighboring countries of the Middle East (Parisinos, 1956). "Psathas" was cultivated at the driest areas of Cyprus (Hadjichristodoulou and Della, 1976), probably due to the earlier heading of this landrace. Landraces originated from the Middle East are characterized by early heading because of the adverse climatic conditions in this area (Royo, Nazco and Villegas, 2014).

It should be stressed that although some entries might share the same ancestors, landraces are dynamic populations and their genetic diversity is modulated by natural and human selections, mutations, out crossings etc. Pagnotta, et al. (2004) stated that despite a possible common origin, it is likely that landraces experience processes of evolution generating divergence. Kabbaj, et al. (2017) postulated that landraces, which might have originated from related seed source and then exposed to similar natural pressures in different geographical areas, could accumulate distinct mutations over time.

1.4.2. Genetic diversity and structure of the "Kyperounda" landrace

The high genetic variability of "Kyperounda" is in agreement with the high phenotypic diversity of this landrace **(Figures 12 and 13)** (Bennet, 1973; Hadjichristodoulou and Della, 1976; Della, Farias and Josephides, 1980). Other studies also revealed the presence of variability within landrace populations of durum (Pagnotta, et al., 2004; Zhang, et al., 2006; Colomba and Gregorini, 2011; Mangini, et al., 2017) and bread wheat (Dreisigacker, et al., 2005). The average number of alleles per locus was higher than those reported by Ribeiro-Carvalho, et al. (2004), and Alsaleh, et al. (2016) who examined the genetic diversity within the landraces "Kunduru" and "Barbela". A high number of unique genotypes was recorded. Most genotypes appeared with low frequency, thus sampling a few individuals per landrace will probably result to low within landrace diversity (Oliveira, et al., 2012). Parisinos (1965) stated that "Kyperounda" was extensively used in early breeding program in Cyprus during the 50s, and a wide number of crosses were made between "Kyperounda" and other local or introduced landraces. Some of these lines were released to the farmers. Probably, these early breeding activities further widened the "Kyperounda" genetic diversity and the introduced of unique alleles.

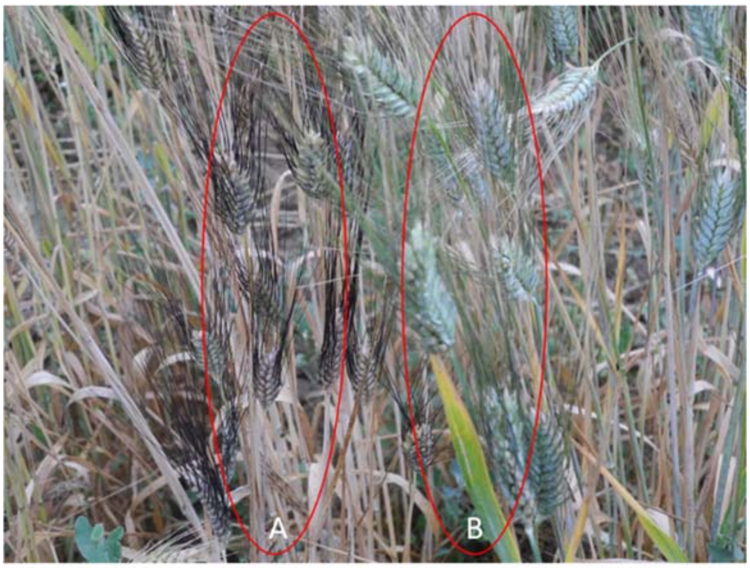


Figure 12: "Kyperounda" sublines depicting early (A) and late (B) maturity.

Figure 13: "Kyperounda" spikes: short beak and slithly colored ear (A), short beak and intermediate colored ear (B) short beak and intesively colored ear (C), presence

of hairiness on the clums and slithly colored ear (D), long beak and slithly coloured ear (E), and long peak and intermdediate colored ear (F).

The genetic diversity was higher within the "Kyprerounda" accessions conserved by the ARI genebank indicating that there is high genetic diversity within this collection. This can be partially attributed to the conservation method used in ARI genebank. Conserving accessions composed of sublines contributes to safeguarding high percentage of genetic variability, nevertheless the cost of this conservation method is a major concern (Dreisigacker, et al., 2005). The sampling method of the two accessions provided by USDA-ARS is unknown. The lower genetic diversity in these accessions can be attributed to the debulking strategy that is followed by genebanks and the expected reduction of genetic diversity with successive regeneration of bulk samples (Dreisigacker, et al., 2005; Skovmand, et al., 2005). Our findings stress that high genetic diversity is still hidden within individual landrace populations conserved as sublines. The exploitation of new high throughput and cost effective technologies such as genotyping by sequencing is urgent to uncover the true extent of genetic diversity hidden in large genebank collections and to rationalize *ex situ* conservation (Vikram, et al., 2016).

The highest diversity was observed in population 2 which was collected from Neo Chorio, Pafos. This site is a remote area located to the western part of the island where traditional farming systems and landraces are still present. Mountainous and remote areas are considered to be the hot spots of diversity (Hadjichristodoulou and Della, 1976; Zhang, et al., 2006). The environmental conditions in Neo Chorio are more favorable compared to Athienou and Pareklishia **(Table 3)**. Adverse climatic conditions contributed to the increase of the genetic variability within the landrace "Haurani" (Pagnotta, et al., 2004) and to the accumulation of high number of rare alleles in creole wheats (Vikram, et al., 2016). In the resent study, the more adverse climatic conditions were in Athienou. Population 1 which was collected from this area showed lower diversity, most likely because Athienou is located to the central plain where intensive agriculture and the use of modern varieties were applied long before the collection of the genetic material. This is further evident from the higher percentage of admixture with dwarf sublines of durum and bread wheat found in this population in the field experiment (data not shown).

The presence of variation for heading within "Kyperounda" was previously reported (Hadjichristodoulou and Della, 1976). Sublines from population 1 were earlier in heading compared with the heading of the sublines of the other two populations. Early heading contributes to drought escape during grain filling in the Mediterranean and landraces originated from drier areas were found to have earlier heading (Royo, Nazco and Villegas, 2014; Soriano, et al., 2016). Sublines from population 1 were collected from Athienou area. This area depicts the most adverse climatic conditions from the three collecting sites. In addition, high percentage of sublines from population 1 group to Cluster 1 and although no clear grouping of the populations was observed in PCoA, some genotypes from the population 1 were group separately on the wright down direction of axis 1. On the other hand, sublines from populations 2 and 3 were collected from areas with similar climatic conditions and were genetically close. These results indicate that environmental conditions can influence, to some extent, the genetic and phenotypic diversity of landraces (Pagnotta, et al., 2004; Ruiz, et al., 2012; Ren, et al., 2013; Royo, Nazco and Villegas, 2014; Soriano, et al., 2016).

Structure analysis did not group "Kyperounda" populations according to their collecting site. Similarly, landraces were not clustered according to their geographical origin. Luck of association between structure analysis and the ecogeographical factors of the wheat germplasm is frequently reported because genetic diversity is greatly impacted by multiple factors, such as environmental conditions, breeding methods and gene flow via human activities, i.e. exchange of seeds between farmers from different geographical areas and unconscious farmers' selections (Zhang, et al., 2006; Ruiz, et al., 2012; Oliveira, et al., 2012; Ren, et al., 2013; Baloch, et al., 2017). This is further evident by the high within group variability depicted by AMOVA in this and other studies (Dreisigacker, et al., 2005; Maccaferri, et al., 2005; Zhang, et al., 2006; Ganeva, et al., 2010; Ruiz, et al., 2012; Ren, et al., 2013; Soriano, et al., 2016; Kabbaj, et al., 2017).

1.4.3. Conclusions

High genetic diversity was detected in the set of 55 durum wheat accessions used in the present study. The genetic variability was higher in landraces than in modern varieties. Furthermore, landraces were grouped in different clusters in structure analysis. Our findings reinforce the potential use of landraces in breeding programs as gene donors to widening the genetic basis of the modern varieties. Cypriot landraces had the highest genetic variability and also depicted high number of private alleles suggesting the existence of unique genetic diversity within this genepool. Accessions of the same landrace were clustered separately signifying that passport data cannot be used as a criterion to eliminate duplications in genebanks. This was further evident by the high genetic diversity depicted within landrace "Kyperounda". Our results also suggest that agro-ecological conditions in the collecting site should be used with caution when linked to expected genetic diversity and adaptation to specific conditions in cultivated species because genetic diversity can be modulated by both climatic conditions and human activities. The genetic diversity in "Kyperounda" was higher in the accessions conserved as sublines in ARI genebank than in the accessions conserved as bulks. These results indicate that further research is needed to reveal the genetic diversity hidden within individual landraces and this research should be conducted in accessions conserved as sublines. Conserving the genetic variability between and within landraces in regional or global level might not be fissile because it will boost conservation cost. Thus, in integrated regional and global conservation systems, national conservation programs should have a predominant role for the *ex situ* conservation of within individual local landraces genetic variability.

2. Chapter 2 – Evaluation of Durum Wheat Modern Varieties Under Diverse Climatic Conditions

2.1. Introduction

Durum wheat is a major crop for the Mediterranean basin (Habash, Kehel and Nachit, 2009). This area is also the most significant import market and the largest consumer of durum wheat products (Royo, et al., 2017). The crop has been closely linked with the diet and the tradition in the area. Further to pasta production, durum wheat is widely used for the preparation of bread, couscous, bourghul and other traditional products (Guzmán, et al., 2016). Durum wheat in the Mediterranean usually grows under rain-fed conditions, where drought and heat stress or the combined effect of these variables results to yield loss (García del Moral, et al., 2003; García del Moral, et al., 2005; Habash, Kehel and Nachit, 2009) and to lesser extent affects the quality (Subira, et al., 2014).

During the recent years, the vast majority of the durum wheat producing areas in the Mediterranean are cultivated with modern varieties. These varieties are released by national breeding programs based, in most cases, on material received from CIMMYT and ICARDA (Boggini, et al., 1997; De Vita, et al., 2007; Royo, et al., 2007; Royo, et al., 2010; Subira, et al., 2014). In the case of Cyprus, national durum wheat breeding program also incorporated genetic material from North America (Josephides and Kyratzis, 2007). The increased grain yields and the related yield components, those are spikes per m^2, seeds per spike, kernel weight, and fixing plant phenology i.e plant height, anthesis date were main goals of the breeding programs (Hadjichristodoulou, 1985; De Vita, et al, 2007; Giunta, Motzo and Pruneddu, 2007; Royo, et al., 2007; Subira, et al., 2015). Moreover, breeding efforts also targeted quality traits that are desirable for the industry, such as yellow pigment concentration, protein content, vitreousness, gluten quality and volume weight (Marchylo, et al., 2001; Motzo, Fois and Giunta, 2004; De Vita, et al, 2007; Har Gil, Bonfil and Svoray, 2011; Ficco, et al., 2014; Sissons, et al., 2014; Subira, et al., 2014; De Santis, et al., 2017; Magallanes-López, et al., 2017). Among the several methods that are used for the assessment of gluten quality, Gluten Index is an internationally accepted indicator of gluten strength. This is a simple analysis method significantly correlated with the physical dough measurements (Marchylo, et al., 2001; Giuliani, et al., 2011) and storage protein composition (De Santis, et al., 2017).

Despite the progress made, breeders need to further increase yields to meet the demand from the arising growing population (Araus, et al., 2008). This is a daunting challenge in drought prone environments like the Mediterranean basin, where the impact of climate change is more severe (Habash, Kehel and Nachit, 2009). The evaluation of genetic material under the environmental conditions of the targeted breeding area for the traits of interest is necessary for the exploitation of the genetic resources in plant breeding (e.g. Hadjichristodoulou, 1987; Lopes, et al., 2012; Lopes, et al., 2014; Del Pozo, et al., 2016, Gonzalez-Navarro, et al., 2016). Several studies employed varieties of different origin. Magallanes-López, et al. (2017) stated that the phenotypic characterization of varieties with different origin increases the genetic diversity of the materials which is useful for finding more genotypic-phenotypic associations. Exotic germplasm can be useful in plant breeding even for areas with long standing tradition for a particular crop which can contribute to the broadening of the existing genetic base (Boggini, et al., 1997).

Stability across environments together with high mean values are desirable in wheat breeding programs (Boggini, et al., 1997; Rharrabti, et al., 2001; Li, et al., 2013), especially in the Mediterranean area due to high annual variation in grain yields and quality traits (Rharrabti, et al., 2003b; Habash, Kehel and Nachit, 2009; Subira, et al., 2015). Plasticity of phenological development merits consideration as a distinct trait influencing crop adaptation, and yield and phenotypic plasticity can contribute to the understanding of GxE interactions of yield in grain and horticultural crops (Sadras, et al., 2009). Breeders can select for or against phenotypic plasticity, thereby tailoring the response to environmental conditions to enhance crop resilience for severe or unpredictable climate scenarios (Grogan, et al., 2016). Similarly, Sadras and Rebetzke (2013) stated that dual focus on traits *per se* and their plasticity is a fruitful approach to understand the phenotype, particularly when GxE interaction is large. The slope of regression model (b) fitted with genotype and environmental means (Finlay and Wilkinson, 1963) is frequently used to estimate the plasticity of agronomic traits (Hadjichristodoulou, 1985; Rharrabti, et al., 2001;

Rharrabti, et al., 2003b; De Vita, et al., 2007; Sadras and Lawson, 2011; Subira, et al., 2014; Grogan, et al., 2016).

Many studies in the past examined the phenotypic characterization of genetic material from the Near East and the Mediterranean and the association between traits. However, in these studies, the inclusion of modern varieties originated from Cyprus is scarce. In addition, very few studies investigated associations between plasticity for important agronomic traits in durum wheat. The main objectives of the present study were: (1) to examine the phenotypic diversity for agronomic and quality traits of a set of modern varieties including varieties widely used in the Mediterranean and varieties released by the national breeding program of Cyprus, (2) to explore the correlations between the traits *per se*, (3) to examine the traits' plasticity, and (4) to investigate the correlations between the traits' plasticity as well as of the traits' plasticity with the traits *per se*.

2.2. Materials and methods

2.2.1. Plant material

Twenty durum wheat varieties (*Triticum turgidum* subsp. *durum*) were selected for the present study (**Table 7**). Six varieties were bred by the Cypriot National Breeding Program and represent the main commercial varieties cultivated in Cyprus for the last forty years. The other fourteen varieties were released by other breeding programs targeting areas with similar climatic conditions. These varieties are widely grown, at least in some regions of the Mediterranean, and some of them have been used as parents to breeding programs.

Table 7: List of the durum wheat varieties used for the phenotypic evaluation.

Name	Year of release	Country of Registration / Origin	Name	Year of release	Country of Registration / Origin
Aronas	1977	Cyprus	Pisti	2008	Greece
Mesaoria	1982	Cyprus	Simeto	1988	Italy
Karpasia	1985	Cyprus	Duilio	1984	Italy
Macedonia	1994	Cyprus	Iride	1996	Italy
Ourania	2006	Cyprus	Claudio	1998	Italy
Hekabe	2003	Cyprus	Svevo	1996	Italy
Anna	2000	Greece	Adnan2		ICARDA
Atlas	1995	Greece	Omrabi5		ICARDA
Matt	2003	Greece	Korifla		ICARDA
Mexikali81	1985	Greece	Waha		ICARDA

2.2.2. Field experimental conditions and design

Experiments were conducted at three locations (Achelia - 34°43'N, 32°29'E, Dromolaxia - 34°53'N, 33°35'E and Athalassa 35°08'N, 33°24'E) for two consecutive years (2011/2013 at Achelia and Dromolaxia and 2012/2014 at Athalassa) (six environments in total). The three experimental locations exhibit different climatic conditions **(Figure 14)**. Achelia has higher temperature on winter and lower temperature on spring, higher precipitation and the deep clay soil favouring high yields. Athalassa has shallow sandy clay loam soil and lower precipitation during crop cycle resulting to drought stress during heading and grain filling. In addition, the higher day temperature in spring and the frequent occurrence of extreme high temperatures during grain filling very often results to heat stress conditions. Dromolaxia also has sandy clay loam soil and it depicts intermediate climatic conditions. Dromolaxia and Achelia represent the main durum wheat producing areas in Cyprus.

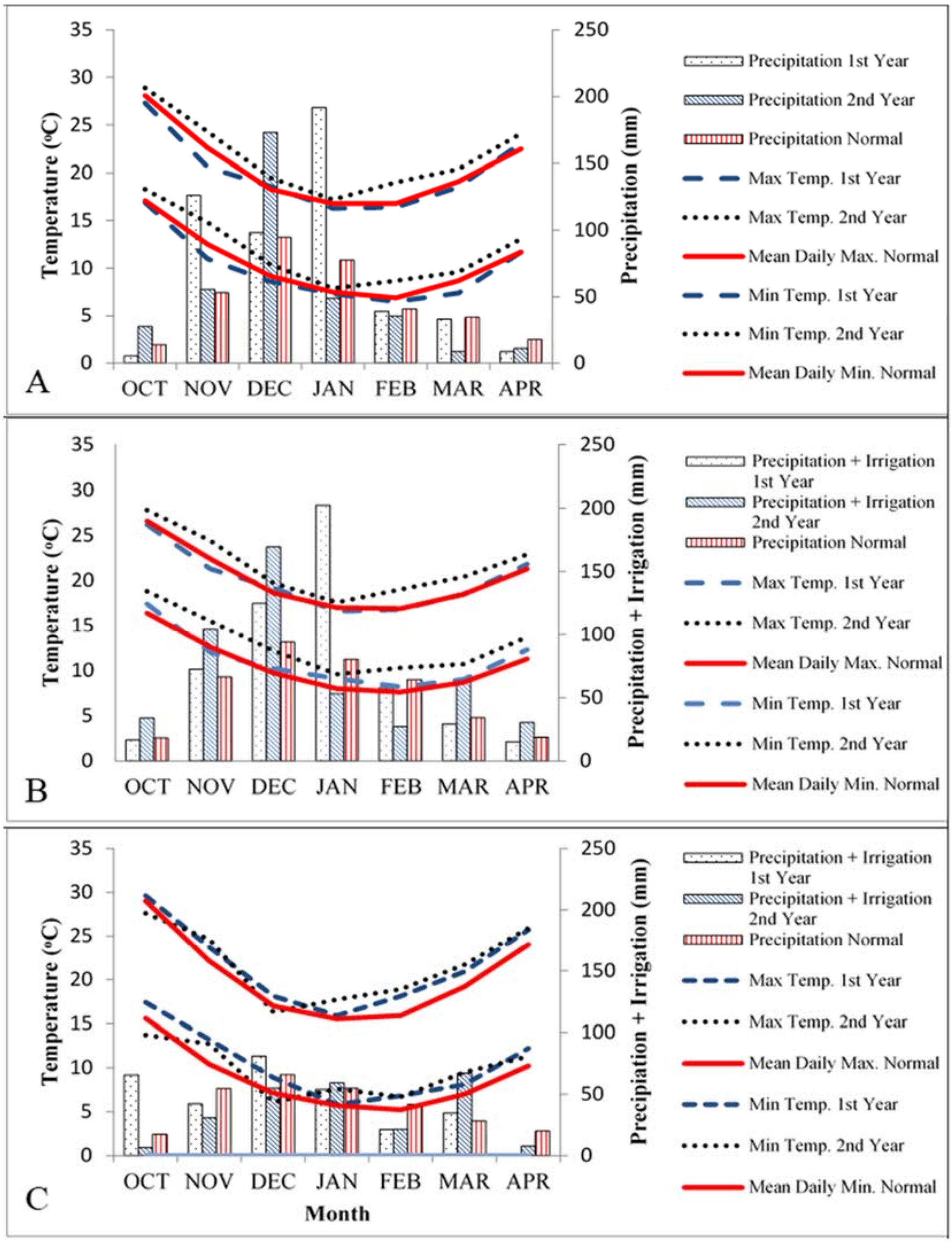


Figure 14: Normal climatic conditions based on historical records and climatic conditions during the experimentation years 2011/2013 in Dromolaxia (A), Achelia (B) and 2012/2014 in Athalassa (C) experimental station.

The experimental design was a randomized complete block with four replications. Each plot was 8m long, and comprised of six rows, spaced apart 0.175m. Seed rate was adjusted to 226 germinating seeds m^{-2}. Experiments were sown at the end of November in Athalassa, and on December in Dromolaxia and Achelia. Due to the exceptionally high precipitation in December during the 2nd year, sowing in Achelia was delayed till the end of December. The fields were fertilized before sowing with 60 Kg ha^{-1} of N_2 and P_2O_5, respectively. In the 1st year, experiments in Achelia and Dromolaxia were additionally top dressed with 40 Kg of N_2 ha^{-1} at tillering. Weeds were chemically controlled at tillering (Atlantis® Bayer, Illoxan® Bayer, Granstar® DuPont). High infestation with the cereal tortricid *Cnephasia pumicana* in Achelia the 1st year was controlled by Valiant® (Agriphar) **(Figure 15)**. Irrigation was applied in Athalassa during booting (30mm) in the 1st year, and during tillering (50mm) and booting (50mm) in the 2nd year. Irrigation was also applied in Achelia during anthesis (60mm) in the 2nd year.

Figure 15: Spike infested by the cereal tortricid *Cnephasia pumicana* in Achelia.

2.2.3. Measurements of agronomic traits

Heading was recorded when the ears of 50% of the tillers had emerged from the flag leaf sheaths for approximately half their length and was expressed as growing degree days from emergence to heading (GDD). Growing degree days were calculated as described by Aparicio, et al. (2000). Plant height (PH) was recorded as an average of three measurements per plot at physiological maturity, excluding awns. Number of fertile tillers per m^2 (NTLSM) was estimated at physiological maturity from four rows, each one 1m long, randomly selected on the 2nd and the 5th rows. Plants from two rows, each one 1m long, were randomly selected on the 2nd and 5th row and hand harvested to estimate the number of seeds per spike (SPS) and the harvest index (HI). The plots were mechanically harvested at the end of May or beginning of June and grain yield was recorder at 12% moisture level (GRYLD). Before harvesting, the two external rows and half meter from both ends of the plots were discarded to avoid the boarding effect **(Figure 16)** (Ceccareli and Grando, 1996). Thousand kernel weight (TKW) was calculated as the mean weight of two samples of 200 seeds per plot and expressed in g. Volume weight (VW) was measured with a 0.5L chondrometer (Seedburo) and expressed as Kg hl^{-1}.

Figure 16: Boarding effect (A) and experimental plots after discarding external rows (B).

Protein content (PRO), yellow pigment content (CAR) and gluten index (GI) were assessed on whole grain flour samples. Grain nitrogen content was determined according to Kjeldahl method. PRO was calculated multiplying the N value by 5.7 and expressed as a percentage on a dry weight base. CAR was determined by AACC method 14-50.01 and expressed in ppm. GI was measured by AACC method 38-12.02, using a Perten Glutomatic with a minimum of two repeats per sample. PRO, CAR and GI were assessed on two samples per variety. Each sample was drawn from the bulk of two replications. Ear length, number of spikelets per spike and ear density were estimated on 20 spikes randomly selected from each plot. Harvest Index, ear length, number of spikelets per spike and ear density were measured only in Dromolaxia and Achelia areas during the 1st year.

2.2.4. Statistical analysis

Combined analysis of variance was conducted for agronomic traits considering genotypes and environments (and years within each location) as fixed factors using GLM procedures in SPSS (IBM, SPSS ver. 22). Kolmogorov-Smirnov test was applied to check the normality of the standardized residuals. Predicted values were plotted against standardized residuals to assess the equality of variance. Homogeneity of mean square errors form separate ANOVAs was checked with Bartlett's test. Means were compared by applying the LSD test at significance level 0.05. Stability was calculated as the slope of the regression line of genotype means on environmental means according to Finlay and Wilkinson (1963). Stability was calculated for all traits. Genotypes for which linear relationships were not obtained, were excluded from further analysis. The comparison of the regression lines was performed as described by Clewer and Scarisbrick (2001). Pearson correlations were computed to estimate associations between traits. Squared Euclidean distances were calculated on standardized Z values, with a mean of 0 and a standard deviation of 1, from mean phenotypic data across environments. Hierarchical cluster analysis was conducted using the furthest neighbor method. Principal Component Analysis (PCA) was performed on environmental means using the direct oblimin rotation method to explore relationships among traits and climatic variables. PCA, Pearson correlations, regression, and cluster analysis were carried out using SPSS (IBM, SPSS ver. 22).

2.3. Results

2.3.1. Environmental conditions

Average maximum and minimum temperatures were similar and higher than normal in Dromolaxia and Achelia during the 1st and the 2nd year, respectively. The 1st year, precipitation in these locations was higher than normal during November, December and particularly January, followed by slightly lower precipitation until April, while the 2nd year, precipitation was exceptionally high on December followed by low precipitation until the end of the growing season. In Achelia, additional irrigation was applied at anthesis during the 2nd year. During both years in Athalassa, average temperatures and precipitation were higher and lower than normal, respectively, particularly the 2nd year. Compared to the 2nd year, the water input in this location was higher until anthesis the 1st year and lower during grain filling **(Figure 14)**.

2.3.2. Phenotypic diversity

Table 8 shows the results of the combined analysis of variance, mean values and the coefficient of variation across all environments. There were statistically high significant differences between varieties for the agronomic traits examined in the present study. Mean genotype values and LSDs are shown to **Table 9** across environments and within locations in appendices I, II and III**.** The differences were also significant between environments **(Table 10)**. Genotype effect explained high percentage of variability for CAR, and to a lesser extent for SPS, GI and PH, while the environmental factor explained very high percentage of variability for GRYLD, PRO, VW, TKW, and to lesser extent for NSSM, NTLSM and GDD. Interactions, although significant, explained less variability than the main effects.

Statistical significant differences between varieties were also observed within locations **(Table 8)** and within each environment **(Table 10)**. The genotype factor consistently explained high variability for PH, SPS, TKW and CAR while, the year factor constantly explained high variability for GDD and GRYLD. The genotype factor explained the high percentage of variability for NSSM, NTLSM and for VW in Dromolaxia and Athalassa but not in Achelia

Table 8: Combined Analysis of Variance over all environments and at each location (% of the total Sum of Squares from ANOVA analysis). Mean values and Coefficient of Variations (CV) are also shown.

	GDD	PH	GRYLD	NTLSM	SPS	NSSM	TKW	VW	CAR	PRO	GI
All environments											
Environment	76.59***	33.01***	91.93***	60.34***	38.02***	63.96***	85.13***	86.50***	16.08***	86.61**	41.91***
Block (Environment)	2.08***	10.79***	2.15***	8.04***	4.09***	6.93***	1.33***	2.04***	0.34**	3.81***	0.14*
Genotype	17.33***	31.46***	0.95***	10.66***	37.66***	11.23***	8.27***	5.47***	77.59***	4.11***	42.79***
Genotype x Environment	2.37***	11.03***	1.94***	6.54**	7.03***	4.81*	3.01***	3.47***	4.36***	3.50***	14.12***
Error	1.63	13.72	3.04	14.42	13.19	13.07	2.27	2.52	1.63	1.98	1.04
Mean	1242	83.33	4118	321.04	30.56	9947.26	38.31	75.54	6.90	17.14	39.63
CV (%)	1.22	4.32	10.81	11.52	9.51	15.06	5.43	1.49	3.89	4.44	10.69
Location: Dromolaxia											
Year	57.72***	32.65**	52.25**	13.12*	0.00	8.92	1.80	0.48	0.95	37.08	51.51**
Block(Year)	1.78***	12.31***	14.29***	9.77***	6.69***	14.06	6.29***	5.55***	0.10	19.08***	0.34**
Genotype	34.12***	39.07***	12.37***	38.34***	73.87***	39.80***	71.58***	67.08***	96.31***	22.44***	33.51***
Genotype x Year	2.27***	3.96*	5.70**	9.14*	3.46	6.49	5.38**	9.53***	1.14	9.95	13.52***
Error	4.12	12.01	15.40	29.63	15.99	30.73	14.94	17.37	1.50	11.45	1.12
Mean	1249	85.04	4515	377.88	31.10	11636.45	39.93	77.89	6.96	15.12	31.50
CV (%)	1.26	3.98	9.58	10.07	8.69	12.65	5.52	1.08	3.55	5.22	14.30
Location: Achelia											
Year	52.45***	3.53*	61.75***	51.37**	0.33	30.32**	15.89***	46.88***	0.00	17.34	37.18***
Block(Year)	1.45***	2.04*	7.07***	11.16***	3.40*	12.21	1.86**	0.80	0.11	9.75***	0.05
Genotype	39.67***	74.77***	7.06**	14.07***	62.40***	23.00***	58.17***	30.97***	96.66***	48.09***	52.48***
Genotype x Year	3.55***	4.95*	7.55***	2.91	8.84**	6.84	15.05***	10.76***	2.51***	11.58	9.36***
Error	2.88	14.71	16.58	20.48	25.03	27.61	9.03	10.60	0.71	13.24	0.93
Mean	1151	86.81	6293	340.23	35.13	11928.35	50.50	80.77	6.19	14.35	46.57
CV (%)	1.30	3.67	8.77	10.54	8.99	14.20	3.61	0.77	2.34	3.53	9.41
Location: Athalassa											
Year	49.06**	1.55	15.83	3.26	16.66*	13.44	5.29	15.08	2.53	0.10	21.23***
Block(Year)	9.15***	29.06***	33.13***	26.96***	8.55***	23.12	20.88***	16.73***	0.88**	31.40***	0.01
Genotype	36.58***	31.76***	12.10**	27.49***	46.65***	24.68***	47.70***	45.45***	90.83***	47.05***	68.48***
Genotype x Year	2.18***	8.83*	9.76*	9.05	8.17**	8.84*	5.71	6.79**	2.64	8.71	8.92***
Error	3.03	28.81	29.18	33.24	19.98	29.92	20.43	15.95	3.12	12.75	1.36
Mean	1325	78.14	1545	244.99	25.46	6276.98	24.52	67.96	7.55	21.95	40.80
CV(%)	1.12	5.34	20.70	15.10	11.15	20.76	8.91	2.42	4.85	4.22	9.27

*p<0.05, **p<0.01, ***p<0.0001 [Grain yield (GRYLD), number of seeds per m^2 (NSSM), number of fertile tillers per m^2 (NTLSM), seeds per spike (SPS), volume weight (VW), thousand kernel weight (TKW), plant height (PH), growing degree days to heading (GDD), protein content (PRO), yellow pigment concentration (CAR), gluten index (GI)]

Table 9: Means and LSDs between varieties over all environments.

Variety	GDD	PH (cm)	GRYLD (Kg/ha)	NTLSM	SPS	NSSM	TKW (g)	VW (Kg/hl)	CAR (ppm)	PRO (%)	GI (%)
Adnan2	1262	82.24	4204	302.02	41.26	12781	32.72	75.39	6.73	15.95	4.12
Anna	1187	85.09	4175	360.77	26.45	9574	40.33	74.62	6.41	16.14	43.43
Aronas	1231	87.12	3920	319.99	28.03	9170	37.27	74.35	3.87	17.25	57.52
Atlas	1278	84.77	3802	307.08	25.72	8161	41.79	77.91	6.50	18.37	19.69
Claudio	1334	83.05	3929	302.02	28.53	8855	40.50	78.40	6.30	17.91	39.43
Duilio	1238	83.15	4135	299.16	30.01	9114	42.30	77.38	5.56	16.39	42.96
Hekabe	1238	86.63	4199	316.60	30.80	9898	39.12	74.68	8.95	17.41	46.74
Iride	1253	78.00	4169	267.44	41.25	11237	34.76	76.21	6.47	16.88	52.90
Karpasia	1275	83.29	4097	315.77	34.13	10967	33.02	74.49	7.39	18.21	6.21
Korifla	1293	82.63	3681	320.95	31.24	10235	35.04	73.75	6.62	17.22	42.33
Macedonia	1222	84.29	4607	370.95	31.98	12022	34.94	75.18	8.76	16.88	50.82
Matt	1191	82.31	4184	320.17	31.78	10345	36.84	75.12	9.70	17.04	75.40
Mesaoria	1189	73.47	4066	324.76	27.91	9287	37.01	72.77	5.67	18.03	7.69
Mexikali81	1195	81.44	4059	339.40	27.71	9555	39.81	74.34	6.85	16.03	46.47
Omrabi5	1253	97.34	4042	332.97	28.45	9559	36.58	76.96	7.05	17.03	24.36
Ourania	1262	84.66	4352	339.94	31.85	11084	36.10	76.76	7.83	17.32	49.15
Pisti	1181	82.11	4475	375.47	27.87	10549	41.04	75.56	6.28	16.23	60.40
Simeto	1311	76.25	4110	288.45	27.97	8336	45.66	75.75	6.70	16.98	56.04
Svevo	1231	86.29	3910	328.63	27.16	9058	39.19	76.39	8.05	18.55	39.47
Waha	1217	82.49	4237	288.09	31.08	9160	42.27	74.70	6.24	16.90	27.28
LSD	8.62	2.04	253	21.00	1.65	851	1.18	0.64	0.22	0.62	3.43

[Grain yield (GRYLD), number of seeds per m^2 (NSSM), number of fertile tillers per m^2 (NTLSM), seeds per spike (SPS), volume weight (VW), thousand kernel weight (TKW), plant height (PH), growing degree days to heading (GDD), protein content (PRO), yellow pigment concentration (CAR), gluten index (GI)]

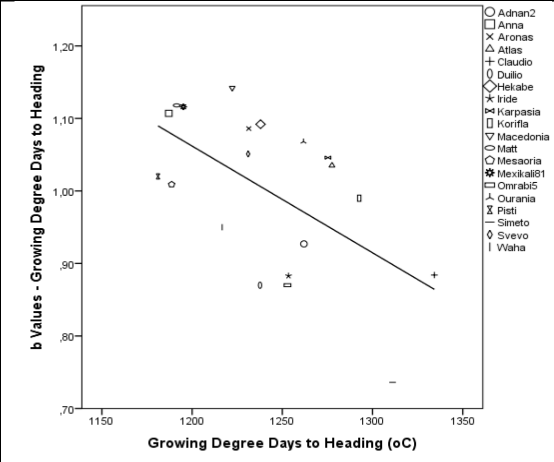
Table 10: Mean values and significant level of the genotype effect within each environment, LSD within each location and LSD between environments.

ENVIROMENT	GDD	PH (cm)	GRYLD (Kg/ha)	NTLSM	SPS	NSSM	TKW (g)	VW (Kg/hl)	CAR (ppm)	PRO (%)	GI (%)
Dromolaxia12	1299 ***	89.75 ***	5187 ***	399.27 ***	31.07 ***	12305 ***	39.28 ***	77.77 ***	6.83 ***	16.10 *	52.54 ***
Dromolaxia 2013	1199 ***	80.34 ***	3842 ***	356.50 ***	31.11 ***	10967 ***	40.58 ***	78.00 ***	7.10 ***	14.15 *	10.46 ***
Achelia 2012	1205 ***	88.12 ***	7193 *	388.18 *	34.83 ***	13426 ***	48.46 ***	81.87 ***	6.18 ***	14.75 ***	65.66 ***
Achelia 2013	1097 ***	85.49 ***	5394 **	292.29 ***	35.44 ***	10430 **	52.54 ***	79.67 ***	6.19 ***	13.95 **	27.48 ***
Athalassa 2013	1275 ***	78.93 ***	1723 **	253.77 ***	27.60 ***	7014 ***	23.56 ***	66.55 ***	7.32 ***	22.01 ***	30.50 ***
Athalassa 2014	1375 ***	77.33 ***	1346 ns	235.21 *	23.28 ***	5539 ***	25.46 ***	69.31 ***	7.78 ***	21.89 **	51.11 ***
LSD years (Dromolaxia)	17.48	5.77	702.75	36.86	ns	ns	ns	ns	ns	ns	10.44
LSD years (Achelia)	17.86	2.00	607.85	44.65	ns	1900	1.39	0.29	ns	ns	4.22
LSD years (Athalassa)	43.40	ns	ns	ns	3.13	ns	ns	ns	ns	ns	3.57
LSD (Environments)	24.83	4.62	542	39.99	2.34	1580	2.31	1.47	0.29	2.52	3.73

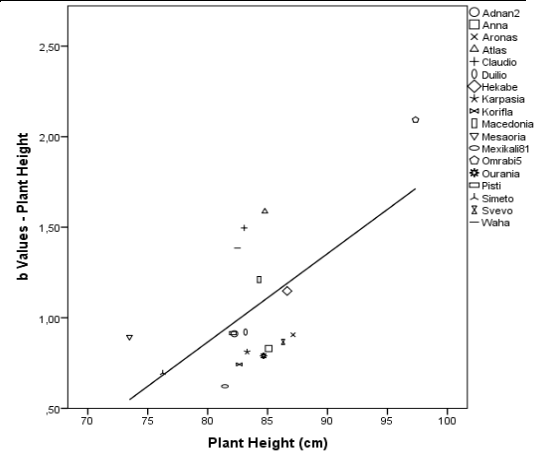
[Grain yield (GRYLD), number of seeds per m^2 (NSSM), number of fertile tillers per m^2 (NTLSM), seeds per spike (SPS), volume weight (VW), thousand kernel weight (TKW), plant height (PH), growing degree days to heading (GDD), protein content (PRO), yellow pigment concentration (CAR), gluten index (GI)] ns (not significant), *$p<0.05$, **$p<0.01$, ***$p<0.0001$

and for PRO and GI in Achelia and Athalassa but not in Dromolaxia. The effect of year was not significant for CAR and PRO, and it was significant for GDD and GI, at all locations.

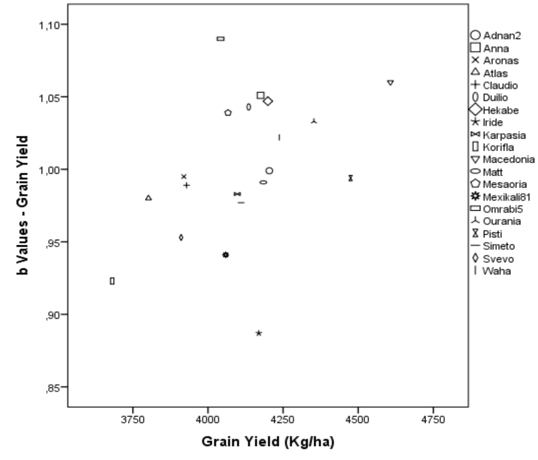
Varieties statistically differed in plasticity (b values) except for GRYLD, NSSM, NTLSM, and SPS. GRYLD showed the lowest variation in b values while CAR and GI had the largest variation. Plasticity was increased with increased average value with the exception of GDD and VW for which an opposite trend was observed **(Figure 17)**. Associations between plasticity and mean values were not significant for GRYLD and yield components except for NSSM. Late heading varieties were more stable than early varieties, nevertheless, there was variation in varieties plasticity throughout all the range of heading. The largest plasticity in PH was recorded for "Omrabi5" which was the tallest variety in the experiments. The same variety showed the highest plasticity for grain yield and "Iride" the lowest, however, none of them were among the highest yielding varieties. "Omrabi5" had good performance in high yielding environments and poor performance in low yielding environments while "Iride" showed the opposite trend. "Adnan 2" had the highest NSSM and the highest plasticity for this trait. "Iride", "Simeto" and "Waha" had the lowest NTLSM, however, "Waha" was able to produce more fertile tillers in more favorable conditions while "Iride" produced more fertile tillers in less favorable conditions. "Pisti" and "Makedonia" had the highest NTLSM. "Pisti" produced more fertile tillers in more productive conditions while "Makedonia" produced more fertile tillers in less productive conditions. "Iride" and "Adnan 2" had the highest number of SPS and they showed the highest plasticity for this trait. "Adnan 2", "Karpasia" and "Iride" had the lowest TKW and "Iride" was the most stable variety. "Simeto" had the largest TKW and was one of the most stable varieties for this trait. The superiority of "Simeto" was more profound to low yielding environments. "Simeto" also had average VW and was the most stable variety for this trait. "Claudio" had the highest VW. "Matt", followed by "Makedonia", "Hekabe" and "Svevo" had the highest CAR, however, "Matt" was consistently the best variety in all environments with the highest response to conditions favoring high CAR. "Svevo" had the highest PRO and high plasticity, while "Simeto" had average PRO and the lowest plasticity.



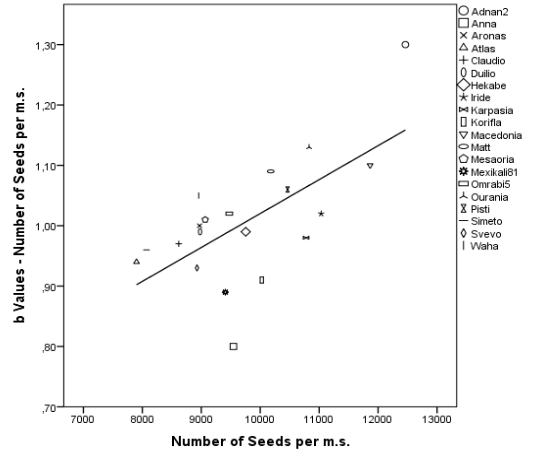
($R^2=0.337$, $p=0.007$) (a)



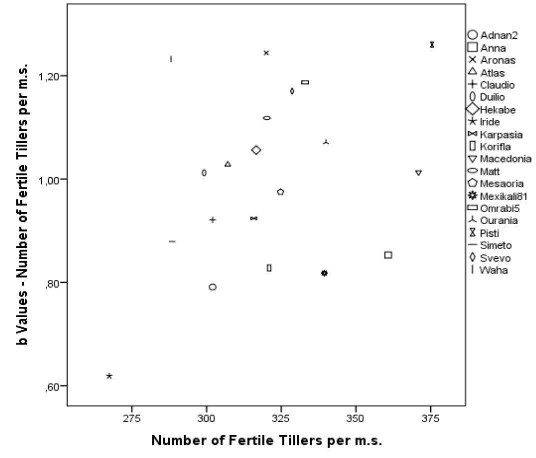
($R^2=0.383$, $p=0.006$) (b)



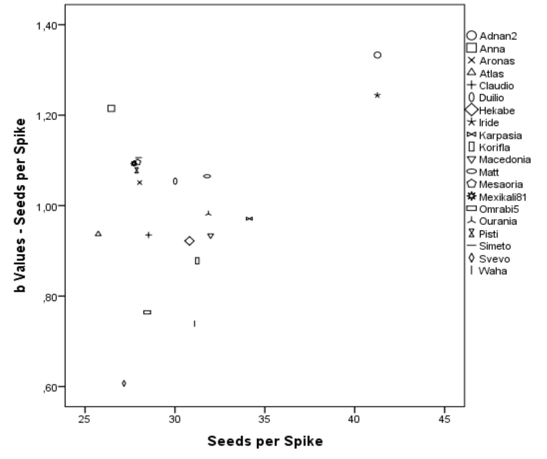
($p=0.063$) (c)



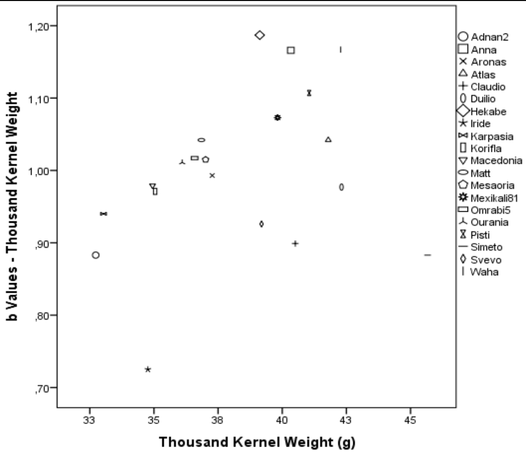
($R^2=0.424$, $p=0.002$) (d)



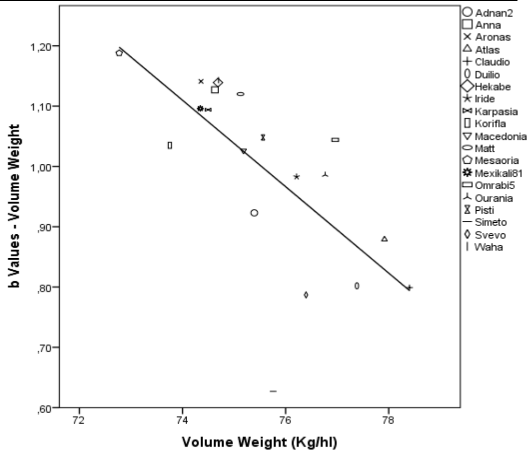
($p=0.129$) (e)



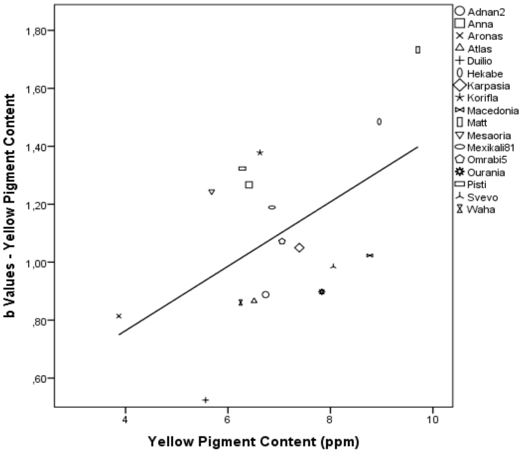
($p=0.06$) (f)



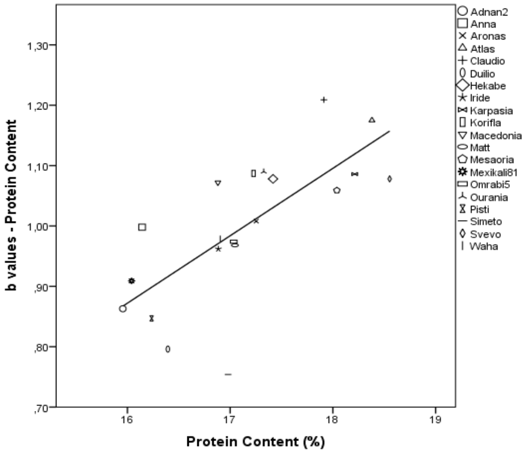
$(p=0.147)$ (g)



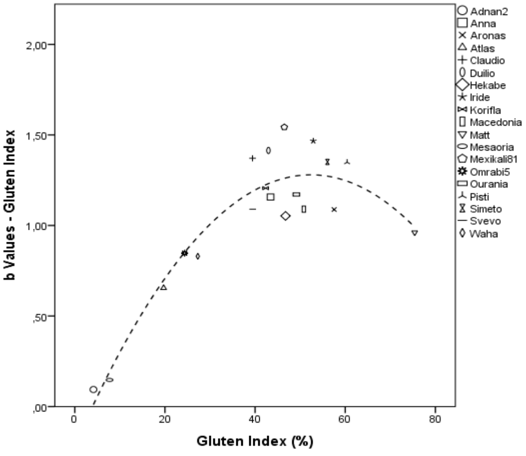
$(R^2=0.465, p<0.001)$ (h)



$(R^2=0.287, p=0.027)$ (i)



$(R^2=0.520, p<0.001)$ (g)



$(R^2=0.875, p<0.001)$ (k)

Figure 17: Associations between varieties plasticity with the respective varieties mean values across environments. R^2 and p values are shown and regression lines are fitted when associations were significant.

The varieties were classified in four categories concerning their plasticity for GI. Varieties with very low GI were the most stable, varieties with average GI around

20 had b values around 0.75 and genotypes with average values between 40 - 60 had b values between 1 - 1.5. The last category was represented by the variety "Matt" which had the highest GI and it was more stable than the former category.

The hierarchical cluster analysis based on the agronomic traits is shown in **Figure 18**. The three varieties released from the national breeding program of Cyprus during the last twenty years were clustered together ("Ourania", "Hekabe", "Makedonia") along with "Matt". Three varieties that were released by the national breeding program of Greece were also clustered together ("Mexikali 81", "Anna" and "Pisti").

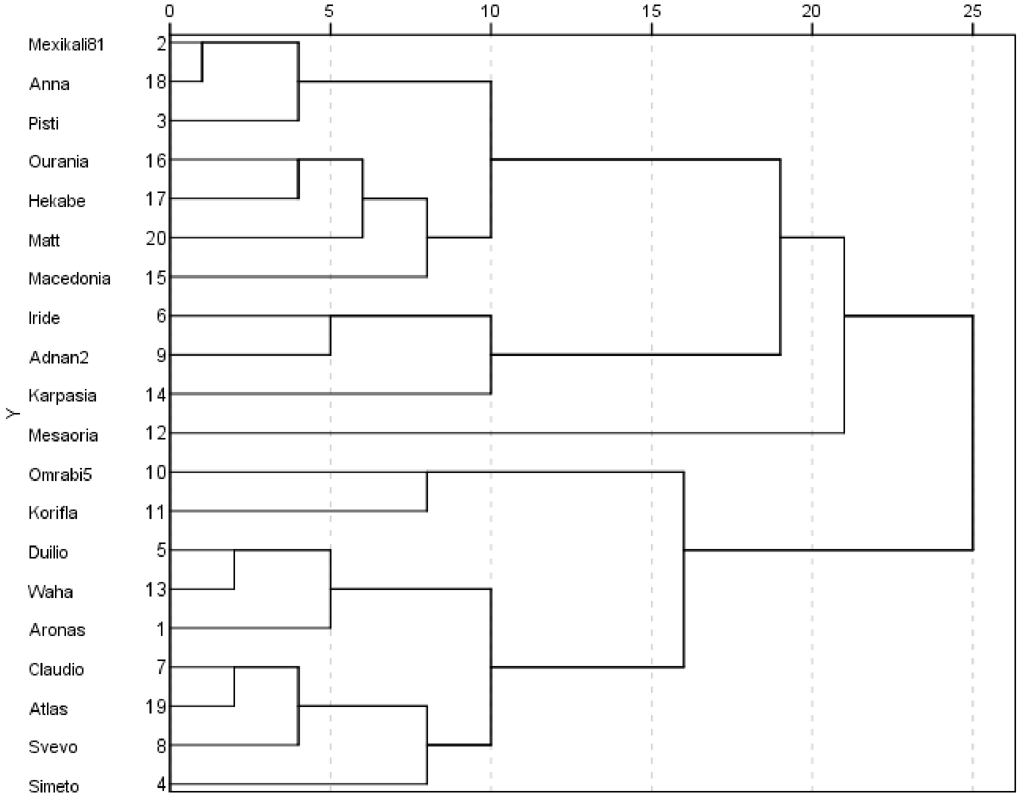
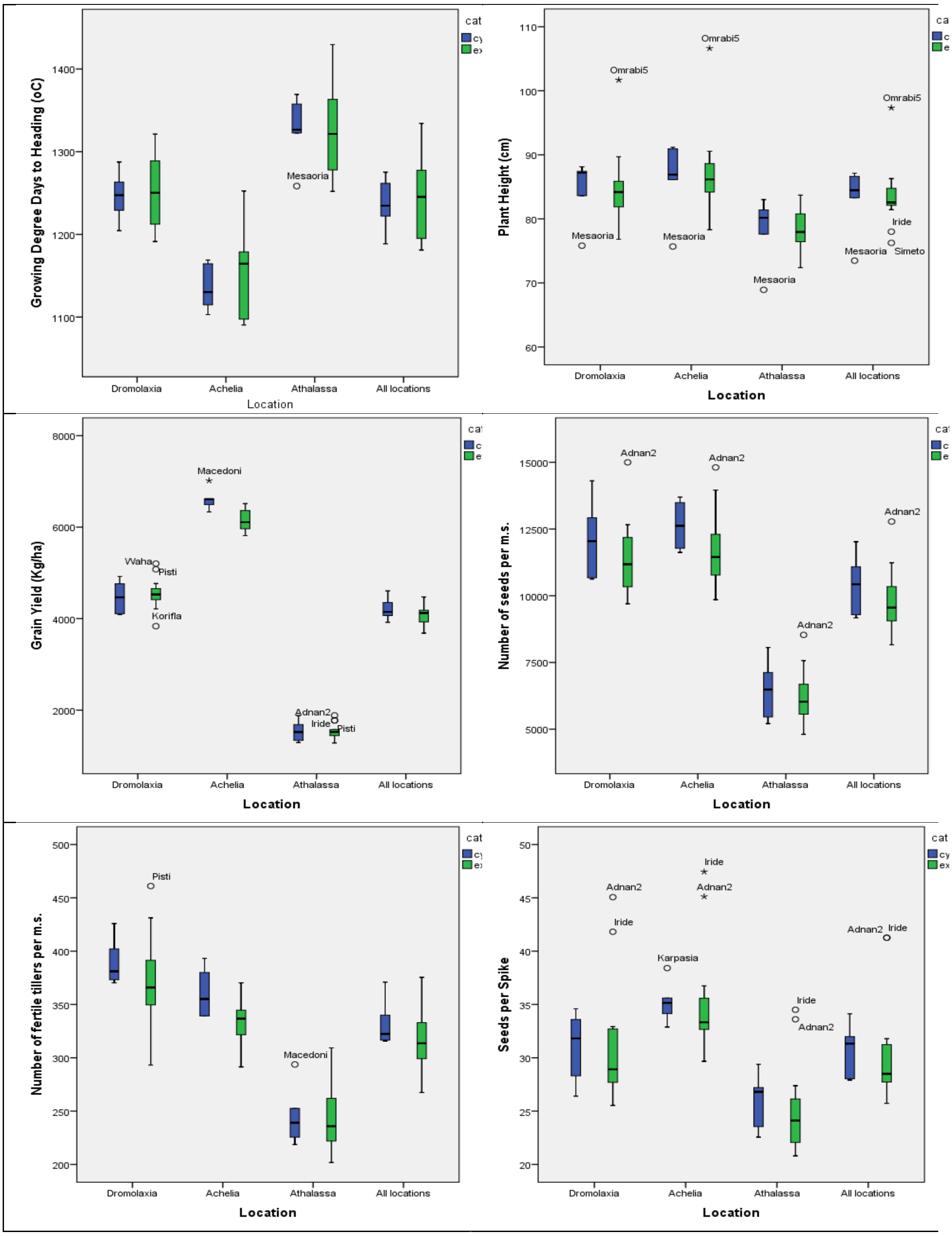


Figure 18: Dendrogram of the 20 varieties based on squared Euclidean distances calculated on standardized mean phenotypic data across environments.

Figure 19 depicts the box plots of the agronomic traits at each location and across environments for the group of varieties originated from the breeding program of Cyprus and from the other breeding programs. Cypriot varieties combined high NTLSM and high number of SPS resulted to high NSSM. On the other hand, they had low TKW and VW. Cypriot varieties had a narrower range of heading, wider range of CAR and wide range of PRO and GI.


Growing Degree Days to Heading (oC)
1100
1200
1300
1400
Mesaoria
Dromolaxia
Achelia
Athalassa
All locations
Location
cat
cy
ex
Plant Height (cm)
60
70
80
90
100
110
Omrabi5
Mesaoria
Iride
Simeto
Location
ca
c
e
Grain Yield (Kg/ha)
2000
4000
6000
8000
Macedoni
Waha
Pisti
Korifla
Adnan2
Iride
Location
cat
c
e
Number of seeds per m.s.
5000
7500
10000
12500
15000
Adnan2
Location
cat
c
e
Number of fertile tillers per m.s.
200
250
300
350
400
450
500
Pisti
Macedoni
Location
cat
cy
ex
Seeds per Spike
20
25
30
35
40
45
50
Adnan2
Iride
Karpasia
Location
cat
cy
ex

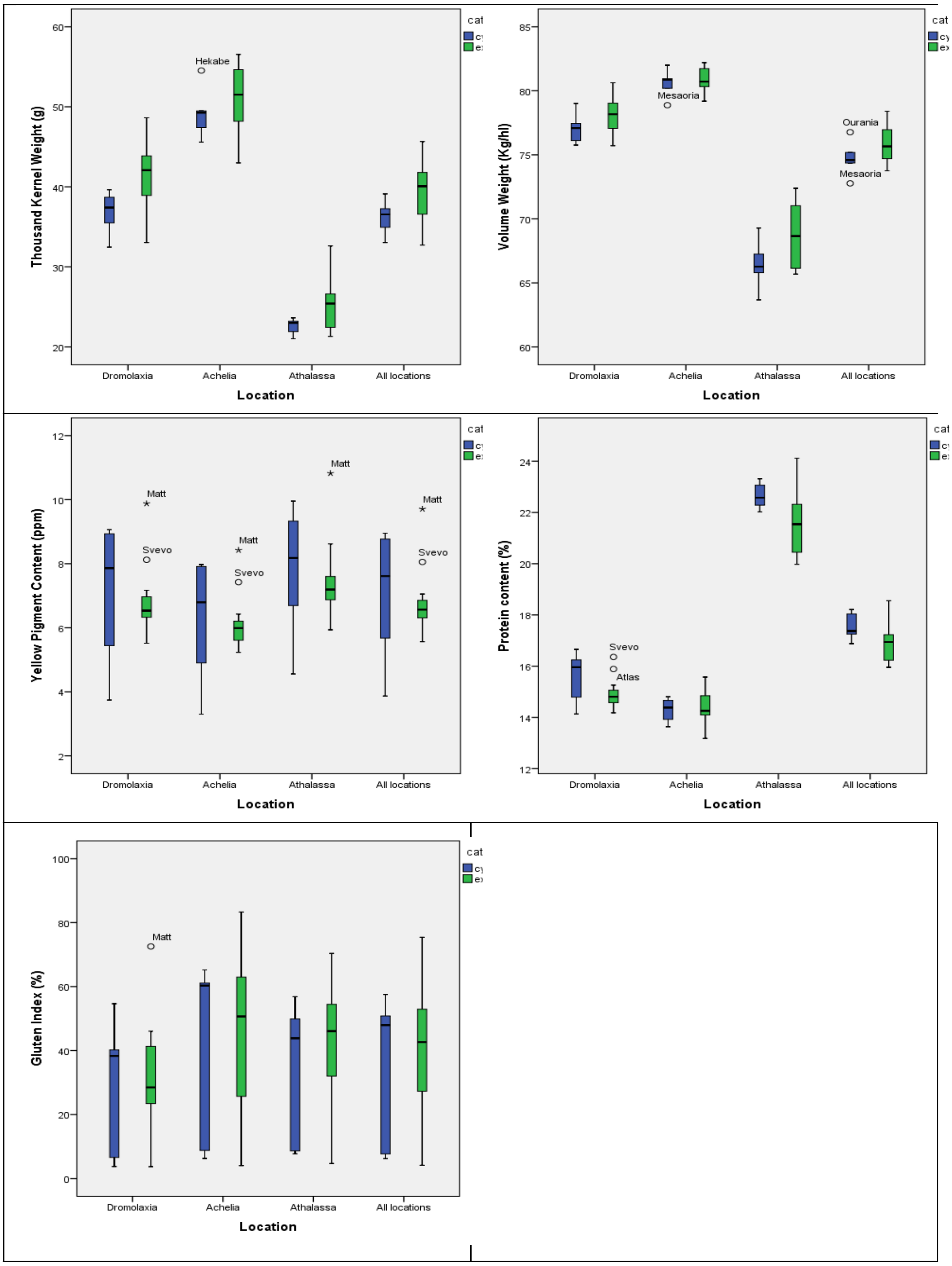


Figure 19: Box plots of the agronomic traits at each location and across environments for the group of varieties originated from the breeding program of Cyprus (blue) and from the other breeding programs (green).

2.3.3. Mean values and correlations with climatic variables

Average GRYLD was 4118 Kg/ha pooled over environments, and 4515, 6293, 1545 Kg/ha in Dromolaxia, Achelia and Athalassa, respectively. Achelia also depicted the highest PH, NSSM, SPS, TKW, VW and GI. GDD, PRO, and CAR were highest in Athalassa. Dromolaxia had the highest NTLSM **(Table 8)**.

The first two axis of the principal component analysis explained 92.34% of the total variability **(Figure 20)**. GRYLD, yield components, PH, VW, and water input during the growing season were strongly and positively related to PC1. Maximum temperatures during March and April were strongly and negatively related to PC1 and to a lesser extent to CAR, PRO, and maximum temperatures during growing season (loading values > 0.7). Average minimum temperature during growing season and average minimum temperature during grain filling were strongly and positively related with PC2 while GDD and GI were negatively related (loading values > 0.7).

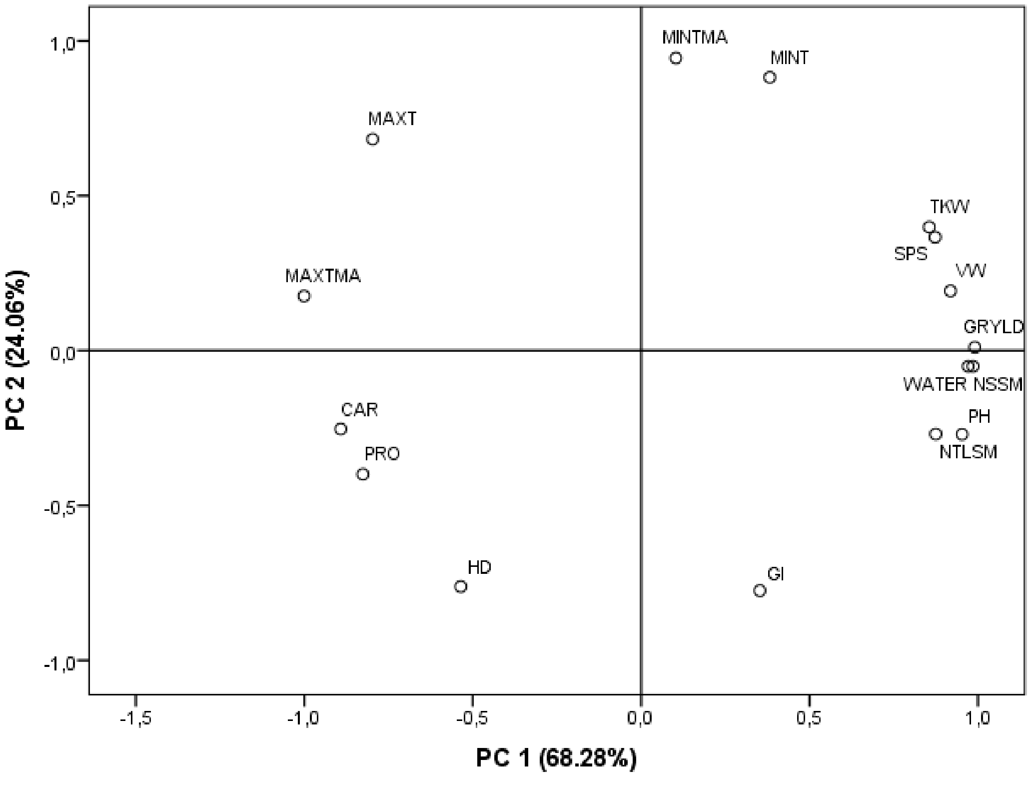


Figure 20: Principal component analysis based on environmental means and climatic variables.

2.3.4. Correlations between traits

Correlations between traits were estimated on genotype means at each environment and across environments **(Table 11)** The trait that was more related with GRYLD was the NSSM, the correlations were stronger in the two low yielding environments. Number of SPS was the trait consistently related with NSSM. Negative correlations between yield components were observed except between NTLSM and TKW. "Pisti" and "Anna" combined high TKW and NTLSM while "Iride" and "Adnan 2" combined low performance for these traits. Positive correlations were also observed between PH and VW, and between GDD and PRO, and negative correlations between GRYLD and PRO in three environments (data not shown). When correlations were estimated on average values across environments, GRYLD was positively related with NSSM and HI. The correlation with HI was stronger ($r=0.616, p<0.001$) when the tall variety "Omrabi-5" was excluded from the analysis.

Varieties had different yielding strategies. For example, when NTLSM were plotted against GRYLD **(Figure 21a)**, the five varieties with the lowest NTLSM had average GRYLD. These varieties, "Iride" and "Adnan 2" had the highest number of SPS and "Simeto", "Duilio" and "Waha" had the highest TKW. The two former varieties had high NSSM, where the three later ones had low NSSM **(Figure 21b)**. The two best yielding varieties had the higher NTLSM. However, "Makedonia" had high number of SPS and low TKW while "Pisti" had the opposite. GRYLD was negatively correlated with PRO ($r=-0.469, p=0.037$, **Figure 21c**). Two groups of varieties were identified when PRO was plotted with yield components **(Figures 21d, e, and f)**. For example, in both groups, PRO was increased with TKW **(Figure 21e)**. The two groups also showed different trend when TKW was plotted against plasticity for protein content **(Figure 22h)**. "Karpasia" was an outlier because this variety combined high PRO, low TKW, average NTLSM and high number of SPS. Eventually, "Karpasia" had the highest yield among the five varieties with the higher protein content **(Figure 21c)**.

Table 11: Pearson correlations between agronomic traits at each environment and across environments.

	GRYLD-NTLSM	**GRYLD -SPS**	**GRYLD -TKW**	**GRYLD -NSSM**	**NTLSM -SPS**	**NTSM-NSSM**	**SPS-TKW**	**SPS-NSSM**	**TKW-NSSM**	**GRYLD -GDD**	**GRYLD -HI**
Dromolaxia 12	-	-	0.479 *	-	-0.461 *	-	-0.556 *	0.721 **	-0.567 **	-	0.646 **
Dromolaxia 13	-	-	-	-	-0.576 **	-	-0.636 **	0.718 **	-0.854 **	-0.644 **	-
Achelia 12	-	-	-	0.503 *	-0.487 *	-	-0.621 **	0.814 **	-0.747 **	-	nm
Achelia 13	0.586 **	-	-	0.672 **	-	0.627 **	-	0.760 **	-	-0.577 **	nm
Athalassa 13	0.533 *	-	-	0.748 **	-	0.585 **	-	0.607 **	-	-	nm
Athalassa 14	-	0.727 **	-	0.790 **	-	0.503 *	-	0.846 **	-0.505 *	-	nm
Across environments	-	-	-	0.576 *	-	-	-	0.767 **	-0.783 **	-	0.504 *

[Grain yield (GRYLD), number of seeds per m^2 (NSSM), number of fertile tillers per m^2 (NTLSM), seeds per spike (SPS), thousand kernel weight (TKW), growing degree days to heading (GDD)] - (not significant), *p<0.05, **p<0.01, ***p<0.0001, nm (not measured)

As previously reported (Mohammadi, 2014), in most cases, associations between plasticities and traits *per se* were not significant, nevertheless some associations were observed. GRYLD was plotted against NSSM plasticity **(Figure 22a)**. Two varieties, "Adnan 2" and "Anna" behaved as outliers because of the different strategies in NTLSM and SPS **(Figures 17d, e and f)**. When the two outliers were removed, a strong linear relationship was present between these two traits. A strong positive correlation was also observed between NTLSM and plasticity for TKW, when the two outliers were removed from the analysis **(Figure 22e)**. The outliers, "Hekabe" and "Waha", combined high TKW and high plasticity for this trait **(Figure 17g)**. In general, the presence of outliers, which were related to the different yield strategies of the varieties, weakens the correlations between traits and their plasticity. Nevertheless, linear relations were obtained for some traits **(Figures 22c, d, f, and g)**. A quadratic relation was observed between plasticity for GRYLD and plasticity for NTLSM **(Figure 22b)**.

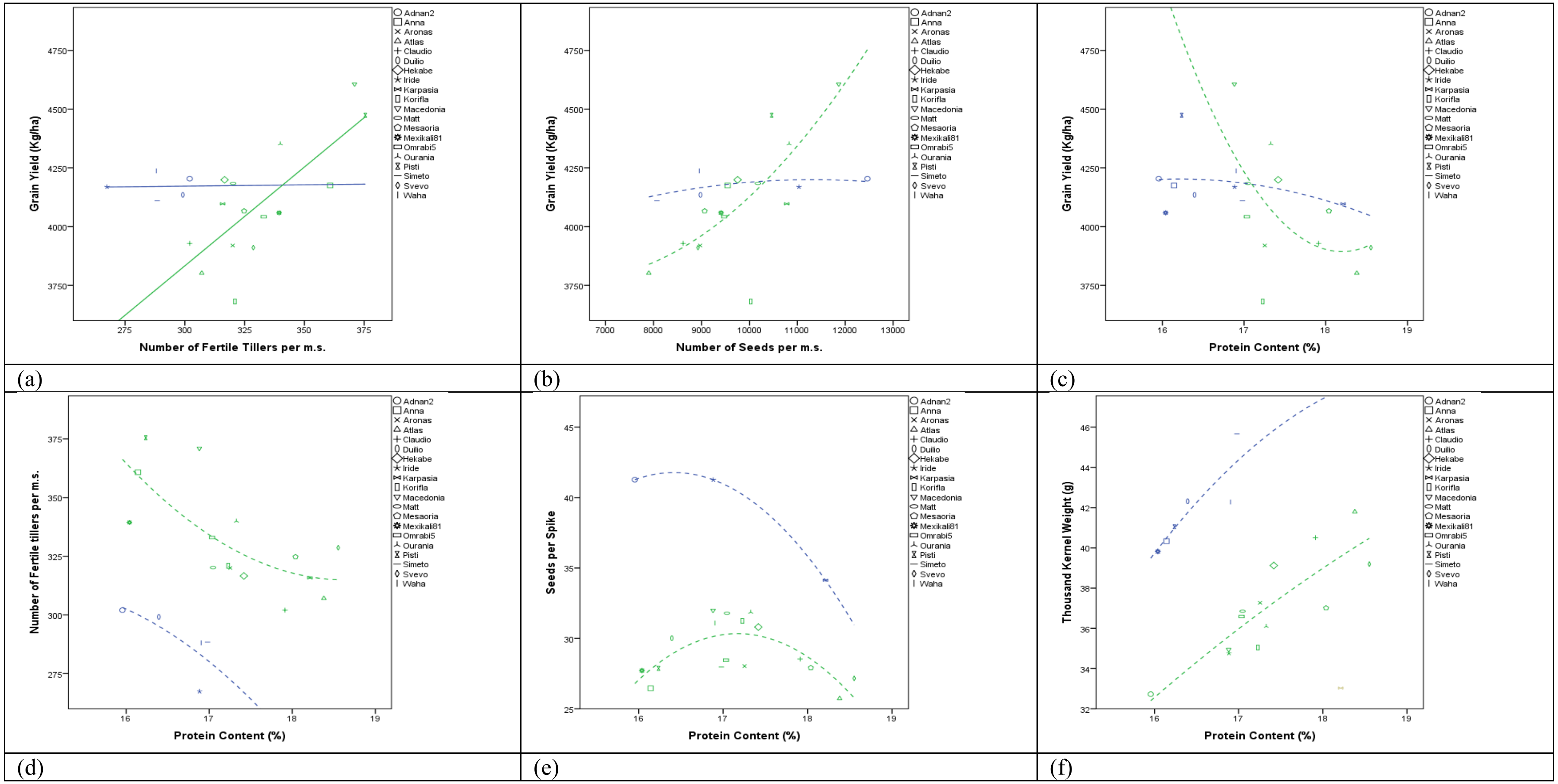
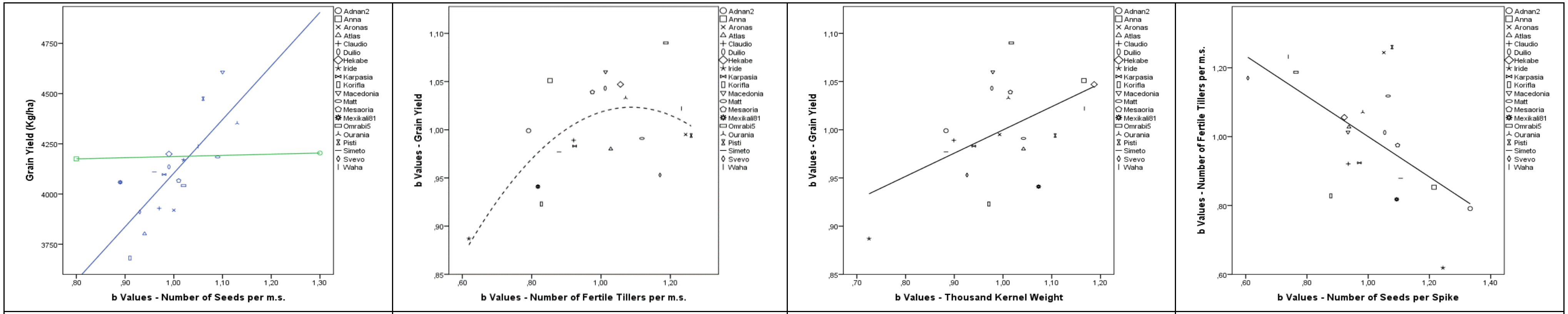
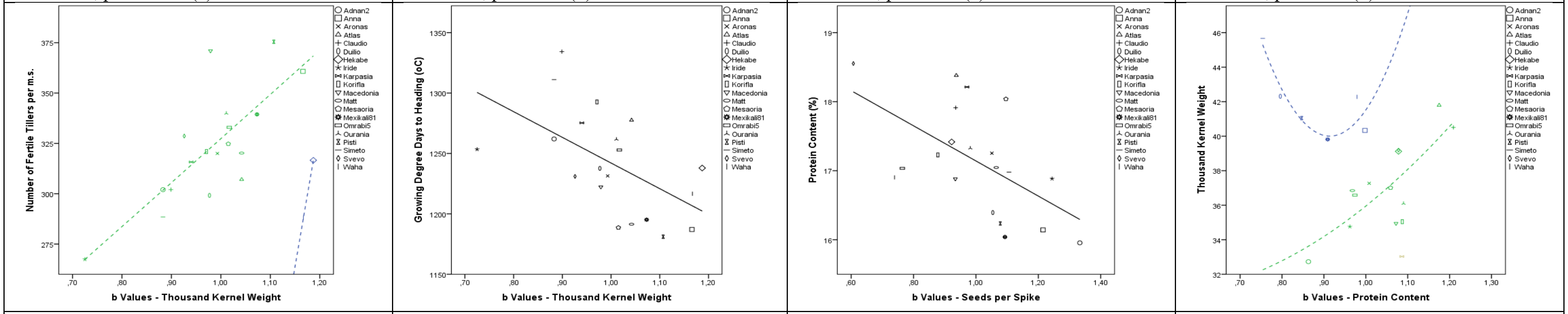


Figure 21: Scatter plots between grain yield with yield components (a) and (b), and protein content (c). Scatter plots between protein content and yield components (d), (e) and (f).



R^2*=0.604, p<0.0001* (a) *R*^{2}*=0.432, p=0.008* (b) *R*^{2}*=0.289, p=0.014* (c) *R*^{2}*=0.347, p=0.006* (d)



R^2*=0.599, p<0.0001* (e) *R*^{2}*=0.305, p=0.012* (f) *R*^{2}*=0.325, p=0.009* (g) (h)

Figure 22: Associations between traits plasticity (b), (c), (d) and between traits plasticity and mean values (a), (e), (f), (g), (h).

2.4. Discussion

Phenotypic characterization of genetic resources has been the cornerstone for plant breeding. In the present study, 20 modern varieties were evaluated for important agronomic and quality traits under diverse climatic conditions in three locations for two years (six environments). **Figure 23** presents an overview of the experiments during the critical stages of heading and grain filling**,** while **Figure 24** shows the two early ("Mesaoria", "Pisti"), intermediate ("Macedonia", "Ourania") and late heading ("Simeto", "Claudio") varieties from heading to physiological maturity at Dromolaxia during 2013.

Figure 23: Overview of the experiments at the three locations during the critical stages of heading (photos 1st row) and grain filling (photos 2nd row). The grain filling period was longer in Axhelia, followed by Dromolaxia.

2.4.1. Phenotypic diversity

There was genetic diversity for all the traits examined in the present study. For most traits, the highest percentage of variance was explained by the environment. Several studies reported the low percentage of variance explained by the genotypic effect for GRYLD (Boggini, et al., 1997; Li, et al., 2013; Subira, et al., 2015; Villegas, et al., 2016; Magallanes-López, et al., 2017; Royo, et al., 2017). In the present study, the percentage was as low as that reported by Royo, et al. (2010). Number of SPS was the yield component showing the larger genetic control. This is in agreement with the results of Subira, et al. (2015). The environmental effect was high for

NSSM, NTLSM, TKW, VW and PC (Rharrabti, et al., 2001; Rharrabti, et al., 2003a; Royo, et al., 2010; Li, et al., 2013; Subira, et al., 2014; Subira, et al., 2015; Guzmán, et al., 2016; Villegas, et al., 2016; Magallanes-López, et al., 2017).

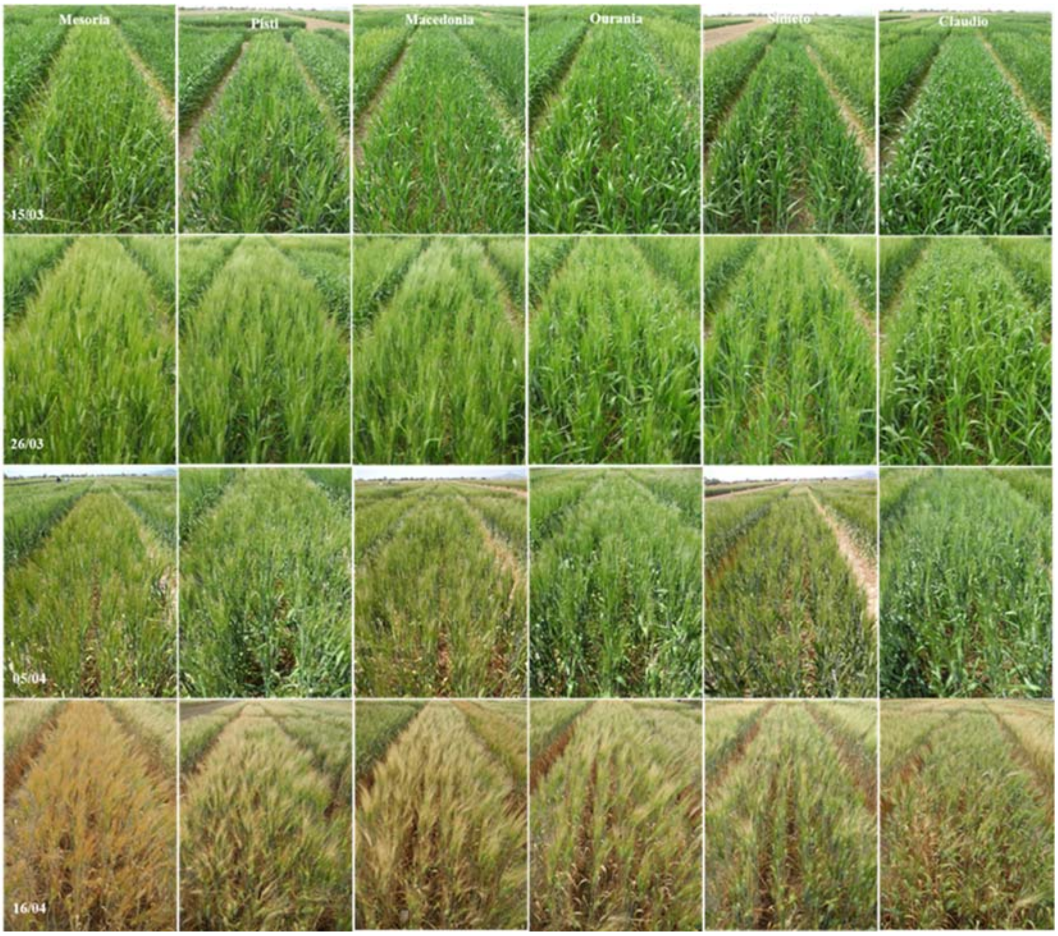


Figure 24: Experimental plots showing two early ("Mesaoria", "Pisti"), intermediate ("Macedonia", "Ourania") and late heading ("Simeto", "Claudio") varieties from heading (15/03) to physiological maturity (16/04) at Dromolaxia during 2013.

High percentage of the variance for GDD was explained by the environmental effect and to a lesser extent from genotypes. Villegas, et al. (2016) also attributed to site effect the highest percentage of variability for growing degree days from sowing to anthesis, however the percentage of variability attributed to genotypes was also high. The high percentage of variance explained by the genotype effect for CAR is in agreement with other studies (Rharrabti, et al., 2003a; Li, et al., 2013; Sissons, et al., 2014; Subira, et al., 2014; Guzmán, et al., 2016; Magallanes-López, et al., 2017) attributable to the high heritability of this trait (Clarke, et al., 2006). Gluten quality is mainly affected by genetic factors, nevertheless environmental conditions play a significant role (Marchylo, et al., 2001; Rharrabti, et al., 2003a; Li, et al., 2013; Sissons, et al., 2014; Subira, et al., 2014; Vida, et al., 2014; Guzmán, et al., 2016; Magallanes-López, et al., 2017). Technological properties are related with high quality alleles (Boggini, et al; 1997; Subira, et al., 2014; Magallanes-López, et al., 2017) and with the differential expression of specific storage proteins (De Santis, et al., 2017). Similar to Canadian durum wheat program (Marchylo, et al., 2001), four variety groups were identified in the present study. These groups are likely to be related with the different quality alleles and the expression of storage proteins.

The lack of significant correlations between grain yield and yield components with the plasticity of the traits implies that there was independence between mean values and plasticity. Positive trend between yield plasticity and GRYLD was reported by several studies (Rharrabti, et al., 2001; De Vita, et al., 2007). The highest yielding genotypes have b values around 1 (Hadjichristodoulou, 1985; De Vita, et al., 2007; Josephides and Kyratzis, 2007). In the present study, average grain yield was not correlated with grain yield plasticity. However, when maximum grain yield was plotted against yield plasticity, a significant quadratic relationship was obtained ($R^2=0.58, p=0.0006$) with the slope getting steeper for varieties with plasticity over 1. Contrary, when minimum grain yield was plotted against yield plasticity, a weak linear negative association was obtained ($R^2= 0.29, p=0.0139$). Grogan, et al. (2016) also reported positive correlations between yield plasticity and maximum grain yield. However, they reported positive correlations between yield plasticity and minimum grain yield, indicating that greater plasticity was favorable under optimal conditions without a penalty under low yielding conditions. The negative correlations in the present study are in agreement with the findings of Sadras, et al. (2009) who stated that high yield plasticity is an undesirable trait as it can be associated with low yield in low yielding environments. Similarly, Mohammadi (2014) found positive and negative correlations between grain yield and stability at high and low yielding environments of durum wheat, respectively. Reduced tillering varieties carrying the *tin* alleles can reduce the plasticity of both ear number and yield (Sadras and Rebetzke, 2013). In the present study, the most stable variety for GRYLD (Iride) combined very low tillering capacity and high stability for this trait.

The genetic control of the flowering time is modulated by the photoperiod sensitivity genes, the vernalization requirements genes and "earliness *per se*" (Royo, et al., 2017). The variation between varieties in heading and stability throughout all the range of heading should be attributed to different allele combinations at these genes. Motzo and Giunta (2007) also reported notable variation in the response of modern dwarf varieties for photoperiod and cold requirements. Late heading genotypes were more stable than early genotypes. Other studies however, reported positive correlations between plasticity in phenophases and their duration until heading (Grogan, et al., 2016) and anthesis (Sadras, et al., 2009). Plasticity of a trait depends on the particular combination of genotypes and environments (Sadras, et al., 2009). Experimenting under Mediterranean conditions, Hadjichristodoulou (1987) reported that early varieties tend to be more variable than late varieties for both barley and durum wheat. The particular combination of genotypes and environments can also justify the significant differences of varieties in plasticity for TKW and VW, contrary to previous works (Subira, et al., 2014).

The high variability in GI stability indicates the different response of genotypes to environmental conditions. Genotypes can show a unique response to the environmental conditions in respect of quality (Li, et al., 2013; Guzmán, et al., 2016, Magallanes-López, et al., 2017). The high GI and stability of the variety "Mat" confirms that selections can be made for genotypes that gluten quality will be mainly under genetic control (Rharrabti, et al., 2003b). Similarly, Vida, et al. (2014) stated that varieties with a strong gluten matrix responded less sensitively to changes in the environmental conditions. This is further evident by the breeding progress of the Italian and Spanish varieties for which gluten traits were increased and plasticity decreased over time (Subira, et al., 2014).

The optimum phenology to maximize yield depends of the targeted environments of the breeding program (Lopes, et al., 2014; Royo, et al., 2017). Both early and late heading cause yield penalties, and for the case of Cyprus, optimum heading was set by Hadjichristodoulou (1987). This setting justifies the low variation in heading between Cypriot varieties. High NTLSM was one of the targets set by the national breeding program of Cyprus (Hadjichristodoulou, 1985) justifying the high tillering capacity which was combined with high number of SPS. On the contrary, Cypriot varieties were inferior compared to the varieties with other origin concerning TKW and VW, indicating that further efforts needs to be taken to improve these traits in the future. The improvement of quality was one of the main targets of the national breeding program through the crossings with genetic material from North America (Josephides and Kyratzis, 2007). This breeding strategy explains the good quality performance of the Cypriot varieties. In general, Cypriot varieties showed similar mean agronomic and quality values thus they were cluster together. However, this is a point for consideration since selecting for the same phenotype will gradually reduce the genetic diversity.

2.4.2. Mean values and correlations with climatic variables

Water stress, high temperatures or the combine effect of these variables limit yields of durum wheat in the Mediterranean (García del Moral, et al., 2003; García del Moral, et al., 2005; Habash, Kehel and Nachit, 2009). In the present study, water input during growing season and average maximum temperature during March and April were strongly correlated with GRYLD and yield components, stressing that it is the combined effect of heat and drought that limits grain yield. Stress symptoms are shown to **Figures 25 and 26**.

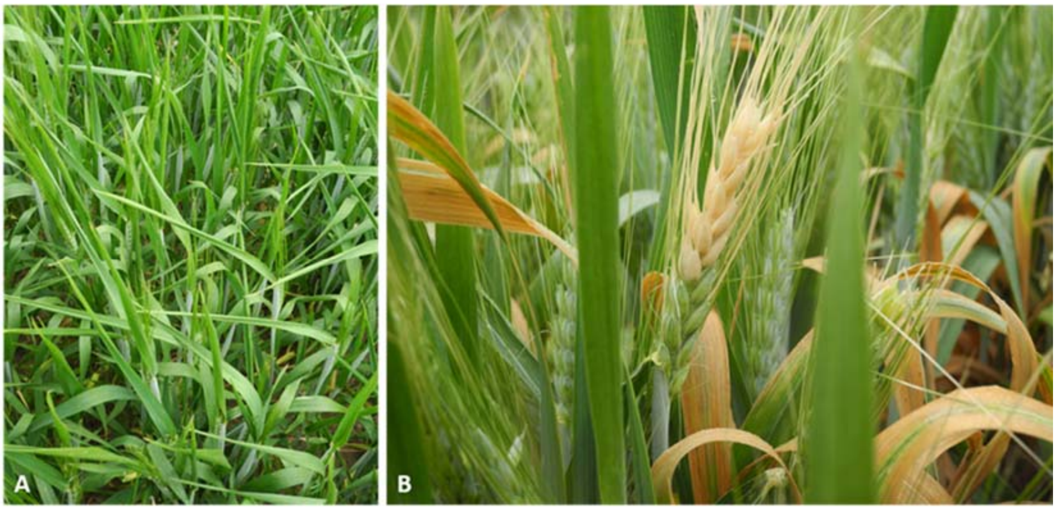


Figure 25: Stress symptoms; Leaf rolling (A), and reduced seed set (B).

Figure 26: Experimental plots showing stress symptoms during booting (A) and grain filling (B).

In several studies conducted under Mediterranean conditions, the reduction of GRYLD under stress was related to the reduction to NSSM or its components (García del Moral, et al., 2003; García del Moral, et al., 2005; Del Pozo, et al., 2016). In the present study, the reduction between the high yielding location (Achelia) and the intermediate yielding location (Dromolaxia) was mainly due to reduction of TKW, while the reduction between Achelia and the lowest yielding environment (Athalassa) was equally attributed to the reduction to NSSM and TKW. The number of NSSM produced in Achelia and Dromolaxia was lower than the optimum NSSM reported to the literature to obtain high yields (Gonzalez-Navaro, et al., 2016; Villegas, et al., 2016). It is likely that the late sowing in Achelia and the conservative seed rate used in this study (Arduini, et al., 2006) did not allow the production of higher NSSM in the more favorable conditions of Achelia. Sowing after mid-December for the conditions of Cyprus, can reduce yield up to 70%, mainly through the reduction of tiller number (Photiades and Hadjichristodoulou, 1984). Within each location, the effect of year on GRYLD was higher in Achelia than in the other locations, due to late sowing in the 2nd year which reduced NTLSM and GRYLD, despite the compensatory effect of higher TKW.

The negative association between GRYLD and PRO was reported by several authors (Boggini, et al., 1997; De Vita, et al., 2007; Magallanes-López, et al., 2017, De Santis, et al., 2017). Previous studies also reported negative correlations between GI and PRO (De Santis, et al., 2017), and between SDS volume and PRO (Boggini, et al., 1997; Rharrabti, et al., 2003a), while others reported positive correlations between gluten quality parameters and PRO (Guzmán, et al., 2016). Non-significant corrections between PRO and gluten quality parameters were observed by Dick and Quick (1983), while Garrido-Lestache, López-Bellido and López-Bellido (2005) reported none significant, positive and negative correlations between GI and PRO, depending on the different nitrogen management scenarios. In the present study, no consistent correlation was obtained between GI and PRO or any other agronomic trait, either on genotype or environmental means, indicating that these traits are independent.

Reduction of CAR to higher yielding environments was previously reported (Li, et al., 2013) and attributed to dilution effect of carotenoids when kernel size increases (Clarke, et al., 2006; Sissons, et al., 2014). Very strong negative correlations between TKW and CAR were found in the present study on environmental means, but not on genotypic means in each environment and across environments (data not shown), suggesting that selecting for high TKW does not affect CAR. Clarke, et al. (2006) reported positive and negative correlations between CAR with average temperature and with precipitation during grain filling, respectively. Rharrabti, et al. (2003a) founded that high seasonal temperatures, increased CAR in grain. The very high CAR in Athalassa, where plants faced adverse climatic conditions, might be related with the increase production of components of the plant defense mechanisms, which includes antioxidant molecules (Ficco, et al., 2014).

Gluten quality can be affected by abiotic stresses and by cultural practices. Positive effects on quality were reported under drought (Giuliani, et al., 2011; Li, et al., 2013; Guzmán, et al., 2016; Magallanes-López, et al., 2017). Negative effects were found under heat stress (Har Gil, Bonfil and Svoray, 2011; Li, et al., 2013; Vida, et al., 2014; Guzmán, et al., 2016), although Giuliani, et al. (2011) reported that high temperatures can increase technological performance. Negative effects were also observed when irrigation is applied (Rharrabti, et al., 2003a; Har Gil, Bonfil and Svoray, 2011; Sissons, et al., 2014), and with nitrogen fertilization (Garrido-Lestache, López-Bellido and López-Bellido, 2005; Har Gil, Bonfil and Svoray, 2011). Vida, et al. (2014) reported a significant effect of nitrogen to GI, although the response was positive or negative between years. Giuliani, et al. (2011) found a positive effect of nitrogen in GI, although not consistent between years. In the present study, GI was not related with climatic variables suggesting that the variety, climatic conditions and cultural practices affects in a complex manner the gluten quality. Similarly, Har Gil, Bonfil and Svoray (2011) concluded that the most important factor influencing wheat quality in Israel was the type of variety, although environmental factors and crop management had also strong effect.

2.4.3. Correlations between traits

NSSM and harvest index were the traits correlated the most with GRYLD. Increased durum wheat yield is related with these traits (Motzo, Fois and Giunta, 2004; De Vita, et al., 2007; Giunta, Motzo and Pruneddu, 2007; Royo, et al., 2007; Villegas, et al., 2016). The correlations between GRYLD and NSSM were stronger at the two low yielding environments since under stress conditions, GRYLD is predominantly associated with this trait (Lopes, et al., 2012). Number of SPS is the yield component most related to yield improvement of durum wheat in the Mediterranean through its contribution to NSSM (De Vita, et al., 2007; Giunta, Motzo and Pruneddu, 2007; Royo, et al., 2007; Subira, et al., 2015). The consistent strong relationships between SPS and NSSM are in line with these works. Many studies reported that GRYLD is less related to TKW under stress conditions (Simane, et al., 1993; García del Moral, et al., 2005) as was the case in the present study. There is evidence that improvement of TKW was not targeted by breeding programs. For example, De Vita, et al. (2007), Giunta, Motzo and Pruneddu (2007), Subira, et al. (2014) and Royo, et al. (2007) found non-significant changes in TKW and VW in a retrospective analysis of Spanish and Italian durum wheat varieties, respectively.

Tradeoffs between yield related traits is frequently reported, and the associations between them are affected by the environmental conditions (Del Pozo, et al., 2016). The compensatory effects for yield components are more pronounced under stress conditions due to the limited resources (Simane, et al., 1993; García del Moral, et al., 2003; García del Moral, et al., 2005). In the present study, the compensatory effect between yield components were significant in the three environments, where favorable conditions during winter increase the NTLSM; however, less favorable conditions during spring likely enhance the competition for assimilates between components. On the contrary, late sowing in Achelia the 2nd year and the adverse climatic conditions in Athalassa resulted in low NTLSM mitigating the compensatory effects.

Many studies reported negative correlations between heading or anthesis and GRYLD (Lopes, et al., 2014; Royo, et al., 2017) especially under moderate stressed environments, where early heading permits drought escape during the sensitive stage of grain filing (Lopes, et al., 2012). Negative correlations between GRYLD and GDD were obtained at Dromolaxia in 2013, where plants experience drought stress due to extremely low precipitation from January and higher temperatures. The same trend, although non-significant, was observed at Dromolaxia in 2012. Negative correlations between GRYLD and GDD were observed at Achelia in 2013. Despite the late sowing at Achelia in 2013, heading was earlier than in 2012, due to higher winter temperatures. The plants did not experience any drought stress during grain filling although temperatures where relatively high. Compared to the earlier sowing in the 1st year in this location, late sowing in the 2nd year caused similar reduction of NTLSM for the five early heading and the four late heading varieties. However, early heading varieties produced more SPS and heavier seeds the 2nd year, compared to the reduction of SPS and the similar TKW of the late heading varieties (data not shown). SPS is determined during the period before and after anthesis, especially during the phase between booting and anthesis, while environmental conditions critically affect ear fertility through the effect on allocation of assimilates to the spike (Isidro, et al., 2011, Dreccer, et al., 2014). Drought and high temperature reduce spike fertility (García del Moral, et al., 2005). The low water input from January to end of March and the higher winter temperature during the 2nd year likely caused progressive increase of stress, affecting more severely ear productivity of late varieties. In some cases, like in heat stress environments, maximizing biomass production before heading might be a very important adaptation trait, thus varieties with late heading can be more productive (Lopes, et al., 2012). The non-significant correlations between GDD and GRYLD in Athalassa can be attributed to the severe stress conditions that plants experienced during grain filling in this location, shortening the duration of the grain filling stage and therefore the post anthesis photosynthesis contribution to grain yield (Royo, et al., 2017). Biomass accumulation before heading is associated with grain yield as it is related to carbohydrate remobilization to grain during the grain filling stage (Villegas, et al., 2001). Recently, Royo, et al. (2017), reported positive correlations between days to anthesis and dry matter at anthesis, and the contribution of the translocation of pre-flowering assimilates to grain yield were higher under temperature stress conditions. In a harsh environment like Athalassa, longer vegetative period might be an advantage because late anthesis genotypes translocate higher percentages of pre-anthesis assimilates to grain (Royo, et al., 2017).

The lack of significant correlations between TKW and GDD confirms that selection can be made for high kernel weight irrespective of heading. Kernel weight under stress conditions is mostly related to grain filling rate rather than grain filling duration (Motzo, Giunta and Deidda, 1996; García del Moral, et al., 2005), although longer grain filling period can result to heavier seeds (Simane, et al., 1993; García del Moral, et al., 2003). Positive correlations between PH and VW were reported by Clarke, et al. (2009), that may be related with the lack of improvement of VW in durum wheat (Subira, et al., 2014). Optimum PH must be determined by the breeding programs because either tall or short varieties could cause reduction of yield (Lopes, et al., 2014). In this study, non-significant correlations between GRYLD and PH were obtained, most likely because there was low variation in PH. Motzo, Giunta and Deidda (1996) found significant positive environmental correlations between PRO and anthesis, which might be due to longer nitrogen uptake for late genotypes, especially under favorable conditions. Giuliani, et al. (2011) reported positive correlations between nitrogen content at anthesis and protein content. In the present study, the positive correlations between GDD and PRO might be due to longer nitrogen uptake till anthesis for late genotypes. Further to the well-established negative correlation between GRYLD and PRO, negative correlations are regularly reported for yield components and PRO (Motzo, Giunta and Deidda, 1996; Motzo, Fois and Giunta, 2004; Blanco, et al., 2012). Such trend was not observed in this study. Two groups of varieties were identified when yield components were plotted against PRO suggesting that associations between yield components and PRO should be further investigated.

Traits and their plasticities are interrelated and adjusting the plasticity of one trait can influence other (Grogan, et al., 2016). Plasticities of related traits can be negatively correlated (Sadras, et al., 2009) since genotypes can be stable for one trait and unstable for another (Rharrabti, et al., 2003b). Previous studies reported significant associations between plasticities of yield and phenology (Sadras, et al., 2009; Mohammadi, 2014; Grogan, et al., 2016). In the present study, plasticity in GDD and GRYLD were not correlated. However, significant correlation was observed between GDD and TKW plasticity, contrary to the non-significant correlations between GDD and TKW *per se*. The grain filing stage is longer for early varieties (Simane, et al., 1993; Motzo, Giunta and Deidda, 1996; De Vita, et al., 2007; Sandras, et al., 2009). Thus, the longer grain filling period of early varieties can make them more responsive to the production of heavier seeds when the conditions are favorable. Plasticity for TKW was also positively related with NTLSM, and with plasticity with GRYLD, although weakly. These results indicate that plasticity for TKW should be investigated into more detail. Sadras and Lawson (2011) reported a two-fold increase in grain size since 1882 in south Australian wheat varieties. As expected, plasticity for NSSM was correlated with GRYLD. Negative correlations between plasticities of ear number and seeds per ear and between their mean values *per se* were previously reported (Sadras and Rebetzke, 2013). The quadratic association of plasticity for NTLSM and GRYLD observed in the present study indicates that extensive plasticity for tillering might not be desirable (Hadjichristodoulou, 1985). The negative correlation between protein content and plasticity for SPS might be related to higher nitrogen demand for genotypes of high SPS plasticity. Motzo, Giunta and Deidda (1996) reported a negative correlation between protein content and seeds per spike attributable to the dilution effect of available nitrogen caused by different ear fertility levels.

In the present study, varieties had different yielding strategies. Different pattern of adaptation to environmental conditions has been reported for durum wheat in the Mediterranean (García del Moral, et al., 2003; Royo, et al., 2010; Subira, et al., 2015) and for hexaploid wheat (Gonzalez-Navaro, et al., 2016). The two highest yielding varieties had high NTLSM. Hadjichristodoulou (1985) stated that under Cyprus conditions, high yielding barley varieties were those with high tillering capacity. NTLSM was significantly correlated with plasticity for TKW indicating that varieties with higher tillering capacity can produce heavier seeds under more favorable conditions. The highest yielding Cypriot varieties combined high NTLSM and SPS, the two components forming the NSSM. High NTLSM and SPS is the main yielding strategy for durum wheat in the Mediterranean. Simane, et al. (1993) stated that grain yield is heavily depended upon spikes per m^2 and kernels per spike. García del Moral, et al. (2005) reported that under stress conditions, the variation in grain yield is mainly due to spikes per m^2 and to a lesser extent due to grains per spike. García del Moral, et al. (2003) reported that genotypes that combined higher spikes per m^2 and seeds per spike were among the best yielding. It is stated that further improvement in wheat grain yields should be obtained by increasing the grain number (Gonzalez-Navaro, et al., 2016) as the plasticity for grain number is much larger than the plasticity of grain weight (Sadras and Slafer, 2012). In this sense, fruiting efficiency has been proposed as an alternative for further increase in grain number and for mitigating the frequently observed tradeoff between grain number and grain weight (Ferrante, Savin and Slafer, 2015).

The two varieties with the highest number of SPS had very low number of NTLSM and TKW and low plasticity for these traits. Genotypes with low tillering and high number of SPS can perform better under stressed conditions (Simane, et al., 1993), however, the low TKW and the low plasticity for this trait should be a concern. Low kernel weight is associated with lower end use quality (Giunta, Motzo and Pruneddu, 2007) and it can be associated with low VW, especially under environments with high abiotic and biotic stress (Clarke, et al., 2009). Small or shriveled seeds are commonly produced under terminal drought stress reducing crop value (Mitchell, et al., 2013). The varieties showing the highest TKW had low NTLSM and SPS. Although these varieties were not among the highest yielding varieties, even in the low yielding environments due to lower NSSM, they managed to retain high TKW. It is likely that these varieties had a yield penalty due to the conservative sowing rate used in the present study (Arduini, et al., 2006). The ability of high TKW varieties with reduced tillering capacity to sustain high kernel weight under stress conditions can be related with higher efficiency to translocate pre-anthesis assimilates to grains. Positive correlations between water soluble carbohydrate content at anthesis and TKW were reported (Del Pozo, et al., 2016). Genotypes with high water soluble carbohydrates have lower NSSM, mainly due to lower number of spikes per m^2, and heavier seeds (Dreccer, van Herwaarden and Chapman, 2009). Reduced tillering genotypes managed to retain high kernel weight and grain yield under stress conditions due to greater anthesis biomass and increased stem water soluble carbohydrates, ensuring more assimilates for translocation to grains (Mitchell, et al., 2013). Genotypes with high water soluble carbohydrates are also positively associated with high number of seeds per spike (Dreccer, van Herwaarden and Chapman, 2009; Del Pozo, et al., 2016), with higher potential to retain high number of seeds per spike in warmer climates (Dreccer, et al., 2014). Reduced tillering genotypes with high water soluble translocation efficiency might improve the efficiency with which resources are used by crop to maintain grain yield and kernel size and increase yield stability under stress conditions (Mitchell, et al., 2013). A stable number of seeds per m^2 and an increase in kernel weight was observed in the south Australian wheat varieties after 1982 (Sadras and Lawson, 2011). Thus, varieties with high and stable TKW and high water soluble translocation efficiency might represent an alternative yield strategy under the Mediterranean conditions for durum wheat production, especially under low yielding environments. Varieties with high grain weight were found to be more stable in Mediterranean environments (Royo, et al., 2008). However, as stressed by Mitchell, et al. (2013), for low tillering genotypes, optimized spike density and therefore seed rate should be determined in each environment.

2.4.4. Conclusions

There was genetic diversity for all the traits, however, for most of them, the highest percentage of variance was explained by the environmental effect. The traits exhibiting the highest and the lowest genetic control were the yellow pigment content and grain yield, respectively. Variation for grain yield plasticity was low while variation for yellow pigment content and gluten index plasticities was high. The differences between varieties for plasticity were significant for the quality traits, growing degree days to heading, thousand kernel weight and plant height.

Number of seed per m^2 was significantly correlated with grain yield in most environments and the correlations were stronger in the low yielding environments. Number of seeds per spike was the traits consistently related with number of seeds per m^2. Trait offs between yield components were recorded, except between number of fertile tillers per m^2 and thousand kernel weight. Varieties had different yield formation strategies. The two highest yielding varieties had the highest number of fertile tillers per m^2 combined with high seeds per spike or high kernel weight.

Growing degree days to heading and number of fertile tillers per m^2 were negatively and positively related with thousand kernel weight plasticity, respectively. The traits *per se* were not related. For particular traits, some varieties had high mean values and they were very stable since they manage to retain high values to all environments (e.g. "Simeto" for thousand kernel weight, "Matt" for gluten index). In some other cases, varieties had high mean values and high plasticity indicating that they are more responsive to the particular trait in the favorable environments (e.g. "Adnan 2" and "Iride" for seeds per spike, "Matt" for yellow pigment content). These findings suggest that traits plasticity merits further consideration from the breeders.

Breeders did not give much attention to kernel weight, because high number of seeds per m^2 was the main way to increase yield of durum wheat varieties in the Mediterranean. Improving simultaneously these two traits is cumbersome because of the frequently observed trait offs. However, the production of shrivel seeds is a major concern for the industry and lowers the seed process. The production of shrivel seeds will be increased with climate change. An alternative breeding strategy for durum wheat in the Mediterranean could be to select varieties with increased water soluble remobilization associated with higher and stable kernel weight.

3. Chapter 3 - Assessment of Vegetation Indices Derived by UAV Imagery for Durum Wheat Phenotyping Under a Stress Environment

3.1. Introduction

Drought stress, as a combination of water deficit and high temperature, is the main constraint limiting grain yield of cereals in the Mediterranean basin (Araus, et al., 2002). This geographic area is expected to face more severe drought and an increase in average temperature in the near future, due to climate change (Giorgi and Lionello, 2008), increasing yield uncertainty of rain-fed crops. Improving crop productivity in drought-prone environments is a daunting challenge. Extensive plant phenotyping and integration of cost effective technologies are considered prerequisites to achieve progress through plant improvement (Reynolds and Tuberosa, 2008). Furthermore, advances in phenotyping are likely to be essential in capitalizing developments in conventional, molecular and transgenic breeding, and ensuring genetic improvement of crops for future food security (Araus and Cairns, 2014).

Remote sensing methods hold great potential as a tool for: a) high throughput phenotyping for plant breeding (Deery, et al., 2014; Sankaran, et al., 2015), b) decision making for precision agriculture (Zhang and Kovacs, 2012; Gago, et al., 2015), c) predicting yields (Son, et al., 2014), and d) predicting spatial field variability in experimental sites (Zaman-Allah, et al., 2015). Their usefulness rely on the fact that they are non-destructive, non-invasive, fast and cost-efficient, well-correlated with agronomical and physiological important crop traits (Reynolds, et al., 2015).

The most common procedure to extract information about crops from remote sensing is through the estimation of Spectral Vegetation Indices (SVI), which are based on formulations fitted with the light reflected by the canopy at different wavelengths (e.g. ratios and differences). The wavelengths are within the visible and the near infrared electromagnetic spectrum. Several SVIs have been proposed and are widely used such as the Normalized Difference Vegetation Index (NDVI), the Simple Ratio (SR) and the Green Normalized Difference Vegetation Index (GNDVI). The existence of genetic variability for SVIs was reported by several authors (Babar, et al., 2006b; Prasad, et al., 2007b; Gutierrez, et al., 2010; Gizaw, Garland-Campbell and Certer, 2016a). SVIs were associated with important traits of cereal crops, such as grain yield under stressed conditions (i.e. ; Bort, et al., 2005; Lobos, et al., 2014; Bowman, et al., 2015; Tattaris, Reynolds and Chapman, 2016; Yousfi, et al., 2016), However, some authors stressed that under severe stressed conditions, SVIs might be less efficient because genotypes are not able to express their yield potentiality (Royo, et al., 2003; Babar, et al., 2006c).

The majority of previous studies were conducted with hand held sensors; however, ground measurements holds several constrains (Chapman, et al., 2014; Deery, et al., 2014; Gago, et al., 2015; Reynolds, et al., 2015; Sankaran, et al., 2015; Tattaris, Reynolds and Chapman, 2016). Some of these constrains can be eliminated using low altitude aerial platforms. Zhang and Kovacs (2012) stated that imagery taken by low altitude aerial systems is promising, given its low cost of operation, high spatial and temporal resolution, and its flexibility in image acquisition programming. Measurements from trials can be taken when they are not accessible to ground platforms e.g. due to water-logged or tall crops (Chapman, et al., 2014). Other advantages are the limited confounded effects caused by environmental drift due to simultaneous data collection and more robust image analysis tools (Reynolds, et al., 2015; Tattaris, Reynolds and Chapman, 2016), wider viewing angle from the air, and absence of physical contact, and hence no mechanical distraction of the growing crop (Liebisch, et al., 2015). Although UAVs can carry lower payload than other aerial vectors, they enable greater flight control and autonomy (Araus and Cairns, 2014) and are less affected by the wind (Deery, et al., 2014; Tattaris, et al., 2014). Recent studies revealed that correlations between SVIs and agronomic traits derived from airborne imagery are similar, or even stronger, than correlations derived from ground measurements (Tattaris, et al., 2014; Zaman-Allah, et al., 2015; Rasmussen, et al., 2016; Tattaris, Reynolds and Chapman, 2016). Measurements can be taken by a wide array of different sensors including conventional digital cameras (Araus and Cairns, 2014; Sankaran, et al., 2015;), that have the advantage of low cost and low weight (Hunt, et al., 2010), and can be easily mounted on UAVs and other aerial vectors (Ball and Konzak, 1993; Lelong, et al., 2008; Liebisch, et al., 2015; Rasmussen, et al., 2016). The fast and cost efficient nature of UAV imagery allows multiple measurements during grain filling. Multiple measurements are necessary because the optimum recording stage is likely to vary with experiment (i.e. Bort, et al., 2005; Bowman, et al., 2015). The efficiency of SVIs is also affected by plant phenology, thus multiple measurements allow the calculation of parameters that are less related with phenology (Lopes and Reynolds, 2012; Montazeaud, et al., 2016).

The successful implementation of such technologies rely on the characteristics of the UAV including stability, safety, control, reliability, positioning, autonomy, sensor mount, controller, sensor characteristics and image and data processing (Chapman, et al., 2014; Sankaran, et al., 2015). It is then necessary to assess the reliability of aerial remote sensing approaches with direct plant-derived data (Lelong, et al., 2008; Gago, et al., 2015; Liebisch, et al., 2015). A number of studies investigated the potential use of imagery derived from sensors mounted on UAVs and other aerial vectors for plant breeding (Ball and Konzak, 1993; Hoyos-Villegas and Fritschi, 2013; Chapman, et al., 2014; Liebisch, et al., 2015; Zaman-Allah, et al., 2015; Rutkoski, et al., 2016) and precision agriculture (Lelong, et al., 2008; Hunt, et al., 2010; Khot, et al., 2016; Rasmussen, et al.,2016). Nevertheless, studies conducted under severely stressed Mediterranean conditions are very limited (Gonzalez-Dugo, et al., 2015).

The main scope of the present work is to investigate the usefulness of SVIs (NDVI, SR and GNDVI) derived from UAV imagery for plant phenotyping under a water limited and heat stressed Mediterranean environment. A fast and cost effective method to estimate SVIs by UAV mounted with digital cameras is described. Genotypic effects of SVIs and agronomic and other physiological traits are presented. Correlations between SVIs and photosynthetic pigments, SPAD measurements, grain yield and other agronomic traits are discussed.

3.2. Materials and methods

3.2.1. Plant material, experimental conditions, field design and measurements of agronomic traits

Experiments were conducted at Athalassa experimental station (35º08΄N, 33º24΄E) for two consecutive growing seasons (2012/2013 - year 1 and 2013/2014 - year 2). Due to the adverse climatic conditions in this area **(Figure 14)**, crop failure and complete loss of yield frequently occurs in this area. Genetic material and experimental conditions were as described to section 2.2, with the exception that, in the 2nd year (2013/2014), measurements were taken in five replications. The plants received no supplementary irrigation or rainfall from heading to physiological maturity during the 1st year, while in the 2nd year received only a negligible amount of rainfall when most of the plants had reached physiological maturity. The measurement of agronomic traits was as described to section 2.2. In addition, physiological maturity was recorded when 50% of the spikes in the plot showed total loss of green color and was expressed as growing degree days from heading to physiological maturity.

3.2.2. Measurements of photosynthetic pigments

Extraction of photosynthetic pigments, chlorophyll *a*, chlorophyll *b*, carotenoids, anthocyanins was carried out as described by Richardson, Duigan and Berlyn (2002), setting the extraction time to 2h. Six discs from three flag leaves were used for the extraction from each experimental plot. The discs were sampled 5 cm and 10 cm apart from the base and the tip of the flag leaf, respectively. The area of each disc was $0.28 cm^2$. The concentrations of the pigments ($g\ L^{-1}$) were calculated according to the equations used by Misra and Dey (2013), which are based on the data published by Lichtenthaler (1987). Sampling was done at milk stage. Chlorophyll content was also measured with a SPAD 502, Konica, Minolta during the 2nd year. Data were recorded the same dates as the UAV flights from six flag leaves randomly selected from each plot. Two measurements were taken from each leaf.

3.2.3. UAV flights, image acquisition and processing

Spectral Vegetation Indices (SVI) (Elvidge and Chen, 1995; Haboudane, et al., 2002) were measured using autonomous UAV. Two flights were carried out during the 1st year when most varieties were at booting and milk stages. The four flights carried out during the 2nd year were performed when most varieties were at heading, anthesis, milk and dough stages.

The autonomous UAVs used for the present study were the fixed wing SwingletCam from Sensfly (1st year) and the multicopterHexa Y from 3D Robotics (2nd year). Both UAVs are fully capable for completely autonomous flight from takeoff to landing, requiring minimum expertise from the operator. Flights can be prepared beforehand, while changes during flight are also possible either through the computer or manual override using the radio control.

Cameras used on board the SwingletCam were provided by SensFly as part of the package. They were a Canon IXUS 220 HS for RGB photos, and a modified near infrared Canon Powershot ELPH 300 HS **(Figure 27)**. Onboard the multicopter, the Canon IXUS 130 IS was used to take RGB photos and a modified near infrared Canon Powershot SX260 HS was used for near infrared photograph **(Figure 28)**. None of the cameras used was radiometrically calibrated as the whole process aims in using simple and cheap equipment that national breeding programs could have access. A field or lab spectroradiometer are expensive equipment which would have raised both the cost and the complexity of the test.

Figure 27: Sample photos from Canon IXUS 220 HS (left) and a modified near infrared Canon Powershot ELPH 300 HS (right).

A Leica Viva dual Global Positioning System (GPS) in Real Time kinematic (RTK) mode was used for ground control point measurements. Prior to the flight, simple white A4 sheets were laid down on the ground as control points. Two flights were performed, one right after the other, with the exact same flight plan, but with different cameras. This method suggests that RGB and NIF photos were not taken simultaneously but with a time gap of 10 to 20 min, depending on plot dispersion.

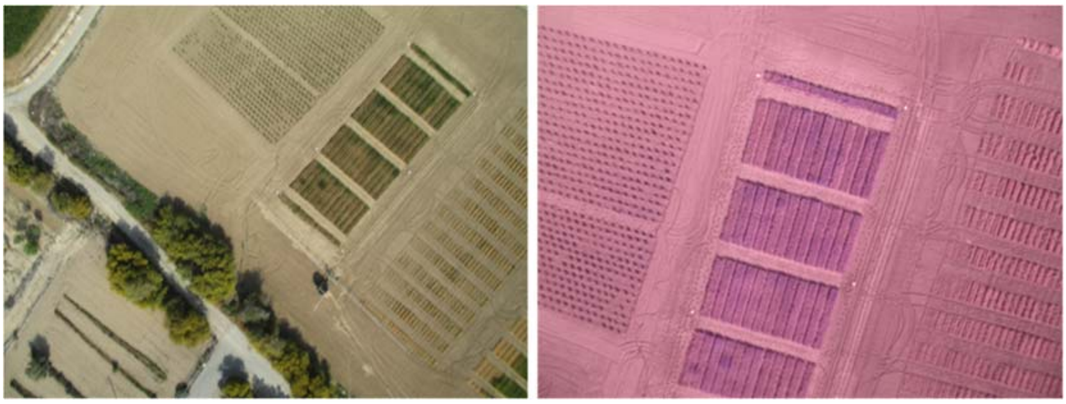


Figure 28: Sample photos from Canon IXUS 130 IS (left) and a modified near infrared Canon PowerShot SX260 (right).

Flights were conducted in varying heights from 72m up to 140m and ground pixel sizes varying from 2.0 cm to 4.3 cm. The variation of ground pixel size is of no importance since the final orthophotos created, for every epoch, had 5.0 cm pixel size, larger than the ones in the original photography. Although the whole area of the crop fields could have been included in a single aerial image from the aforementioned flying height, using a 5.0 cm pixel size, was necessary to capture multiple photos in order to create a Digital Elevation Model (DEM), necessary for the orthorectification and georeferencing process.

All photos were processed using Agisoft'sPhotoscan (version 1.0) to produce georeferenced real color and near infrared orthophotos. During this process the original imagery is orthorectified and georeferenced to ensure that each pixel, at every epoch, of the real color and near infrared orthophotos correspond perfectly to each other. As a byproduct of the process, a Digital Elevation Model (DEM) of the ground and the canopy of the crop surface was produced **(Figure 29)**.

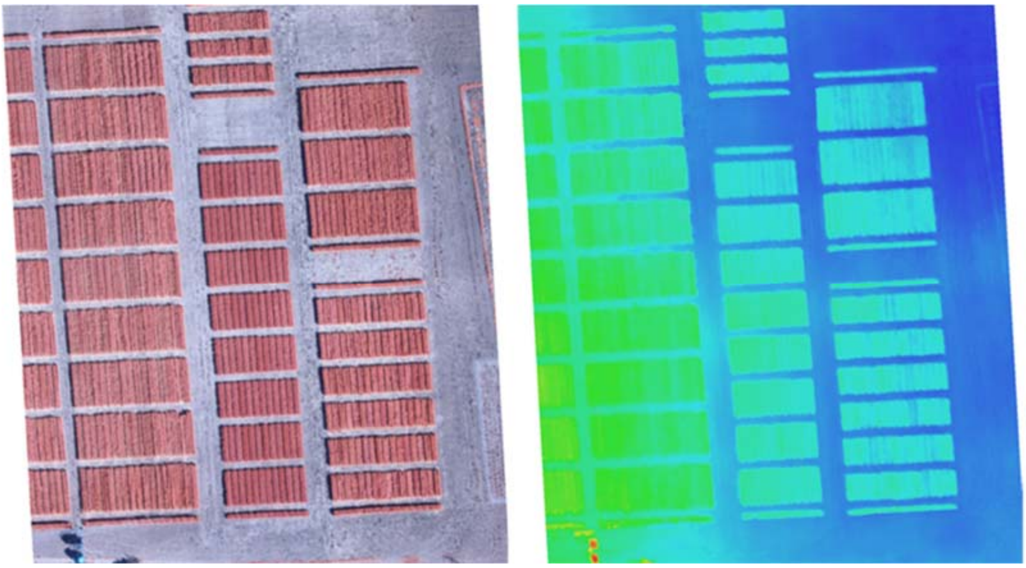


Figure 29: Near infrared orthophoto and corresponding DEM from the first flight (Booting) the first year.

Slight color differentiation is likely to happen, even among photos, from the same camera because of light conditions, camera settings, sun reflection and camera angles. Mosaicking of photos during the last phase of orthophotomosaicing process, produces misalignments and color shifting due to automatic software color matching and correction. In order to avoid the color changes, a single photo from each set (true color or near infrared), covering the crop area was selected to create the final orthophotos. Orthophotos were created with user specified coordinates values, so that to ensure full correspondence over overlapping pixels. Final orthophotos had the exact same number of pixels and three channels each **(Figure 30)**.

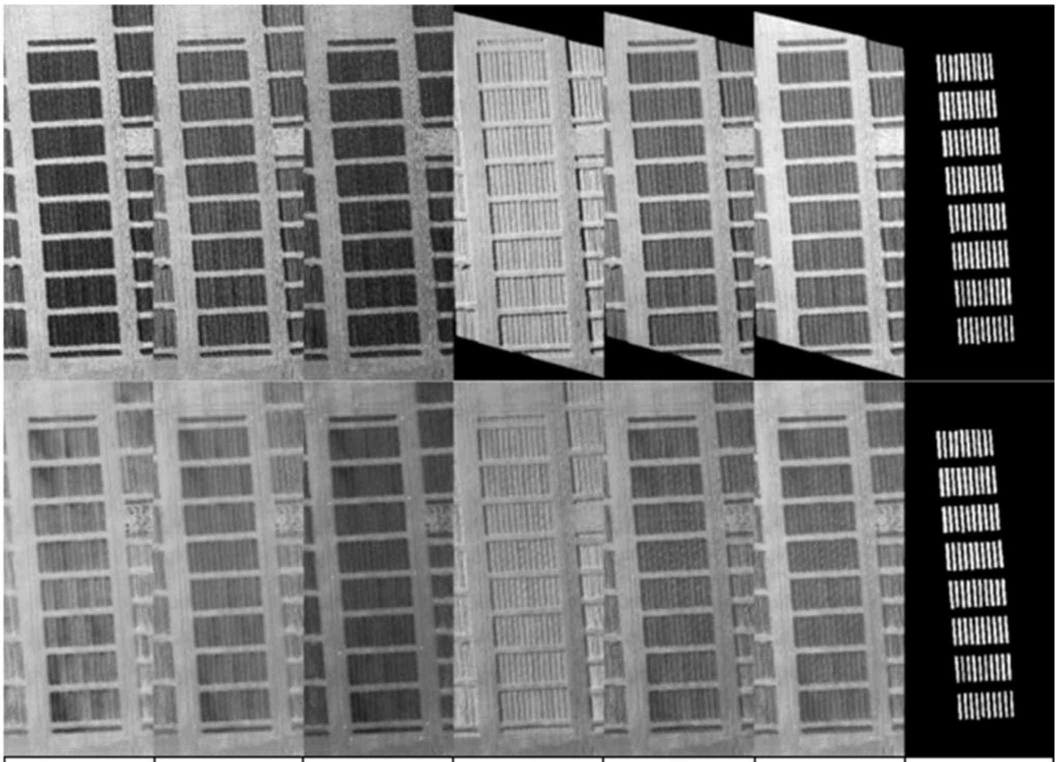
After the creation of the true color and near infrared orthophotos, they were loaded into Matlab software (version 12) for further processing. Within Matlab they were stacked to form a six channel photo, according to **Figure 30**. With arithmetical functions among the pixels, several SVIs were calculated. Once the multispectral orthophotos were created, masks over each crop were manually created. The masks were concentrated over the crop's main body, excluding the crop's edges where mixing with the next variation might have caused misleading results. Nevertheless, even by reducing extend and pixels of each plot, there is an average of 9350 pixels left per plot. Based on the manually collected masks (**Figure. 30**, on the far right), they were combined in each experimental plot. The following indices (Agapiou, Hadjimitsis and Alexakis, 2012) were calculated for each experimental plot:

$NDVI = (R_{NIR} - R_{red}) / (R_{NIR} + R_{red})$

$GNDVI = (R_{NIR} - R_{green}) / (R_{NIR} + R_{green})$

$SR = R_{NIR} / R_{red}$

The 2nd year, GNDVI was calculated only for the first two flights, i.e. at heading and anthesis.



Channel	1	2	3	4	5	6
Ch/Cam	R/RGB	G/RGB	B/RGB	NIR/NIR	G/NIR	B/NIR

Figure 30. Channel integration, from the two flights (booting and milk stages) over the 1st year. The last image on the right, shows all experiments' masks combined, similar in both cases.

3.2.4. Statistical analysis

Combined analysis over years was conducted for agronomic traits considering genotypes and years as fixed factors. One-way ANOVA was conducted for agronomic traits, SVIs, SPAD measurements and photosynthetic pigments for each growth stage and year. ANOVA was also conducted for SVIs and SPAD measurements considering all the growth stages together during each year. The significant differences among genotype mean values were calculated by Tukey's test. Pearson correlation coefficients on genotype means were estimated between agronomic traits, photosynthetic pigments, SPAD measurements and SVIs. Correlations between NDVIs, SPAD values, and chlorophyll *b* recorded at Athalassa with grain yield recorded from experiments at Achelia and Dromolaxia are also presented. Principal Component Analysis was performed using the direct oblimin rotation method to explore relationships among variables. The PCs with eigenvalues greater than 1 were selected and coefficients greater than 0.3 are shown. Stepwise regression analysis was performed on genotype means to investigate SVIs, SPAD measurements and photosynthetic pigments contribution to grain yield. All analyses were carried out using SPSS (IBM, SPSS ver 22).

3.3. Results

3.3.1. Genotypic effects

Analysis of Variance F-values for different traits among genotypes including means, maximum and minimum values, and the Coefficient of Variations (CVs) for the two years and the combined analysis over years for the agronomic traits are shown in **Table 12**. There was genetic variability between genotypes, except for grain yield at the 2nd year and at the combined analysis over years. Environmental conditions affected seeds per spike, volume weight, thousand kernel weight and growing degree days to heading. The interactions were weaker or non-significant. Statistically significant differences between genotypes were also observed for SVIs, except for SR at milk stage the 2nd year, SPAD values at all stages and for photosynthetic pigments **(Table 13)**. The SVIs differences were more profound during the 1st year. NDVI and SR explained more variation at milk stage, contrary to GNDVI, which explained more variation at booting stage during the 1st year. Comparison of genotypes mean values are depicted to **Table 14**.

NDVIs and SR means were lower during the 1st year, particularly at milk stage. Since different digital cameras were used during the 2nd year, there is variation among various digital cameras due to the different sensor sensitivity at various spectral reflectances and the different lens filters used. For example, Li, et al. (2010) found close relations between vegetation indices derived from three different digital cameras and canopy cover, however the magnitude of the estimated canopy cover varied with camera. Thus, the results of the two years were analyzed independently.

There were significant differences between genotypes and growth stages when measurements from all stages were analyzed together for SVIs and for SPAD **(Table 15)**. Both SVIs and SPAD values were progressively reduced as plants were reaching maturity. However, differences between milk and dough stages were not significant for NDVI and SR at the 2nd year. Furthermore, SPAD values at heading and anthesis did not differ significantly. Significant interactions between genotypes and growth stages were observed for NDVIs and GNDVIs the 1st year and for SPAD values the 2nd year.

Table 12: Analysis of Variance F-values for genotypes, Coefficients of Variation (CV), means, minimum (Min) and maximum (Max) values of grain yield (GRYLD), number of fertile tillers per m^2 (NTLSM), seeds per spike (SPS), volume weight (VW), thousand kernel weight (TKW), plant height (PH), growing degree days to heading (GDDHD) and growing degree days from heading to physiological maturity (GDDPM).

	GRYLD (Kg/ha)	**NTLSM**	**SPS**	**VW (Kg/hl)**	**TKW (g)**	**PH (cm)**	**GDDHD**	**GDDPM**
				1st year				
F genotype	2.443**	4.371***	15.814***	13.973***	20.537***	5.202***	50.912***	-
CV	16.59	14.55	8.20	2.11	6.39	5.10	1.13	-
Mean	1723	252.71	27.60	66.55	23.56	78.93	1275	-
Min	1247	206.79	22.42	62.35	19.24	68.88	1203	-
Max	2126	337.50	38.81	71.05	33.50	87.25	1398	-
				2nd year				
F genotype	1.162	2.772**	14.051***	5.709***	4.010***	5.111***	23.542***	3.128***
CV	25.72	15.00	11.42	2.87	12.19	5.30	1.33	7.47
Mean	1651	258.49	24.00	70.34	27.03	81.30	1360	558.40
Min	995	172.38	18.13	65	21.29	70.73	1296	443.58
Max	2013	312.50	36.53	75	35.35	89.80	1437	606.33
				Combined analysis				
F genotype	1.260	4.679***	25.267***	11.461***	8.868***	6.751***	43.288***	-
F year	0.017	0.288	50.007***	78.109***	13.216***	0.019	540.147**	-
F genotype x year	1.768*	1.919*	2.717***	1.754*	1.307	1.657	2.344**	-
Mean	1685	255.79	25.69	68.57	25.41	80.19	1320	-
CV	21.82	15.62	9.91	2.89	11.70	5.71	1.52	-

*p<0.05, **p<0.01, p<0.001***

Table 13: Analysis of Variance F-values for genotypes, Coefficients of Variation (CV), means, minimum (Min) and maximum (Max) values for SVIs, SPAD values and photosynthetic pigments.

	1st year	2nd year		1st year	2nd year		2nd year	
	NDVI booting	**NDVI heading**	**NDVI anthesis**	**NDVI milk**	**NDVI milk**	**NDVI dough**	**SPAD heading**	**SPAD anthesis**
F genotype	8.086***	2.464**	2.245**	9.937***	2.042*	2.261**	9.036***	5.447***
CV	4.00	8.39	12.32	27.32	14.72	8.54	3.02	3.26
Mean	0.49536	0.50460	0.41274	0.11633	0.30512	0.28772	55.89	56.42
Min	0.43407	0.44609	0.34481	0.04668	0.23476	0.25044	51.68	51.94
Max	0.55610	0.56472	0.50076	0.25669	0.38853	0.34017	59.82	59.06
	SR booting	**SR heading**	**SR anthesis**	**SR milk**	**SR milk**	**SR dough**	**SPAD milk**	**SPAD dough**
F genotype	6.009***	3.036***	2.171*	8.417***	1.659	2.083*	5.297***	4.144***
CV	6.46	10.25	13.41	7.46	12.24	6.02	14.44	37.07
Mean	3.09	3.16	2.54	1.27	1.95	1.82	45.86	25.96
Min	2.61	2.67	2.12	1.10	1.63	1.67	28.69	10.59
Max	3.68	3.67	3.11	1.70	2.33	2.04	59.51	49.86
				1st year				
	Anthocyanin (g/l)	**Chlorophyll *b* (g/l)**	**Chlorophyll *a* (g/l)**	**Carotenoids (g/l)**	**Total chlorophyll (g/l)**	**GNDVI booting**	**GNDVI milk**	
F genotype	6.001***	13.956***	14.118***	11.084***	14.208***	15.346***	9.942***	
CV	10.40	19.61	17.47	13.65	17.62	2.72	4.92	
Mean	0.004234	0.001045	0.008761	0.004207	0.009806	0.26742	0.21992	
Min	0.003419	0.000385	0.003651	0.002515	0.004036	0.24178	0.18053	
Max	0.005427	0.001770	0.014283	0.006068	0.016053	0.29556	0.25394	
				2nd year				
	Anthocyanin (g/l)	**Chlorophyll *b* (g/l)**	**Chlorophyll *a* (g/l)**	**Carotenoids (g/l)**	**Total chlorophyll (g/l)**	**GNDVI heading**	**GNDVI anthesis**	
F genotype	2.293**	4.318***	4.945***	3.492***	4.885***	2.440**	2.078*	
CV	15.30	27.64	24.46	20.94	24.77	8.36	10.67	
Mean	0.003599	0.001121	0.007922	0.002952	0.009044	0.23066	0.21854	
Min	0.002999	0.000721	0.005149	0.002026	0.005871	0.19939	0.18468	
Max	0.004529	0.001934	0.013303	0.004187	0.015237	0.25480	0.24731	

*p<0.05, **p<0.01, p<0.001***

Table 14: Comparison of genotype means for NDVI and GNDVI at booting during the 1st year, NDVI and GNDVI at heading and anthesis, respectively, during the 2nd year, chlorophyll pigments and SPAD values at milk stage.

	1st year NDVI Booting	**2nd year NDVI Heading**	**1st year GNDVI Booting**	**2nd year GNDVI Anthesis**	**1st year Chlorophyll *a* (g/l)**	**2nd year Chlorophyll *a* (g/l)**	**1st year Chlorophyll *b* (g/l)**	**2nd year Chlorophyll *b* (g/l)**	**2nd year SPAD Milk**
Aronas	0.46678abc	0.53148ab	0.25076ab	0.23027ab	0.005511abcd	0.006721abc	0.000637abcd	0.000996abc	46.025bcd
Mesaoria	0.47959abcd	0.44609a	0.26136bcd	0.18468a	0.005493abcd	0.005149a	0.000587abc	0.000721a	28.690a
Karpasia	0.49610bcde	0.54065ab	0.26632bcde	0.24016ab	0.007851bcde	0.008608abcd	0.000943bcdfg	0.001211abcd	47.318bcd
Macedonia	0.50984bcdef	0.48019ab	0.27340cde	0.20329ab	0.008101bcde	0.005958abc	0.000966bcdfg	0.000833abc	35.437ab
Ourania	0.50484bcdef	0.51110ab	0.27216cde	0.23215ab	0.009587ef	0.007910abc	0.001155dfgh	0.001090abc	45.100abcd
Hekabe	0.48076abcd	0.53472ab	0.25720abc	0.23371ab	0.007516abcde	0.005652ab	0.000830abcdf	0.000804abc	46.183bcd
Anna	0.48917bcde	0.49358ab	0.25997abcd	0.21465ab	0.004337ab	0.006435abc	0.000490ab	0.000903abc	44.318abcd
Atlas	0.50070bcde	0.52222ab	0.26857bcde	0.23065ab	0.010133ef	0.009429abcd	0.001174dfgh	0.001317abcd	45.516bcd
Matt	0.43407a	0.44810a	0.24177a	0.18573a	0.004857abc	0.005619ab	0.000542ab	0.000754ab	34.400ab
Mexikali81	0.46210ab	0.48587ab	0.24943ab	0.21573ab	0.003650a	0.006612abc	0.000384a	0.000971abc	42.239abc
Pisti	0.50235bcde	0.48517ab	0.26729bcde	0.20638ab	0.009454def	0.00623abc	0.001115cdfgh	0.000874abc	40.741abc
Simeto	0.52317def	0.48977ab	0.27891defg	0.20934ab	0.011319efg	0.010694cd	0.001448ghi	0.001562cd	54.143cd
Duilio	0.48180abcde	0.49154ab	0.26282bcde	0.21143ab	0.008536cdef	0.008243abc	0.000991bcdfgh	0.001107abc	48.193bcd
Iride	0.51762cdef	0.49268ab	0.28121efg	0.21165ab	0.012443fg	0.010219bcd	0.001534hi	0.001528bcd	54.491cd
Claudio	0.50682bcdef	0.56311b	0.27158cde	0.24730b	0.014282g	0.0133029d	0.001770i	0.001934d	59.511d
Svevo	0.46283ab	0.51071ab	0.24981ab	0.22686ab	0.010162ef	0.008093abc	0.001091cdfgh	0.000986abc	42.133abc
Adnan 2	0.53418ef	0.51211ab	0.29556g	0.22536ab	0.011392efg	0.007770abc	0.001480ghi	0.001100abc	48.254bcd
Omrabi 5	0.55609f	0.49668ab	0.29340fg	0.22086ab	0.008519cdef	0.009061abcd	0.000978bcdfg	0.001269abcd	54.911cd
Korifla	0.50860bcdef	0.56472b	0.27296cde	0.23603ab	0.011247efg	0.008384abc	0.001439ghi	0.001219abcd	47.308bcd
Waha	0.49332bcde	0.46125ab	0.27533cdef	0.19503ab	0.010657efg	0.006837abc	0.001304fghi	0.000930abc	45.891bcd

Table 15: Analysis of Variance F-values for genotypes, growth stage, genotype x growth stage, Coefficient of Variation (CV) and means of NDVI, SR, GNDVI and SPAD values.

	1st year			2nd year			
	NDVI	**SR**	**GNDVI**	**NDVI**	**SR**	**GNDVI**	**SPAD**
F genotype	9.012***	3.363***	9.224***	4.866***	4.434***	3.282***	4.937***
F growth stage	5302.67***	2011.58***	494.78***	316.69***	267.18***	11.91**	240.90***
F genotype x stage	3.324***	1.425	1.775*	0.263	0.433	0.222	1.514*
CV	10.69	11.67	5.51	14.02	14.74	10.92	18.91
Mean	0.305846	2.184715	0.243669	0.37755	2.37	0.22460	46.03

*p<0.05, **p<0.01, p<0.001***

3.3.2. Associations between SVI indices and photosynthetic pigments

Correlations between SVIs, SPAD values and photosynthetic pigments during the 1st year and the 2nd year, respectively are shown at **Tables 16 and 17**. There were very strong correlations between SVIs recorded at each growth stage and at different growth stages. SPAD values at milk stage were highly correlated with SPAD values at dough stage. Weaker, although significant correlations were also observed between SPAD values at heading and SPAD values at anthesis and at milk stage. SPAD values at milk and dough stages showed significant correlations with SVIs. The correlations were stronger at dough stage. There were significant correlations between chlorophyll pigments and carotenoids with SVIs with the exception of GNDVI at heading for the 2nd year. Anthocyanin correlations were non-significant or were weak. SPAD values were significantly correlated with chlorophyll pigments and carotenoids, except from SPAD at anthesis.

Table 16: Pearson correlations between SVIs and photosynthetic pigments during the 1st year.

	NDVI booting	**SR booting**	**GNDVI booting**	**NDVI milk**	**SR milk**	**GNDVI milk**
SR booting	0.988**					
GNDVI booting	0.960**	0.940**				
NDVI milk	0.498*	0.458*	0.555*			
SR milk	0.464*	ns	0.524*	0.966**		
GNDVI milk	0.680**	0.673**	0.689**	0.875**	0.853**	
Anthocyanin	ns	ns	0.445*	0.827**	0.809**	0.709**
Chlorophyll *b*	0.604**	0.563**	0.693**	0.885**	0.864**	0.806**
Chlorophyll *a*	0.573**	0.528*	0.655**	0.871**	0.848**	0.780**
Carotenoids	0.579**	0.532*	0.641**	0.885**	0.860**	0.804**
Total chlorophyll	0.577**	0.533*	0.660**	0.873**	0.851**	0.784**

ns (not significant), $*p<0.05$, $**p<0.01$

There were significant correlations between SVIs recorded at milk stage the 1st year with SVIs, SPAD values at milk and dough stages and photosynthetic pigments recorded the 2nd year **(Table 18)**. SVIs recorded at booting the 1st year were significantly related only with chlorophyll pigments, carotenoids and SPAD values at heading, anthesis and milk stages.

Table 17: Pearson correlations between SVIs, SPAD values and photosynthetic pigments during the 2nd year.

	NDVI heading	**GNDVI heading**	**SR heading**	**SPAD heading**	**NDVI anthesis**	**GNDVI anthesis**	**SR anthesis**	**SPAD anthesis**	**NDVI milk**	**SR milk**	**SPAD milk**	**NDVI dough**	**SR dough**	**SPAD dough**
GNDVI heading	0.873**													
SR heading	0.985**	0883**												
SPAD heading	ns	ns	ns											
NDVI anthesis	0.956**	0.828**	0.959**	ns										
GNDVI anthesis	0.958**	0.889**	0.963**	ns	0.960**									
SR anthesis	0.921**	0.801**	0.949**	ns	0.985**	0.941**								
SPAD anthesis	ns	ns	ns	0.766**	ns	ns	ns							
NDVI milk	0.874**	0.660**	0.886**	ns	0.947**	0.894**	0.942**	ns						
SR milk	0.853**	0.634**	0.875**	ns	0.928**	0.880**	0.941**	ns	0.989**					
SPAD milk	0.583**	0.449*	0.573**	0.579**	0.671**	0.614**	0.630**	ns	0.710**	0.658**				
NDVI dough	0.774**	0.553*	0.789**	ns	0.870**	0.803**	0.874**	ns	0.958**	0.957**	0.632**			
SR dough	0.763**	0.535*	0.781**	ns	0.857**	0.792**	0.868**	ns	0.952**	0.960**	0.632**	0.998**		
SPAD dough	0.617**	ns	0.633**	ns	0.709**	0.626**	0.712**	ns	0.806**	0.777**	0.845**	0.805**	0.798**	
Anthocyanin	ns	ns	ns	ns	ns	ns	ns	0.476*	ns	ns	0.526*	0.532*	0.527*	0.665**
Chlorophyll *b*	0.533*	ns	0.538*	0.578**	0.658**	0.530*	0.656**	ns	0.752**	0.722**	0.853**	0.767**	0.757**	0.931**
Chlorophyll *a*	0.526*	ns	0.544*	0.528*	0.649**	0.539*	0.658**	ns	0.751**	0.730**	0.841**	0.776**	0.773**	0.933**
Carotenoids	ns	ns	ns	0.637**	0.458*	ns	0.452*	0.476*	0.550*	0.508*	0.757**	0.584*	0.569**	0.813**
Total chlorophyll	0.528*	ns	0.544*	0.535*	0.651**	0.538*	0.659**	ns	0.752**	0.730**	0.844**	0.776**	0.772**	0.934**

ns (not significant), *p<0.05, **p<0.01

Table 18: Pearson correlations of SVIs, SPAD values and photosynthetic pigments recorded at different years.

		1st year										
		NDVI booting	**SR booting**	**GNDVI booting**	**NDVI milk**	**SR milk**	**GNDVI milk**	**Anthocyanin**	**Chlorophyll *b***	**Chlorophyll *a***	**Carotenoids**	**Total chlorophyll**
2nd year	**NDVI heading**	ns	ns	ns	0.616**	0.629**	0.562**	ns	ns	ns	ns	ns
	GNDVI heading	ns	ns	ns	0.537*	0.544**	0.557*	ns	ns	ns	ns	ns
	SR heading	ns	ns	ns	0.614**	0.623**	0.559*	ns	0.450*	0.454*	0.468*	0.454*
	SPAD heading	0.511*	0.520*	0.505*	ns	ns	0.579**	ns	0.474*	0.444*	ns	0.448*
	NDVI anthesis	ns	ns	ns	0.619**	0.635**	0.576**	ns	ns	ns	ns	ns
	GNDVI anthesis	ns	ns	ns	0.560*	0.563**	0.561*	ns	ns	ns	ns	ns
	SR anthesis	ns	ns	ns	0.609**	0.622**	0.547*	ns	ns	0.444*	0.455*	ns
	SPAD anthesis	0.565**	0.549*	0.561*	ns	ns	ns	0.466*	ns	ns	ns	ns
	NDVI milk	ns	ns	ns	0.637**	0.650**	0.565**	ns	0.458*	0.466*	0.481*	0.465*
	SR milk	ns	ns	ns	0.616**	0.626**	0.532*	ns	ns	0.457*	0.472*	0.456*
	SPAD milk	0.559*	0.577**	0.549*	0.625**	0.613**	0.736**	0.534*	0.656**	0.641**	0.651**	0.644**
	NDVI dough	ns	ns	ns	0.606**	0.624**	0.491*	0.498*	ns	0.448*	0.470*	0.446*
	SR dough	ns	ns	ns	0.607**	0.623**	0.485*	0.505*	ns	0.455*	0.476*	0.453*
	SPAD dough	ns	ns	ns	0.722**	0.720**	0.635**	0.599**	0.711**	0.722**	0.703**	0.721**
	Anthocyanin	ns	ns	ns	0.672**	0.670**	0.566**	0.823**	0.603**	0.620**	0.668**	0.619**
	Chlorophyll *b*	0.489*	0.476*	0.499*	0.766**	0.769**	0.735**	0.685**	0.740**	0.728**	0.727**	0.730**
	Chlorophyll *a*	0.464*	0.447*	0.478*	0.787**	0.784**	0.754**	0.723**	0.753**	0.755**	0.760**	0.755**
	Carotenoids	0.488*	0.454*	0.495*	0.677**	0.666**	0.720**	0.636**	0.616**	0.603**	0.618**	0.605**
	Total chlorophyll	0.468*	0.451*	0.481*	0.786**	0.783**	0.753**	0.719**	0.753**	0.753**	0.757**	0.753**

ns (not significant), *$p<0.05$, **$p<0.01$

3.3.3. Associations with agronomic traits

Significant correlations were obtained with NDVIs and GNDVIs at booting the 1st year and with GNDVIs at anthesis the 2nd year **(Table 19)**. The correlations were higher the 1st year, when genetic variation in grain yield was also significant. According to stepwise regression results, GNDVI at booting and at anthesis explained 31.8 and 21.5% of grain yield variability for the 1st year and the 2nd year, respectively. Standardized beta coefficients were positive in both cases **(Table 20)**.

Table 19: Pearson correlations between SVIs and grain yield at different growth stages.

Year	Growth stage	Correlations
1st year	NDVI booting	0.526*
	GNDVI booting	0.564**
	SR booting	0.461
	NDVI milk stage	0.418
	GNDVI milk stage	0.419
	SR milk stage	0.384
2nd year	NDVI heading	0.426
	GNDVI heading	0.318
	SR heading	0.410
	NDVI anthesis	0.438
	GNDVI anthesis	0.464*
	SR anthesis	0.413
	NDVI milk stage	0.402
	SR milk stage	0.382
	NDVI dough stage	0.361
	SR dough stage	0.346

*p<0.05, **p<0.01, n=20

The correlations between NDVIs at different growth stages, SPAD values at milk and dough stages, and chlorophyll *b* with grain yield, from Achelia and Dromolaxia experiments, are presented in **Table 21**.

Table 20: Stepwise regression between grain yield and anthocyanin, carotenoids, chlorophyll *b*, NDVI and GNDVI at booting and milk stage for the 1st year and anthocyanin, carotenoids, chlorophyll *b*, SPAD at milk and dough stages, NDVI at heading and milk stage and GNDVI at heading and anthesis for the 2nd year.

Year	**Variable enter**	**Model R^2**	**Standardized Beta**	**F change**	**Probability**
1st year	GNDVI booting	0.318	0.564	8.384	0.010
2nd year	GNDVI anthesis	0.215	0.464	4.938	0.039

Negative correlations were obtained between NDVI and grain yield in Dromolaxia for two consecutive years. Negative correlations between NDVI and grain yield were also observed in Achelia for one year while for the other year, NDVI was positively related. The best recording stage varied with experiment. Significant negative correlations were also obtained between SPAD values and chlorophyll *b* with grain yield, although in most cases were weaker than the NDVI.

Table 21: Pearson correlations between NDVI at different stages, SPAD at milk and dough stages and chlorophyll *b* with grain yield from the same set of genotypes grown at different years and locations.

	Dromolaxia 2011/12	**Achelia 2011/12**	**Athalassa 2012/13**	**Dromolaxia 2012/13**	**Achelia 2011/12**	**Athalassa 2013/14**
NDVI booting Athalassa 2012/13	ns	0.557*	-	ns	ns	ns
NDVI milk Athalassa 2012/13	ns	ns	-	-0.450*	-0.478*	ns
Chlorophyll *b* Athalassa 2012/13	ns	ns	-	ns	-0.491*	ns
NDVI heading Athalassa 2013/14	-0.615**	ns	ns	-0.536*	ns	-
NDVI anthesis Athalassa 2013/14	-0.547*	ns	ns	-0.574**	ns	-
NDVI milk Athalassa 2013/14	-0.537*	ns	ns	-0.653**	ns	-
NDVI dough Athalassa 2013/14	-0454*	ns	ns	-0.660**	-0.449*	-
SPAD milk Athalassa 2013/14	ns	ns	ns	ns	-0.613**	-
SPAD dough Athalassa 2013/14	ns	ns	ns	-0.543*	-0.641**	-
Chlorophyll *b* Athalassa 2013/14	ns	ns	ns	-0.569**	-0.566**	-

ns (not significant), *p<0.05, **p<0.01, n=20

Principal Component Analysis was conducted to investigate the combinations of traits that best explained the variability. The first three PCs explained 78.46% and

79.97% of the total variance during the 1st and 2nd years, respectively **(Table 22)**. For both years, the PC1 was strongly and positively associated with chlorophyll *b*, carotenoids, anthocyanin, SVIs at milk stage, growing degree days to heading and volume weight. The associations of SVIs recorded at earlier stages with PC1 were weaker, especially the 2nd year. Grain yield was positively related to PC1 the 1st year and to PC2 for both years. Strong and positive associations with PC2 were also observed for number of tillers per m^2 for both years and weaker for SVIs at booting, heading and anthesis, and plant height. Growing degree days to heading were negatively related to PC2 the 1st year. Growing degree days from heading to physiological maturity were positively related to PC2 the 2nd year.

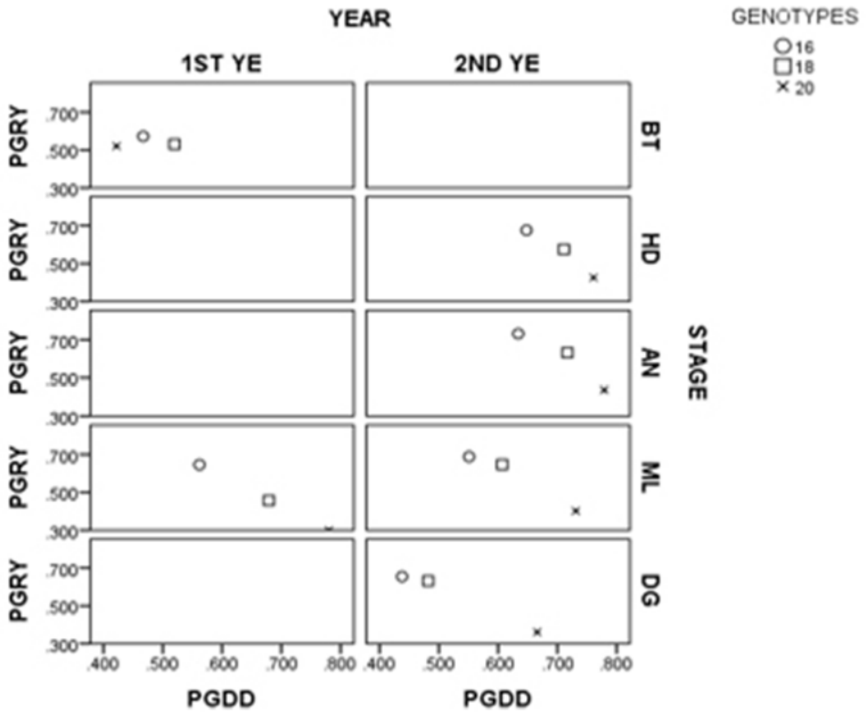
Table 22: Pattern matrix of the PCA analysis. PCA was based on agronomic traits, anthocyanin, carotenoids, chlorophyll *b*, NDVI and GNDVI at booting and milk stage for the 1st year and on the agronomic traits, anthocyanin, carotenoids, chlorophyll *b*, SPAD at milk stage, NDVI at heading and milk stage and GNDVI at heading and anthesis for the 2nd year.

Pattern matrix							
1st year				2nd year			
	PC1	PC2	PC3		PC1	PC2	PC3
Chlorophyll *b*	.963			Carotenoids	.938		
Carotenoids	.952			Chlorophyll *b*	.933		
NDVI milk	.921			Anthocyanin	.887		
GNDVI milk	.895			VW	.837		
Anthocyanin	.861		.313	SPAD milk	.784		
GDDHD	.781	-.389		NDVI milk	.625	.360	-.356
VW	.778		.383	NTLSM		.924	
GNDVI booting	.716	.370	-.382	GRYLD		.849	
NDVI booting	.657	.478		PH		.764	
NTLSM		.917	.322	GDDPM	.560	.679	
GRYLD	.417	.645		GNDVI heading		.332	-.784
PH	-.377	.452		TKW	.520		.765
SPS			-.888	GDDHD	.561		-.699
TKW	.491		.682	NDVI heading	.336	.416	-.623
				GNDVI anthesis	.361	.473	-.586
				SPS			-.584
Cumulative variance (%)	50.01	66.52	78.46	Cumulative variance (%)	46.56	66.33	79.97

[Grain yield (GRYLD), number of tillers per m^2 (NTLSM), seeds per spike (SPS), volume weight (VW), thousand kernel weight (TKW), plant height (PH), growing degree days to heading (GDDHD) and growing degree days from heading to physiological maturity (GDDPM)]

3.3.4. Implications with phenology

In order to examine the implications of plant phenology in the ability of NDVI to predict yield, Pearson correlations between grain yield and NDVI were plotted against the correlations between growing degree days to heading and NDVI for each year and recording stage **(Figure31)**. Each point represents the correlations when all genotypes were taken into account, and when the two and four late heading genotypes were excluded. There were significant correlations between NDVI measurements and grain yield at all growth stages and years when the two and the four late heading genotypes were excluded. The correlations between grain yield and NDVI were stronger when the correlations between NDVI with growing degree days to heading were weaker.



1st YE – 1st year, 2nd YE – 2nd year, BT – Booting, HD – Heading, AN – Anthesis, ML – milk stage, DG – Dough stage

Figure 31: Correlation coefficients between growing degree days to heading with NDVI (PGDD) plotted against coefficients between grain yield and NDVI (PGRY) for each year and recording stage. Each point represents the correlations when all genotypes were taken into account (n=20), when the two late heading genotypes were excluded (n=18) and when the four late heading genotypes were excluded (n=16).

3.4. Discussion

There is growing interest for using SVIs derived by UAV imagery as a fast and cost efficient tool for plant phenotyping. The development of such tools are of paramount importance to continue progress through plant breeding, especially in drought prone and heat stressed environments where climate change is expected to increase yield uncertainty. Studies conducted under Mediterranean environment are limited and the intent of the present study is to elucidate the usefulness of such tools under these harsh environmental conditions.

3.4.1. Genotypic effects

Grain yields were similar (Aparicio, et al., 2000; Gutierrez, et al., 2010; Lopos, et al., 2014) or lower from average yields reported in experiments under stressed conditions for rainfed cereal crops (Babar, et al., 2006c; Lopes and Reynolds, 2012; Bowman, et al., 2015; Gonzalez-Dugo, et al., 2015; Gizaw, Garland-Campbell and Certer, 2016a) indicating the severe stress that plants experience during their growing cycle.

The significant differences between genotypes for SVIs are in line with previous reports (i.e. Aparicio, et al., 2000; Babar, et al., 2006c; Prasad, et al, 2007b; Gutierrez, et al., 2010). The differences in the present study were more profound during the 1st year. Regarding the 2nd year, the discriminate ability of SVIs was affected by the higher experimental error due to the unusual drought conditions during the vegetative stage. Soil heterogeneity becomes more apparent under drought conditions (Masuka, et al., 2012) increasing the experimental error and undermining field screenings, including phenotyping with SVIs (Zaman-Allah, et al., 2015).

During the 1st year, there was less variation among genotypes for NDVI and SR at booting. This is in agreement with the findings of Royo, et al. (2003), Babar, et al. (2006b) and Prasad, et al. (2007a). The maximum Leaf Area Index (LAI) for wheat grown under Mediterranean conditions occurs at booting. The usefulness of SR and NDVI for estimating grain yield and other important agronomic traits is limited to LAI values lower than 3 to 4 (Aparicio, et al., 2000; Aparicio, et al., 2002). Contrary, GNDVI explained more variation at booting during the 1st year indicating that is less affected by high LAI values. Gitelson, et al. (2002) reported that R_{red} sensitivity was at least three time lower than R_{green} when vegetation fraction was more than 60%, thus vegetation indices using green wavelength are likely to perform better at high LAI values.

The variability explained by the growth stage was much higher by the variation explained by genotypes for SVIs and SPAD values as it is deduced in **Table 15**. These findings are in agreement with the results of Aparicio, et al. (2002), Bort, et al. (2005), Babar, et al. (2006b), Babar, et al. (2006c) and Prasad, et al. (2007b). SVI mean values progressively reduced from booting to dough stage as was shown to previous studies (i.e. Babar, et al., 2006a; Prasad, et al., 2007a; Gizaw, Garland-Campbell and Certer, 2016b). The non-significant reduction from milk to dough stage in this study is justified by the fact that severe leaf senescence was present when plants were at milk stage.

Previous studies reported significant interactions between genotypes and growth stages under irrigated and stressed conditions (Babar, et al., 2006b; Babar, et al., 2006c; Prasad, et al., 2007b; Gutierrez, et al., 2010; Gizaw, Garland-Campbell and Certer, 2016a). Those authors pointed out that the interactions of growth stages and indices indicate that care must be taken to identify a suitable growth stage at which the indices will be applied to discriminate most effectively among genotypes in breeding trials. In the present study, the high correlation between SVIs recorded at different stages in the 2nd year is consistent with the non-significant interactions between growth stage and genotypes. During the 1st year, the correlations between SVIs recorded at booting and milk stage were weaker, justifying the existence of significant interactions. The interactions observed during the 1st year can be attributed to the noise induced to the data from the 1st recording stage at booting, when LAI values are at maximum. SR is less affected by the saturation effect of LAI greater than 3 compared with NDVI (Serrano, Filella and Peñuelas, 2000; Aparicio, et al., 2002) which might explain the lack of significant interactions for SR. Aparicio, et al. (2002) reported significant interactions between genotypes and recording stage for NDVI but not for SR. Montazeaud, et al. (2016) stated that NDVI saturation is not easily attained in the rainfed conditions of low yielding environments. During the 2nd year, measurements were taken at heading and onwards, when NDVI saturation effect is getting less significant, reducing the noise in the data. These results indicate that, under severe stress, and for SVI measurements taken after heading, the interactions between growth stage and genotypes are likely to be low or non-significant.

3.4.2. Associations between SVI indices and photosynthetic pigments

Several authors stressed the strong relationship between SVIs (i.e. Bort, et al., 2005; Gizaw, Garland-Campbell and Certer, 2016b). Previous studies reported associations between SVIs recorded at different growth stages in the same environment and between SVIs recorded at different environments under more favourable (Babar, et al., 2006b; Prasad, et al., 2007b) and stressed conditions (Babar, et al., 2006c). The results of this study are in agreement with the previously mentioned observations.

The strong positive correlations between SVIs with SPAD values and photosynthetic pigments confirm the close associations between SVIs and canopy greenness. Serrano, Filella and Peñuelas (2000) also observed significant correlations between NDVI/SR and chlorophyll *a*. Non-significant or very weak correlations were observed between SVIs and SPAD values at heading and anthesis, contrary to the significant positive correlations at milk and dough stages, due to SPAD values above 50, which are less reliable (Minolta SPAD502 plus manual). This is further justified by the weak or non-significant correlations between SPAD values at heading and anthesis with the values recorded at milk and dough stages. Previous studies found non-significant or very weak correlations between SPAD and NDVI measurements for bread and durum wheat under Mediterranean conditions (Yousfi, et al., 2016) or negative correlations for maize (Liebisch, et al., 2015). Contrary, in the present study, positive significant correlations were found between SPAD values with SVIs at both milk and dough stages. This is in line with the significant positive correlations between SPAD values and photosynthetic pigments. Similarly, Babar, et al. (2006a) found positive correlations between reflectance spectral indices (RARS), which are associated with photosynthetic pigments and SPAD measurements.

3.4.3. Associations with agronomic traits

The significant correlations between grain yield and SVIs are in agreement with previous studies proposing SVIs as a mean for estimating important traits such as grain yield under heat and/or drought conditions (Aparicio, et al., 2000; Bort, et al., 2005; Gutierrez, et al., 2010; Lobos, et al., 2014; Tattaris, et al. 2014; Bowman, et al., 2015; Zaman-Allah, et al., 2015; Gizaw, Garland-Campbell and Certer, 2016a; Gizaw, Garland-Campbell and Certer, 2016b; Tattaris, Reynolds and Chapman, 2016). The weak or non-significant correlations of grain yield with SPAD values and photosynthetic pigments confirm the superiority of SVIs compared to SPAD measurements as predictors of grain yield under stressed conditions (Lopes and Reynolds, 2012; Yousfi, et al., 2016).

Other authors postulated that SVIs are likely to be more successful under moderate rather than under severe stressed conditions (Babar, et al., 2006c), where genotypes are able to express their yield potential (Royo, et al., 2003). These studies were conducted with hand-held sensors. Gonzalez-Dugo, et al., (2014) reported non-significant correlations between grain yield and vegetation indices under Mediterranean conditions, derived by hyper spectral camera mounted on manned aircraft. In their study, there was only one sampling date during the critical period of grain filling. Our results indicate that SVIs derived by UAV imagery are likely to be useful in severe stressed Mediterranean conditions, with average grain yield as low as 1700Kg/ha. In drought stressed conditions, small variability in soil depth and texture have increasingly large effects on variability (Ceccareli and Grando, 1996), thus whole plot measurements derived by UAV imagery are likely to be more representative than hand-held measurements. This also justifies the higher correlations between SVIs and grain yield compared with SPAD values and photosynthetic pigments. Multiple sampling dates are necessary from booting to physiological maturity since significant correlations with grain yield might be obtained only in one growth stage that can vary with experiment. The need for multiple measurements during the crop cycle was already stressed by several authors. For example, repeated measurements on the same genotypes over different growth stages accumulate information on the respective health of genotypes through time, thus average values across growth stages can give better predictions of yield (i.e. Babar, et al., 2006c; Prasad, et al., 2007b; Gizaw, Garland-Campbell and Certer, 2016b).

An association between indices measured in one site and the yield of the same genotypes in another site, would mean that the indices could be used to predict yield in diverse environments. Significant correlations between indices measured in one environment and yield measured in a different environment were previously reported (Bort, et al., 2005; Gutierrez, et al., 2010; Gizaw, Garland-Campbell and Certer, 2016a). In the present study, both positive and negative significant correlations were observed between NDVI and grain yield for the same set of genotypes grown at different environments **(Table 21)**. Negative correlations between NDVI and grain yield were observed when negative correlations between grain yield and growing degree days to heading were recorded. Positive correlations were observed when the correlations between grain yield and growing degree days to heading were non-significant **(Table 11)**. The majority of the previous studies reported positive relations between grain yield and vegetation indices. For example, Lopos, et al. (2014) and Gizaw, Garland-Campbell and Certer (2016b) reported positive correlations between NDVI and grain yield under severe water stress, and non-significant correlations between grain yield and days to heading. However, negative correlations were reported under severe stress conditions, where negative correlations between SVIs and grain yield coexisted with negative correlations between days to heading and grain yield (Lopes, et al., 2014; Rutkoski, et al., 2016). Early maturing genotypes are likely to be more productive in stressed environments (Bort, et al., 2005). The superiority of early maturing genotypes in their study justified the negative associations between NDVI and SR at the latest recording stage. They concluded that the changes in the values and the signs of the correlations between grain yield and reflectance indices reflect genotypic differences in response to high temperature and drought during late grain filling. The results of the present study are in agreement with their findings.

The variation explained by the first three PCA components was similar to the variation recently reported by Gizaw, Garland-Campbell and Certer (2016b). The first component was highly correlated with SVIs and volume weight for both years. Other studies showed less consistent correlations between SVIs and volume weight.

For example, Arguello, et al. (2016) reported volume weight and NDVI in the same clustering of a PCA analysis conducted under water logged conditions, but not under normal conditions. Gizaw, Garland-Campbell and Certer (2016b) did not find any close association between volume weight and vegetation indices. In the present study, the consistent correlations between SVIs and volume weight can be attributed to the fact that late heading genotypes had higher volume and higher SVI values. Number of tillers per m^2, plant height, thousand kernel weight and number of seeds per spike, were less correlated to SVIs. Previous studies also reported lower and inconsistent relations between spectral reflectance indices and the above mentioned agronomic traits (Aparicio, et al., 2002; Babar, et al., 2006b; Lopos, et al., 2014; Gizaw, Garland-Campbell and Certer, 2016b).

3.4.4. Implications with phenology

The implications between plant phenology and SVIs can affect the correlations between SVIs and agronomic traits, particularly grain yield (Lopes and Reynolds, 2012; Tattaris, Reynolds and Chapman, 2016). Principal component analysis showed a consistent strong correlation between SVIs and growing degree days to heading, as has been previously reported (Lopes, et al., 2014; Lopos, et al., 2014; Elazab, et al., 2015; Gizaw, Garland-Campbell and Certer, 2016b). Plant phenology affected the ability of SVI to predict yield, as it is deduced by the negative trend between correlations of NDVI with grain yield and correlations between NDVI with growing degree days to heading.

Rate of senesce, estimated as the slope of the NDVI decay against thermal time, and stay green, as an estimation of NDVI at physiological maturity, can give an independent measurement of stay green without the confounding effect of phenology (Lopes and Reynolds, 2012). In the present study, the rate of senescence and stay green were not related with grain yield (data not shown). On the contrary, NDVI values at the intercept of the slope with the Y axis, which estimates NDVI values at the end of booting-beginning of heading, were significantly and positively related with grain yield ($r=0.583, p<0.01$). The correlations were similar when the four late heading genotypes were excluded ($r=0.545, p=0.029$). The intercept NDVI was not related with growing degree days to heading implying that it is not related with phenology. Montazeaud, et al. (2016) reported positive correlations between grain yield and maximum greenness, as estimated by NDVI measurements. Maximum greenness coexists with booting, when water is relatively available and the temperatures are still not high.

In stressed environments, biomass accumulation before heading is associated with grain yield as it is related to carbohydrate remobilization to grain during the grain filling stage (Villegas, et al., 2001). Significant correlations between SVIs and biomass have been reported (Aparicio, et al., 2002; Babar, et al., 2006a). The positive correlation with grain yield that was observed for GNDVI recorded at booting the 1st year, and for intercept NDVI at the 2nd year might be associated with genotypes that manage to accumulate high biomass before heading. The SVI measurements at this stage were independent of phenology as it is shown by the non-significant correlations with days to heading. SVI measurements at later stages were depended from phenology and they were not associated with grain yield because late heading genotypes had higher SVI values, but they were less productive. Lopes, et al. (2014) confirmed that NDVI measurements after booting are related to plant greenness and selecting for high NDVI after booting, late flowering genotypes will be selected which are low yielding. When late heading genotypes were excluded, high positive correlations were obtained implying that within a narrower range of heading, stay-green genotypes were more productive. In environments where days to heading were negatively associated with grain yield, significant negative correlations were obtained between NDVIs and grain yield. SVIs are predictors of canopy greenness (Aparicio, et al., 2000), thus early maturing genotypes were associated with low SVI values.

3.4.5. Conclusions

The results of the present work highlight the potential use of SVIs derived by UAV imagery for durum wheat phenotyping at low yielding Mediterranean conditions. The optimum recording stage varied with experiment. The ability of SVIs as yield predictors was affected by plant phenology. The implications between plant phenology and SVIs derived by UAV imagery should be investigated in future studies, employing parameters that are less related to plant phenology. Other indices, such as water indices (Babar, et al., 2006c; Gutierrez, et al., 2010) and/or RGB indices (Elazab, et al., 2015; Vergara-Diaz, et al., 2016) were found to be superior compared to SVIs in field phenotyping. Additional research should be conducted in the future, addressing the performance of these indices derived from UAV imagery.

Summary of Findings/ Recommendations

The main objectives of this doctoral dissertation were: (1) to characterize durum wheat genetic resources, including modern varieties from different breeding programs and landraces originated from Cyprus and other Mediterranean areas using SSRs, (2) to evaluate a subset of twenty modern varieties originated from different breeding programs for grain yield and other important agronomic traits under diverse climatic conditions, and (3) to assess the usefulness of high throughput, remote sensing techniques for plant phenotyping in the subset of the twenty modern varieties grown under a water limited and heat stressed environment.

Genetic Characterization of Durum Wheat Genetic Resources

Genetic characterization enhances the development of rational conservation strategies and the utilization of germplasm to plant breeding programs. Despite that several studies were conducted over the last years aimed at the genetic characterization of durum wheat genetic resources, there is still large genetic variation within *in situ* and *ex situ* collections, especially in landraces, that remain undiscovered. For example, only a very limited number of studies were designed to investigate the genetic diversity within individual landraces. Concerning Cyprus, there is no robust information available about the genetic constituents within Cypriot durum wheat landraces and modern varieties. In the present study, 22 modern varieties from different breeding programs and 33 landraces originated from Cyprus and other Mediterranean areas were characterized with SSRs. The genetic diversity and structure of the Cypriot landrace "Kyperounda" was further investigated.

Nineteen microsatellites revealed the existence of high genetic diversity. In total, 224 alleles were detected with an average of 11.79 alleles per locus. The average discriminating power (Dj) was 0.84. Landraces formed distinct groups and depicted higher genetic variability reinforcing their potential use to plant breeding. The highest genetic variability was observed in Cypriot landraces and 36 alleles were detected only in this germplasm. These results suggest the existence of unique genetic diversity within Cypriot landraces genepool.

High genetic diversity was detected within Cypriot landrace "Kyperounda" and 50 unique genotypes were recorded out of 217 sublines / plants examined. Fifty-six alleles were recorded with an average of 11.2 alleles per locus. The genetic diversity was higher within the "Kyprerounda" accessions conserved as sublines than in accessions conserved as bulks. Our findings indicate that high genetic diversity is still hidden within individual landrace accessions conserved as sublines.

The genetic differentiation between "Kyprerounda" accessions and the different clustering of landrace accessions conserved under the same name signifies that passport data is not a reliable method to eliminate duplications.

Cypriot landraces were not grouped together and "Kyprerounda" accessions were not clustered according to their collecting site (or accession number) in structure analysis. In addition, AMOVA attributed the higher percentage of variability within groups. The genetic diversity is greatly impacted by multiple factors, such as environmental conditions, breeding methods and gene flow via human activities, i.e. exchange of seeds between farmers from different geographical areas and unconscious farmers' selections.

Further research is needed to uncover the genetic diversity that it is hidden within *ex situ* collections. The implementation of cost efficient and fast high throughput techniques such as SNPs and Genotyping by Sequencing, will allow to unhide the genetic diversity inside large collections, to screen for Quantitative Trait Loci (QTLs) throughout the genome, and to construct association maps with agronomic traits.

Evaluation of Durum Wheat Modern Varieties Under Diverse Climatic Conditions

Despite the progress made, breeders need to further increase yields to meet the demand from the growing population. This is a daunting challenge in drought prone environments like the Mediterranean, where the impact of climate change is more severe. Phenotypic evaluation under diverse environments allows the assessment of variability for important agronomic and physiological traits and to evaluate their correlations with yield. In the present study, twenty modern varieties, originated from different breeding programs, were evaluated for important agronomic and quality traits under diverse climatic conditions in three locations for two years (six environments).

There was genetic diversity for all the traits, however, for most of them, the highest percentage of variance was explained by the environmental effect. The traits exhibiting the highest and the lowest genetic control were the yellow pigment content and grain yield, respectively. Variation for grain yield plasticity was low while variation for yellow pigment content and gluten index plasticities was high. The differences between varieties for plasticity were significant for gluten index, yellow pigment content, protein content, growing degree days to heading, thousand kernel weight and plant height.

Number of seed per m^2 was significantly correlated with grain yield in most cases and the correlations were stronger in the low yielding environments. Number of seeds per spike was the traits consistently related with number of seeds per m^2. Trait offs between yield components were recorded, except between number of fertile tillers per m^2 and thousand kernel weight. Varieties had different yield formation strategies. The two highest yielding varieties had the highest number of fertile tillers per m^2 combined with high number of seeds per spike or high kernel weight.

Breeders did not give much attention to kernel weight, because high number of seeds per m^2 was the main way to increase yield of durum wheat in the Mediterranean. Improving simultaneously these two traits is cumbersome because of the frequently observed trait offs. However, the production of shrivel seeds is a major concern for the industry and lowers the seed value. The production of shrivel seeds will be increased with climate change. An alternative breeding strategy for durum wheat in the Mediterranean that needs further investigation is to select varieties with increase water soluble carbohydrate remobilization associated with higher and stable kernel weight.

Growing degree days to heading and number of fertile tillers per m^2 were negatively and positively related with thousand kernel weight plasticity, respectively. The traits *per se* were not related. For particular traits, some varieties had high average values and they were very stable since they manage to retain high values to all environments (e.g. "Simeto" for thousand kernel weight, "Matt" for gluten index). In some other cases, varieties had high average values and high plasticity indicating that they are more responsive to the particular trait in the favorable environments (e.g. "Adnan 2" and "Iride" for seeds per spike, "Matt" for yellow pigment content). These findings suggest that traits plasticity merits further consideration from the breeders.

In future studies, physiological traits associated with grain yield, especially under stress conditions should be further investigated in this set of varieties. Such traits are the water soluble carbohydrate concentration and remobilization efficiency and the transpiration efficiency using carbon isotopes and canopy temperature.

Assessment of Vegetation Indices Derived by UAV Imagery for Durum Wheat Phenotyping Under a Stress Environment

There is growing interest for using Spectral Vegetation Indices derived by Unmanned Aerial Vehicle imagery as a fast and cost-efficient tool for plant phenotyping. The development of such tools are of paramount importance to continue progress through plant breeding, especially in the Mediterranean basin, where climate change is expected to further increase yield uncertainty. A number of studies investigated the potential use of imagery derived from sensors mounted on UAVs and other aerial vectors for plant breeding and precision agriculture. Nevertheless, studies conducted under severely stressed Mediterranean conditions are very limited. In the present thesis, the usefulness of SVIs (NDVI, SR and GNDVI) derived from UAV imagery for plant phenotyping under a water limited and heat stressed Mediterranean environment was investigated.

Statistically significant differences between varieties were observed for SVIs. GNDVI explained more variability than NDVI and SR, when recorded at booting. GNDVI was significantly correlated with grain yield when recorded at booting and anthesis during the 1st and 2nd year, respectively, while NDVI was correlated to grain yield when recorded at booting, but only for the 1st year. These results suggest that GNDVI has a better discriminating efficiency and can be a better predictor of yield when recorded at early reproductive stages.

The predictive ability of SVIs was affected by plant phenology. Correlations of grain yield with SVIs were stronger as the correlations of SVIs with heading were weaker or not significant. NDVIs recorded at the experimental site were significantly correlated with grain yield of the same set of genotypes grown in other environments. Both positive and negative correlations were observed indicating that the environmental conditions during grain filling can affect the sign of the correlations. These findings highlight the potential use of SVIs derived by UAV imagery for durum wheat phenotyping at low yielding Mediterranean conditions.

The implications between plant phenology and SVIs derived by UAV imagery should be investigated in future studies, employing parameters that are less related to plant phenology. Other indices, such as water indices and/or RGB indices were found to be superior compared to SVIs in field phenotyping. Additional research should be conducted in the future, addressing the performance of these indices derived from UAV imagery.

CONCLUSIONS

Nineteen microsatellites revealed the existence of high genetic diversity in a set of 22 modern varieties from different breeding programs and 33 landraces originated from Cyprus and other Mediterranean areas. Landraces formed distinct groups and depicted higher genetic variability than modern varieties reinforcing their potential use to plant breeding. The highest genetic variability was observed in Cypriot landraces and 36 alleles were detected only in this germplasm suggesting the existence of unique genetic diversity within this genepool. The genetic diversity of the Cypriot landrace "Kyperounda" was investigated in three accessions conserved as sublines in ARI genebank and in two accessions conserved as bulks in USDA-ARS. High genetic diversity was detected suggesting the existence of genetic variability within individual landraces. The genetic diversity was higher in accessions conserved as sublines indicating that high genetic diversity is still hidden within individual landrace accessions conserved with this method. The genetic differentiation between "Kyprerounda" accessions and the different clustering of landrace accessions conserved under the same name, signifies that passport data is not a reliable method to eliminate duplications.

A subset of twenty modern varieties, originated from different breeding programs, were evaluated for important agronomic and quality traits under diverse climatic conditions in three locations for two years (six environments). There was genetic diversity for all the traits, however, for most of them, the highest percentage of variance was explained by the environmental effect. Number of seed per m^2 was significantly correlated with grain yield in most cases, and the correlations were stronger in the low yielding environments. Number of seeds per spike was the trait consistently related with number of seeds per m^2. Trait offs between yield components were recorded, except between number of fertile tillers per m^2 and thousand kernel weight. Varieties had different yield formation strategies. The two highest yielding varieties had the highest number of fertile tillers per m^2 combined with high number of seeds per spike or high kernel weight. Growing degree days to heading and number of fertile tillers per m^2 were negatively and positively related with thousand kernel weight plasticity, respectively. The traits *per se* were not related. For particular traits, some varieties had high average values and they were very stable since they manage to retain high values to all environments. In some other cases, varieties had average values and high plasticity indicating that they are more responsive to the particular trait in the favorable environments. These findings suggest that traits plasticity merits further consideration from the breeders.

The usefulness of SVIs (NDVI, SR and GNDVI) derived from UAV imagery for plant phenotyping was investigated under a water limited and heat stressed Mediterranean environment. Statistically significant differences between varieties were observed for SVIs. GNDVI explained more variability than NDVI and SR, when recorded at booting. GNDVI was significantly correlated with grain yield when recorded at booting and anthesis during the 1st and 2nd year, respectively, while NDVI was correlated to grain yield when recorded at booting, but only for the 1st year. The predictive ability of SVIs was affected by plant phenology. Correlations of grain yield with SVIs were stronger as the correlations of SVIs with heading were weaker or not significant. NDVIs recorded at the experimental site were significantly correlated with grain yield of the same set of genotypes grown in other environments. Both positive and negative correlations were observed indicating that the environmental conditions during grain filling can affect the sign of the correlations. These findings highlight the potential use of SVIs derived by UAV imagery for durum wheat phenotyping at low yielding Mediterranean conditions.

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Appendix I: Means and LSDs between varieties in Dromolaxia.

Variety	GDD	PH (cm)	GRYLD (Kg/ha)	NTLSM	SPS	NSSM	TKW (g)	VW (Kg/hl)	CAR (ppm)	PRO (%)	GI (%)
Adnan2	1268	83.81	4432	332.50	45.06	14999	33.03	77.05	6.96	14.70	3.67
Anna	1191	85.87	4525	431.25	25.52	11004	42.32	77.32	6.51	14.25	38.47
Aronas	1243	87.43	4087	402.14	26.40	10676	38.69	77.07	3.74	16.10	54.62
Atlas	1289	87.31	4213	364.82	26.71	9797	44.55	80.45	6.41	15.88	11.39
Claudio	1321	85.75	4218	349.64	28.21	9890	44.55	80.63	6.32	15.06	26.65
Duilio	1231	84.56	4564	355.00	29.28	10338	43.30	79.03	5.51	14.82	23.91
Hekabe	1252	87.31	4519	373.21	30.27	11317	39.63	77.10	8.93	15.81	40.19
Iride	1262	81.68	4533	293.21	41.81	12182	36.64	78.43	6.49	14.97	41.29
Karpasia	1288	83.62	4107	370.35	34.59	12775	32.47	76.11	7.51	16.65	3.75
Korifla	1297	83.62	3836	361.96	32.92	11969	35.90	75.71	6.56	15.26	27.29
Macedonia	1229	88.12	4924	425.89	33.58	14306	35.50	77.45	9.06	14.13	37.41
Matt	1199	81.31	4619	389.64	32.49	12629	38.92	78.38	9.88	14.57	72.53
Mesaoria	1204	75.81	4414	374.82	28.30	10625	38.65	75.76	5.44	16.24	6.59
Mexikali81	1212	82.43	4656	391.42	28.12	11016	41.84	76.95	7.17	14.26	36.79
Omrabi5	1262	101.68	4414	399.82	29.02	11339	39.91	80.21	6.93	14.87	23.40
Ourania	1263	87.06	4762	387.32	33.36	12924	36.16	79.01	8.20	14.79	39.20
Pisti	1201	81.87	5203	461.07	27.69	12661	42.56	77.96	6.32	14.17	44.53
Simeto	1318	76.81	4768	335.35	28.80	9697	48.62	77.06	6.89	14.70	46.08
Svevo	1239	89.68	4421	391.25	26.95	10547	41.43	78.37	8.11	16.35	29.65
Waha	1221	85.06	5077	366.96	32.70	12039	43.86	77.66	6.26	14.79	22.54
Mean	1249	85.04	4515	377.88	31.10	11636.45	39.93	77.89	6.96	15.12	31.50
LSD	15.62	3.35	428	37.71	2.68	1458	2.19	0.84	0.35	1.13	6.45

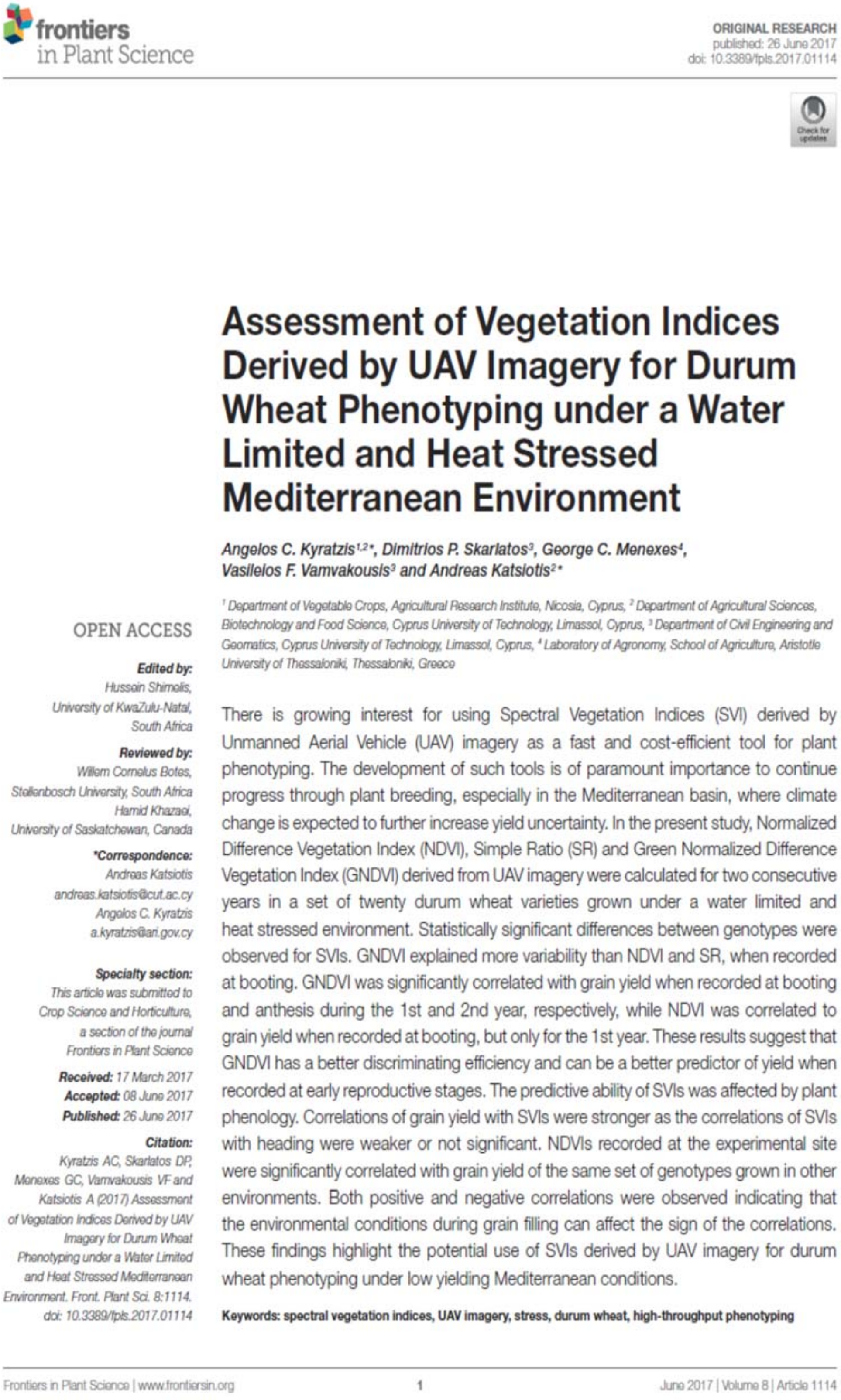
Appendix II: Means and LSDs between varieties in Achelia.

Variety	GDD	PH (cm)	GRYLD (Kg/ha)	NTLSM	SPS	NSSM	TKW (g)	VW (Kg/hl)	CAR (ppm)	PRO (%)	GI (%)
Adnan2	1176	84.25	6405	323.03	45.12	14809	43.83	80.67	5.99	13.18	4.02
Anna	1094	88.62	6513	341.78	32.66	11049	54.53	80.58	5.50	13.19	46.65
Aronas	1129	90.93	6333	339.28	34.15	11626	49.48	80.20	3.30	13.63	61.14
Atlas	1179	90.56	5911	330.17	29.67	9881	53.92	82.06	5.98	15.13	23.88
Claudio	1252	90.12	6049	334.46	34.01	11457	50.34	82.20	6.20	14.84	59.68
Duilio	1162	86.06	6314	321.60	35.58	11444	54.71	81.72	5.22	14.09	53.56
Hekabe	1131	91.18	6609	351.07	34.94	12243	54.53	80.86	7.96	13.92	60.93
Iride	1167	78.31	6090	291.42	47.44	13958	42.99	81.33	6.11	14.26	62.97
Karpasia	1169	86.68	6613	339.10	38.41	13003	45.59	80.93	6.56	14.66	6.26
Korifla	1204	86.62	5813	338.92	34.65	11698	47.23	79.63	5.71	14.10	59.98
Macedonia	1115	87.12	7017	393.21	35.35	13697	47.40	80.85	7.90	14.22	65.18
Matt	1097	83.62	6363	340.35	36.74	12396	49.16	80.56	8.42	14.77	83.30
Mesaoria	1103	75.68	6493	359.10	32.88	11777	49.33	78.87	4.89	14.80	8.77
Mexikali81	1097	84.18	5964	352.85	32.96	11597	52.69	79.95	5.90	13.40	47.72
Omrabi5	1174	106.62	6340	344.64	32.34	11197	48.19	81.72	6.42	14.54	23.42
Ourania	1164	86.12	6613	380.00	35.59	13489	49.19	82.00	7.03	14.54	59.67
Pisti	1090	86.25	6446	370.35	33.45	12302	54.63	80.96	5.34	14.17	67.45
Simeto	1253	79.56	5988	304.10	33.19	10317	55.75	79.18	6.20	15.57	69.31
Svevo	1139	88.00	5868	353.57	30.31	10774	49.98	80.32	7.42	15.56	41.76
Waha	1131	85.62	6127	295.53	33.18	9855	56.55	80.75	5.60	14.25	25.66
Mean	1151	86.81	6293	340.23	35.13	11928.35	50.50	80.77	6.19	14.35	46.57
LSD	14.81	3.15	546.82	35.53	3.13	1678	1.80	0.62	0.20	0.72	6.27

Appendix III: Means and LSDs between varieties in Athalassa.

Variety	GDD	PH (cm)	GRYLD (Kg/ha)	NTLSM	SPS	NSSM	TKW (g)	VW (Kg/hl)	CAR (ppm)	PRO (%)	GI (%)
Adnan2	1342	78.64	1775	250.53	33.61	8534	21.32	68.45	7.23	19.97	4.67
Anna	1276	80.77	1487	309.28	21.18	6670	24.13	65.97	7.21	20.98	45.16
Aronas	1323	83.00	1339	218.57	23.55	5209	23.63	65.80	4.56	22.02	56.81
Atlas	1365	76.43	1282	226.24	20.80	4807	26.90	71.23	7.12	24.11	23.80
Claudio	1429	73.27	1519	221.96	23.36	5217	26.62	72.38	6.39	23.82	31.97
Duilio	1320	78.83	1528	220.89	25.18	5560	28.91	71.38	5.93	20.26	51.42
Hekabe	1330	81.39	1470	225.53	27.20	6134	23.20	66.10	9.95	22.51	39.11
Iride	1332	74.00	1885	217.67	34.50	7569	24.65	68.86	6.82	21.41	54.45
Karpasia	1369	79.56	1572	237.85	29.39	7123	21.02	66.43	8.09	23.31	8.62
Korifla	1377	77.64	1395	261.96	26.14	7038	22.00	65.91	7.60	22.31	39.71
Macedonia	1323	77.62	1880	293.74	27.01	8064	21.92	67.25	9.33	22.28	49.86
Matt	1278	82.00	1570	230.53	26.12	6009	22.45	66.42	10.82	21.78	70.36
Mesaoria	1258	68.91	1293	240.35	22.55	5459	23.04	63.67	6.69	23.06	7.72
Mexikali81	1276	77.68	1556	273.92	22.05	6051	24.91	66.13	7.49	20.44	54.90
Omrabi5	1323	83.70	1372	254.46	23.99	6141	21.64	68.95	7.81	21.67	26.25
Ourania	1358	80.79	1683	252.49	26.60	6839	22.94	69.28	8.25	22.64	48.57
Pisti	1252	78.20	1775	295.00	22.46	6683	25.93	67.76	7.17	20.34	69.24
Simeto	1363	72.37	1575	225.89	21.93	4993	32.62	71.02	7.02	20.65	52.72
Svevo	1315	81.18	1442	241.07	24.22	5853	26.16	70.48	8.61	23.73	46.99
Waha	1298	76.77	1508	201.78	27.37	5588	26.41	65.68	6.87	21.66	33.63
Mean	1325	78.14	1545	244.99	25.46	6277	24.52	67.96	7.55	21.95	40.80
LSD	14.67	4.11	316.81	36.64	2.81	1291	2.17	1.63	0.52	1.33	5.30

Appendix IV: Publication



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Assessment of Vegetation Indices Derived by UAV Imagery for Durum Wheat Phenotyping under a Water Limited and Heat Stressed Mediterranean Environment

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There is growing interest for using Spectral Vegetation Indices (SVI) derived by Unmanned Aerial Vehicle (UAV) imagery as a fast and cost-efficient tool for plant phenotyping. The development of such tools is of paramount importance to continue progress through plant breeding, especially in the Mediterranean basin, where climate change is expected to further increase yield uncertainty. In the present study, Normalized Difference Vegetation Index (NDVI), Simple Ratio (SR) and Green Normalized Difference Vegetation Index (GNDVI) derived from UAV imagery were calculated for two consecutive years in a set of twenty durum wheat varieties grown under a water limited and heat stressed environment. Statistically significant differences between genotypes were observed for SVIs. GNDVI explained more variability than NDVI and SR, when recorded at booting. GNDVI was significantly correlated with grain yield when recorded at booting and anthesis during the 1st and 2nd year, respectively, while NDVI was correlated to grain yield when recorded at booting, but only for the 1st year. These results suggest that GNDVI has a better discriminating efficiency and can be a better predictor of yield when recorded at early reproductive stages. The predictive ability of SVIs was affected by plant phenology. Correlations of grain yield with SVIs were stronger as the correlations of SVIs with heading were weaker or not significant. NDVIs recorded at the experimental site were significantly correlated with grain yield of the same set of genotypes grown in other environments. Both positive and negative correlations were observed indicating that the environmental conditions during grain filling can affect the sign of the correlations. These findings highlight the potential use of SVIs derived by UAV imagery for durum wheat phenotyping under low yielding Mediterranean conditions.

Keywords: spectral vegetation indices, UAV imagery, stress, durum wheat, high-throughput phenotyping

INTRODUCTION

Drought stress, as a combination of water deficit and high temperature, is the main constraint limiting grain yield of cereals in the Mediterranean basin (Araus et al., 2002). This geographic area is expected to face more severe drought and an increase in average temperature in the near future, due to climate change (Giorgi and Lionello, 2008), increasing yield uncertainty of rain-fed crops. Improving crop productivity in drought-prone environments is a daunting challenge. Extensive plant phenotyping and integration of cost effective technologies are considered prerequisites to achieve progress through plant improvement (Reynolds and Tuberosa, 2008). Furthermore, advances in phenotyping are likely to be essential in capitalizing developments in conventional, molecular and transgenic breeding, and ensuring genetic improvement of crops for future food security (Araus and Cairns, 2014).

Remote sensing methods hold great potential as a tool for: (a) high throughput phenotyping for plant breeding (Deery et al., 2014; Sankaran et al., 2015), (b) decision making for precision agriculture (Zhang and Kovacs, 2012; Gago et al., 2015), (c) predicting yields (Son et al., 2014), and (d) predicting spatial field variability in experimental sites (Zaman-Allah et al., 2015). Their usefulness rely on the fact that they are non-destructive, non-invasive, fast and cost-efficient, well-correlated with agronomical and important physiological crop traits (Reynolds et al., 2015).

The most common procedure to extract information about crops from remote sensing is through the estimation of Spectral Vegetation Indices (SVI), which are based on formulations fitted with the light reflected by the canopy at different wavelengths (e.g., ratios and differences). The wavelengths are within the visible and the near infrared electromagnetic spectrum. Several SVIs have been proposed and are widely used, such as the Normalized Difference Vegetation Index (NDVI), the Simple Ratio (SR) and the Green Normalized Difference Vegetation Index (GNDVI). The existence of genetic variability for SVIs was reported by several authors (Babar et al., 2006b; Prasad et al., 2007b; Gutierrez et al., 2010; Gizaw et al., 2016a). SVIs were associated with important traits of cereal crops, such as grain yield under stressed conditions (i.e., Bort et al., 2005; Lobos et al., 2014; Bowman et al., 2015; Tattaris et al., 2016; Yousfi et al., 2016). However, some authors argued that under severe stress conditions, SVIs might be less efficient because genotypes are not able to express their yield potentiality (Royo et al., 2003; Babar et al., 2006c).

The majority of previous studies were conducted with hand held sensors; however, ground measurements face several constrains (Chapman et al., 2014; Deery et al., 2014; Gago et al., 2015; Reynolds et al., 2015; Sankaran et al., 2015; Tattaris et al., 2016). Some of these constrains can be eliminated using low altitude aerial platforms. Zhang and Kovacs (2012) stated that imagery taken by low altitude aerial systems is promising, given its low cost of operation, high spatial and temporal resolution, and its flexibility in image acquisition programming. Measurements from trials can be taken when they are not accessible to ground platforms, e.g., due to water-logged or tall crops (Chapman et al., 2014). Other advantages are the limited confounded effects caused by environmental drift due to simultaneous data collection and more robust image analysis tools (Reynolds et al., 2015; Tattaris et al., 2016), wider viewing angle from the air, and absence of physical contact, hence no mechanical distraction of the growing crop (Liebisch et al., 2015). Although UAVs can carry lower payload than other aerial vectors, they enable greater flight control and autonomy (Araus and Cairns, 2014) and are less affected by the wind (Deery et al., 2014; Tattaris et al., 2014). Recent studies revealed that correlations between SVIs and agronomic traits derived from airborne imagery are similar, or even stronger, than correlations derived from ground measurements (Tattaris et al., 2014, 2016; Zaman-Allah et al., 2015; Rasmussen et al., 2016). Measurements can be taken by a wide array of different sensors including conventional digital cameras (Araus and Cairns, 2014; Sankaran et al., 2015), that have the advantage of low cost and low weight (Hunt et al., 2010), and can be easily mounted on UAVs and other aerial vectors (Ball and Konzak, 1993; Lelong et al., 2008; Liebisch et al., 2015; Rasmussen et al., 2016). The fast and cost efficient nature of UAV imagery allows multiple measurements during grain filling. Multiple measurements are necessary because the optimum recording stage is likely to vary with experiment (i.e., Bort et al., 2005; Bowman et al., 2015). The efficiency of SVIs is also affected by plant phenology, thus multiple measurements allow the calculation of parameters that are less related with phenology (Lopes and Reynolds, 2012; Montazeaud et al., 2016).

The successful implementation of such technologies relies on the characteristics of the UAV including stability, safety, control, reliability, positioning, autonomy, sensor mount, controller, sensor characteristics and image and data processing (Chapman et al., 2014; Sankaran et al., 2015). It is then necessary to assess the reliability of aerial remote sensing approaches with direct plant-derived data (Lelong et al., 2008; Gago et al., 2015; Liebisch et al., 2015). A number of studies investigated the potential use of imagery derived from sensors mounted on UAVs and other aerial vectors for plant breeding (Ball and Konzak, 1993; Hoyos-Villegas and Fritschi, 2013; Chapman et al., 2014; Liebisch et al., 2015; Zaman-Allah et al., 2015; Rutkoski et al., 2016) and precision agriculture (Lelong et al., 2008; Hunt et al., 2010; Khot et al., 2016; Rasmussen et al., 2016). Nevertheless, studies conducted under severely stressed Mediterranean conditions are very limited (Gonzalez-Dugo et al., 2015).

The main scope of the present work is to investigate the usefulness of SVIs (NDVI, SR and GNDVI) derived from UAV imagery for plant phenotyping under a water limited and heat stressed Mediterranean environment. Durum wheat, a predominant stable crop cultivated in the Mediterranean basin, was selected for this study. A fast and cost effective method to estimate SVIs by UAV mounted with digital cameras is described. Genotypic effects of SVIs and agronomic and other physiological traits are presented. Correlations between SVIs and photosynthetic pigments, SPAD measurements, grain yield and other agronomic traits are discussed.

MATERIALS AND METHODS

Plant Material

Twenty durum wheat varieties (*Triticum turgidum* subsp. *durum*) were selected for the present study. Six varieties were bred by the Cypriot National Breeding Program and represent the main commercial varieties cultivated in Cyprus for the last 40 years. The other 14 varieties were released by other breeding programs targeting areas with similar climatic conditions (**Table 1**).

Experimental Conditions and Field Design

Experiments were conducted at Athalassa experimental station (35°08′N, 33°24′E) for two consecutive growing seasons (2012/2013 – year 1 and 2013/2014 – year 2). Athalassa has shallow sandy clay loam soil and rather low precipitation during crop cycle, resulting to drought stress during heading and grain filling. In addition, the rather high day temperature in spring and the frequent occurrence of extreme high temperatures during grain filling very often result to heat stress conditions (**Figure 1**). Crop failure and complete loss of yield frequently occurs in this area.

The experimental design was a randomized complete block with four and five replications the 1st year and the 2nd year, respectively. Six row plots, 8 m long, spaced apart 0.175 m were used. Seed rate was adjusted to 226 germinating seeds m^{-2}. Experiments were sown at the end of November and 60 Kg ha^{-1} of N_2 and P_2O_5 were applied before sowing. Weeds were chemically controlled at tillering (Atlantis®, Bayer, Illoxan®, Bayer, Granstar®, DuPont). Additional irrigation was applied during booting (30 mm) in the 1st year, and during tillering (50 mm) and booting (50 mm) in the 2nd year. The plants received no supplementary irrigation or rainfall from heading to physiological maturity during the 1st year, while in the 2nd year received only a negligible amount of rainfall when most of the plants had reached physiological maturity.

Measurements of Agronomic Traits

Heading date was recorded when the ears of 50% of the tillers had emerged from the flag leaf sheaths for approximately half their length and was expressed as growing degree days from emergence to heading. Physiological maturity was recorded when 50% of the spikes in the plot showed total loss of green color and was expressed as growing degree days from heading to physiological maturity. Growing degree days were calculated as described by Aparicio et al. (2000). Plant height was recorded as an average of three measurements per plot at physiological maturity, excluding awns. Number of fertile tillers per m^2 was estimated at physiological maturity from four rows, each one 1 m long, randomly selected on the 2nd and the 5th rows. Plants from two rows, each one 1 m long, were randomly selected on the 2nd and 5th row and hand harvested to estimate the number of seeds per spike. The plots were mechanically harvested on May and grain yield was recorder at 12% moisture level. Before harvesting, the two external rows and half meter from both ends of the plots were discarded to avoid the boarding effect (Ceccareli and Grando, 1996). Thousand kernel weight was calculated as the mean weight of two samples of 200 seeds per plot and expressed in g. Volume weight was measured with a 0.5L chondrometer (Seedburo) and expressed as Kg hl^{-1}.

Measurements of Photosynthetic Pigments

Extraction of photosynthetic pigments, chlorophyll a, chlorophyll b, carotenoids, anthocyanins was carried out as described by Richardson et al. (2002), setting the extraction time to 2 h. Six disks from three flag leaves were used for the extraction from each experimental plot. The disks were sampled 5 and 10 cm apart from the base and the tip of the flag leaf, respectively. The area of each disk was 0.28 cm^2. The concentrations of the pigments (g L^{-1}) were calculated according to the equations used by Misra and Dey (2013), which are based on the data published by Lichtenthaler (1987). Sampling was done at milk stage. Chlorophyll content was also measured with a SPAD 502,

TABLE 1 | List of the durum wheat varieties used in the present study.

Name	Year of release	Country of registration/Origin	Name	Year of release	Country of registration/Origin
Aronas	1977	Cyprus	Pisti	2008	Greece
Mesaoria	1982	Cyprus	Simeto	1988	Italy
Karpasia	1985	Cyprus	Duilio	1984	Italy
Macedonia	1994	Cyprus	Iride	1996	Italy
Ourania	2006	Cyprus	Claudio	1998	Italy
Hekabe	2003	Cyprus	Svevo	1996	Italy
Anna	2000	Greece	Adnan2		ICARDA*
Atlas	1995	Greece	Omrabi5		ICARDA*
Matt	2003	Greece	Korifla		ICARDA*
Mexikali81	1985	Greece	Waha		ICARDA*

*International Center for Agricultural Research in the Dry Areas.

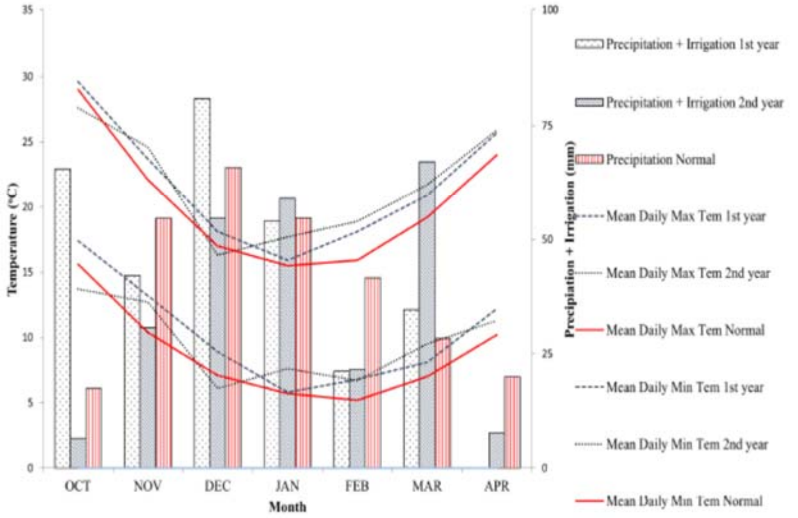

FIGURE 1 | Environmental conditions during the test years and normal conditions at Athalassa experimental station.

Konica, Minolta during the 2nd year. Data were recorded the same dates as the UAV flights from six flag leaves randomly selected from each plot. Two measurements were taken from each leaf.

UAV Flights, Image Acquisition and Processing

Spectral Vegetation Indices (Elvidge and Chen, 1995; Haboudane et al., 2002) were measured using autonomous UAV. Two flights were carried out during the 1st year when most varieties were at booting and milk stages. The four flights carried out during the 2nd year were performed when most varieties were at heading, anthesis, milk and dough stages.

The autonomous UAVs used for the present study were the fixed wing SwingletCam from Sensfly (1st year) and the multicopterHexa Y from 3D Robotics (2nd year). Both UAVs are fully capable for completely autonomous flight from takeoff to landing, requiring minimum expertise from the operator. Cameras used on board the SwingletCam were provided by SenseFly as part of the package. They were a Canon IXUS 220 HS for RGB photos, and a modified near infrared Canon Powershot ELPH 300 HS. Onboard the multicopter, the Canon IXUS 130 IS was used to take RGB photos and a modified near infrared Canon Powershot SX260 HS was used for near infrared photograph. Two flights were performed, one right after the other, with the exact same flight plan, but with different cameras. This method suggests that RGB and NIF photos were not taken simultaneously but with a time gap of 10 to 20 min, depending on plot dispersion. A Leica Viva dual Global Positioning System (GPS) in Real Time Kinematic (RTK) mode was used for ground control point measurements. Prior to the flight, simple white A4 sheets were laid down on the ground as control points.

Flights were conducted at varying heights from 72 up to 140 m and ground pixel sizes varying from 2.0 to 4.3 cm. The variation of ground pixel size is of no importance since the final orthophotos created, for every epoch, had 5.0 cm pixel size, larger than the ones in the original photography. Although the whole area of the crop fields could have been included in a single aerial image from the aforementioned flying height, using a 5.0 cm pixel size, was necessary to capture multiple photos in order to create a Digital Elevation Model (DEM), necessary for the orthorectification and georeferencing process.

All photos were processed using Agisoft'sPhotoscan (version 1.0) to produce georeferenced real color and near infrared orthophotos. During this process the original imagery is orthorectified and georeferenced to ensure that each pixel, at every epoch, of the real color and near infrared orthophotos correspond perfectly to each other. As a byproduct of the process, a DEM of the ground and the canopy of the crop surface was produced. Slight color differentiation is likely to happen, even among photos, from the same camera because of light conditions, camera settings, sun reflection and camera angles. Mosaicking of photos during the last phase of orthophoto mosaicking process, produces misalignments and color shifting due to automatic software color matching and correction. In order to avoid the color changes, a single photo from each set (true color or near infrared), covering the crop area was selected to create the final orthophotos. Orthophotos were created with user specified coordinates values, as to ensure full correspondence over overlapping pixels. Final orthophotos had the exact same number of pixels and three channels each (**Figure 2** and Supplementary Table S1 for the position of individual varieties in **Figure 2**). After the creation of the true color and near infrared

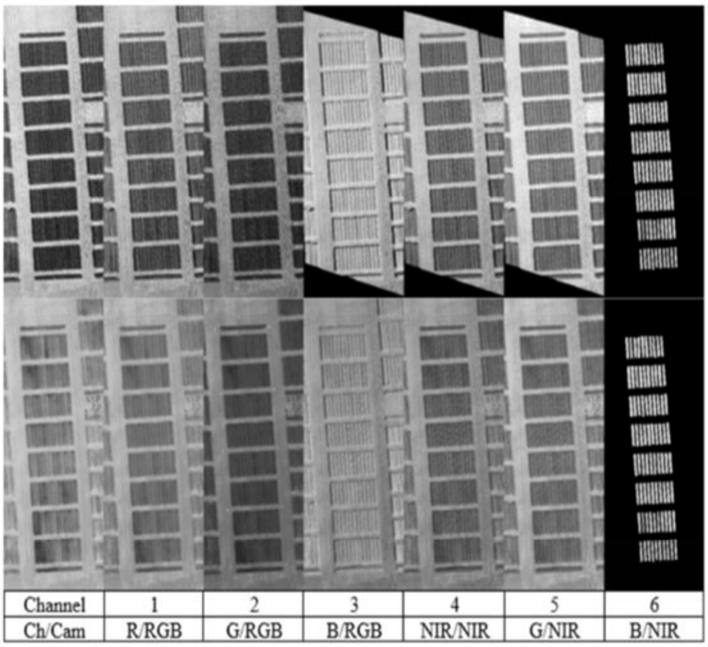

FIGURE 2 | Channel integration, from the two flights (booting and milk stages) over the 1st year. The last image on the right, shows all experiments' masks combined, similar in both cases.

orthophotos, they were loaded into Matlab software (version 12) for further processing. Within Matlab they were stacked to form a six channel photo, according to **Figure 2**. With arithmetical functions among the pixels, several SVIs were calculated. Once the multispectral orthophotos were created, masks over each crop were manually created. The masks were concentrated over the crop's main body, excluding the crop's edges where mixing with the next variation might have caused misleading results. Nevertheless, even by reducing extend and pixels of each plot, an average of 9350 pixels were left per plot. Based on the manually collected masks (**Figure 2**, on the far right), they were combined in each experimental plot. The following indices (Agapiou et al., 2012) were calculated for each experimental plot:

$$\mathrm{NDVI} = (R_{\mathrm{NIR}} - R_{\mathrm{red}}) \big/ (R_{\mathrm{NIR}} + R_{\mathrm{red}})$$

$$\mathrm{GNDVI} = \left(R_{\mathrm{NIR}} - R_{\mathrm{green}}\right) \big/ \left(R_{\mathrm{NIR}} + R_{\mathrm{green}}\right)$$

$$\mathrm{SR} = R_{\mathrm{NIR}} \big/ R_{\mathrm{red}}$$

The 2nd year, GNDVI was calculated only for the first two flights, i.e., at heading and booting.

Statistical Analysis

Combined analysis over years was conducted for agronomic traits considering genotypes and years as fixed factors. One-way ANOVA was conducted for agronomic traits, SVIs, SPAD measurements and photosynthetic pigments for each growth stage and year. ANOVA was also conducted for SVIs and SPAD measurements considering all the growth stages together during each year. Pearson correlation coefficients on genotype means were estimated between agronomic traits, photosynthetic pigments, SPAD measurements and SVIs. Correlations between NDVIs, SPAD values, and chlorophyll b recorded at Athalassa with grain yield recorded in experiments with the same set of genotypes grown at different locations (Achelia and Dromolaxia experimental stations, Cyprus) are also presented. Principal Component Analysis was performed using the direct oblimin rotation method to explore relationships among variables. The PCs with eigenvalues greater than 1 were selected and coefficients greater than 0.3 are shown. Stepwise regression analysis was performed on genotype means to investigate SVIs, SPAD measurements and photosynthetic pigments contribution to grain yield. All analyses were carried out using SPSS (IBM, SPSS ver 22).

RESULTS

Genotypic Effects

Analysis of Variance *F*-values for different traits among genotypes including means, maximum and minimum values, and the

Coefficient of Variations (CVs) for the 2 years and the combined analysis over years for the agronomic traits are shown in **Table 2**. There was genetic variability between genotypes, except for grain yield in the 2nd year and for the combined analysis over years. Environmental conditions affected seeds per spike, volume weight, thousand kernel weight and growing degree days to heading. The interactions were weaker or non-significant. Statistically significant differences between genotypes were also observed for SVIs, except for SR at milk stage the 2nd year, SPAD values at all stages and for photosynthetic pigments (**Table 3**). The SVIs differences were more profound during the 1st year. NDVI and SR explained more variation at milk stage, contrary to GNDVI, which explained more variation at booting stage during the 1st year.

Normalized Difference Vegetation Indices and SR means were lower during the 1st year, particularly at milk stage. Since different digital cameras were used during the 2nd year, there is variation among various digital cameras due to the different sensor sensitivity at various spectral reflectances and the different lens filters used. For example, Li et al. (2010) found close relations between vegetation indices derived from three different digital cameras and canopy cover, however, the magnitude of the estimated canopy cover varied with camera. Thus, the results of the two years were analyzed independently.

There were significant differences between genotypes and growth stages when measurements from all stages were analyzed together for SVIs and for SPAD (**Table 4**). Both SVIs and SPAD values were progressively reduced as plants were reaching maturity. However, differences between milk and dough stages were not significant for NDVI and SR in the 2nd year. Furthermore, SPAD values at heading and anthesis did not differ significantly. Significant interactions between genotypes and growth stages were observed for NDVIs and GNDVIs the 1st year and for SPAD values the 2nd year.

Associations between SVI Indices and Photosynthetic Pigments

Correlations between SVIs, SPAD values and photosynthetic pigments during the 1st year and the 2nd year, respectively, are shown at Supplementary Tables S2, S3. There were very strong correlations between SVIs recorded at each growth stage and at different growth stages. SPAD values at milk stage were highly correlated with SPAD values at dough stage. Weaker, although significant correlations were also observed between SPAD values at heading and SPAD values at anthesis and at milk stage. SPAD values at milk and dough stages showed significant correlations with SVIs. The correlations were stronger at dough stage. There were significant correlations between chlorophyll pigments and carotenoids with SVIs with the exception of GNDVI at heading for the 2nd year. Anthocyanin correlations were non-significant or were weak. SPAD values were significantly correlated with chlorophyll pigments and carotenoids, except from SPAD at anthesis.

There were significant correlations between SVIs recorded at milk stage the 1st year with SVIs, SPAD values at milk and

TABLE 2 | Analysis of Variance *F*-values for genotypes, Coefficients of Variation (CV), means, minimum (Min) and maximum (Max) values of grain yield (GRYLD), number of tillers per m^2 (NTLSM), seeds per spike (SPS), volume weight (VW), thousand kernel weight (TKW), plant height (PH), growing degree days to heading (GDDHD) and growing degree days from heading to physiological maturity (GDDPM).

				1st year				
	GRYLD (Kg/ha)	**NTLSM**	**SPS**	**VW (Kg/hl)**	**TKW (g)**	**PH (cm)**	**GDDHD**	**GDDPM**
F genotype	2.443**	4.371***	15.814***	13.973***	20.537***	5.202***	50.912***	–
CV	16.59	14.55	8.20	2.11	6.39	5.10	1.13	–
Mean	1723	252.71	27.60	66.55	23.56	78.93	1275	–
Min	1247	206.79	22.42	62.35	19.24	68.88	1203	–
Max	2126	337.50	38.81	71.05	33.50	87.25	1398	–
				2nd year				
F genotype	1.162	2.772**	14.051***	5.709***	4.010***	5.111***	23.542***	3.128***
CV	25.72	15.00	11.42	2.87	12.19	5.30	1.33	7.47
Mean	1651	258.49	24.00	70.34	27.03	81.30	1360	558.40
Min	995	172.38	18.13	65	21.29	70.73	1296	443.58
Max	2013	312.50	36.53	75	35.35	89.80	1437	606.33
				Combined analysis				
F genotype	1.260	4.679***	25.267***	11.461***	8.868***	6.751***	43.288***	–
F year	0.017	0.288	50.007***	78.109***	13.216***	0.019	540.147**	–
F genotype x year	1.768*	1.919*	2.717***	1.754*	1.307	1.657	2.344**	–
Mean	1685	255.79	25.69	68.57	25.41	80.19	1320	–
CV	21.82	15.62	9.91	2.89	11.70	5.71	1.52	–

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

TABLE 3 | Analysis of Variance *F*-values for genotypes, Coefficients of Variation (CV), means, minimum (Min) and maximum (Max) values for SVIs, SPAD values and photosynthetic pigments.

	1st year	2nd year		1st year	2nd year		2nd year	
	NDVI booting	**NDVI heading**	**NDVI anthesis**	**NDVI milk**	**NDVI milk**	**NDVI dough**	**SPAD heading**	**SPAD anthesis**
F genotype	8.086***	2.464**	2.245**	9.937***	2.042*	2.261**	9.036***	5.447***
CV	4.00	8.39	12.32	27.32	14.72	8.54	3.02	3.26
Mean	0.49536	0.50460	0.41274	0.11633	0.30512	0.28772	55.89	56.42
Min	0.43407	0.44609	0.34481	0.04668	0.23476	0.25044	51.68	51.94
Max	0.55610	0.56472	0.50076	0.25669	0.38853	0.34017	59.82	59.06
	SR booting	**SR heading**	**SR anthesis**	**SR milk**	**SR milk**	**SR dough**	**SPAD milk**	**SPAD dough**
F genotype	6.009***	3.036***	2.171*	8.417***	1.659	2.083*	5.297***	4.144***
CV	6.46	10.25	13.41	7.46	12.24	6.02	14.44	37.07
Mean	3.09	3.16	2.54	1.27	1.95	1.82	45.86	25.96
Min	2.61	2.67	2.12	1.10	1.63	1.67	28.69	10.59
Max	3.68	3.67	3.11	1.70	2.33	2.04	59.51	49.86

	1st year						
	Anthocyanin (g/l)	**Chlorophyll b (g/l)**	**Chlorophyll a (g/l)**	**Carotenoids (g/l)**	**Total chlorophyll (g/l)**	**GNDVI booting**	**GNDVI milk**
F genotype	6.001***	13.956***	14.118***	11.084***	14.208***	15.346***	9.942***
CV	10.40	19.61	17.47	13.65	17.62	2.72	4.92
Mean	0.004234	0.001045	0.008761	0.004207	0.009806	0.26742	0.21992
Min	0.003419	0.000385	0.003651	0.002515	0.004036	0.24178	0.18053
Max	0.005427	0.001770	0.014283	0.006068	0.016053	0.29556	0.25394
	2nd year						
	Anthocyanin (g/l)	**Chlorophyll b (g/l)**	**Chlorophyll a (g/l)**	**Carotenoids (g/l)**	**Total chlorophyll (g/l)**	**GNDVI heading**	**GNDVI anthesis**
F genotype	2.293**	4.318***	4.945***	3.492***	4.885***	2.440**	2.078*
CV	15.30	27.64	24.46	20.94	24.77	8.36	10.67
Mean	0.003599	0.001121	0.007922	0.002952	0.009044	0.23066	0.21854
Min	0.002999	0.000721	0.005149	0.002026	0.005871	0.19939	0.18468
Max	0.004529	0.001934	0.013303	0.004187	0.015237	0.25480	0.24731

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

dough stages and photosynthetic pigments recorded the 2nd year (Supplementary Table S4). SVIs recorded at booting the 1st year were significantly correlated only with chlorophyll pigments, carotenoids and SPAD values at heading, anthesis and milk stages.

Associations with Agronomic Traits

Significant correlations were obtained between grain yield with NDVIs and GNDVIs at booting the 1st year and with GNDVIs at anthesis the 2nd year (**Table 5**). The correlations were higher the 1st year, when genetic variation in grain yield was also significant.

TABLE 4 | Analysis of Variance *F*-values for genotypes, growth stage, genotype × growth stage, Coefficient of Variation (CV) and means of NDVI, SR, GNDVI and SPAD values.

	1st year			2nd year			
	NDVI	**SR**	**GNDVI**	**NDVI**	**SR**	**GNDVI**	**SPAD**
F genotype	9.012***	3.363***	9.224***	4.866***	4.434***	3.282***	4.937***
F growth stage	5302.67***	2011.58***	494.78***	316.69***	267.18***	11.91**	240.90***
F genotype x stage	3.324***	1.425	1.775*	0.263	0.433	0.222	1.514*
CV	10.69	11.67	5.51	14.02	14.74	10.92	18.91
Mean	0.30585	2.18	0.24367	0.37755	2.37	0.22460	46.03

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

TABLE 5 | Pearson correlations between SVIs and grain yield at different growth stages.

Year	Growth stage	Correlations
1st year	NDVI booting	0.526*
	GNDVI booting	0.564**
	SR booting	0.461
	NDVI milk stage	0.418
	GNDVI milk stage	0.419
	SR milk stage	0.384
2nd year	NDVI heading	0.426
	GNDVI heading	0.318
	SR heading	0.410
	NDVI anthesis	0.438
	GNDVI anthesis	0.464*
	SR anthesis	0.413
	NDVI milk stage	0.402
	SR milk stage	0.382
	NDVI dough stage	0.361
	SR dough stage	0.346

*$p < 0.05$, **$p < 0.01$, $n = 20$.

According to stepwise regression results, GNDVI at booting and at anthesis explained 31.8 and 21.5% of grain yield variability for the 1st year and the 2nd year, respectively. Standardized beta coefficients were positive in both cases (**Table 6**).

The correlations between NDVIs at different growth stages, SPAD values at milk and dough stages, and chlorophyll b with grain yield, from the same set of genotypes grown in different years and locations, are presented in **Table 7**. Negative correlations were obtained between NDVI and grain yield in Dromolaxia for two consecutive years. Negative correlations between NDVI and grain yield were also observed in Achelia for one year while for the other year, NDVI was positively correlated. The best recording stage varied with experiment. Significant negative correlations were also obtained between SPAD values and chlorophyll b with grain yield, although in most cases were weaker than the NDVI.

Principal Component Analysis was conducted to investigate the combinations of traits that best explained the variability. The first three PCs explained 78.46 and 79.97% of the total variance during the 1st and 2nd years, respectively (**Table 8**). For both years, the PC1 was strongly and positively associated with chlorophyll b, carotenoids, anthocyanin, SVIs at milk stage, growing degree days to heading and volume weight. The associations of SVIs recorded at earlier stages with PC1 were weaker, especially the 2nd year. Grain yield was positively related to PC1 the 1st year and to PC2 for both years. Strong and positive associations with PC2 were also observed for number of tillers per m^2 for both years and weaker for SVIs at booting, heading and anthesis, and plant height. Growing degree days to heading were negatively related to PC2 the 1st year. Growing degree days from heading to physiological maturity were positively related to PC2 the 2nd year.

TABLE 6 | Stepwise regression between grain yield and anthocyanin, carotenoids, chlorophyll b, NDVI and GNDVI at booting and milk stage for the 1st year and anthocyanin, carotenoids, chlorophyll b, SPAD at milk and dough stages, NDVI at heading and milk stage and GNDVI at heading and anthesis for the 2nd year.

Year	Variable enter	Model R^2	Standardized Beta	*F* Change	Probability
1st year	GNDVI booting	0.318	0.564	8.384	0.010
2nd year	GNDVI anthesis	0.215	0.464	4.938	0.039

Implications with Phenology

In order to examine the implications of plant phenology in the ability of NDVI to predict yield, Pearson correlations between grain yield and NDVI were plotted against the correlations between growing degree days to heading and NDVI for each year and recording stage (**Figure 3**). Each point represents the correlations when all genotypes were taken into account, and when the two and four late heading genotypes were excluded. There were significant correlations between NDVI measurements and grain yield at all growth stages and years when the two and the four late heading genotypes were excluded. The correlations between grain yield and NDVI were stronger when the correlations between NDVI with growing degree days to heading were weaker.

DISCUSSION

There is growing interest for using SVIs derived by UAV imagery as a fast and cost efficient tool for plant phenotyping. The development of such tools is of paramount importance to continue progress through plant breeding, especially in drought prone and heat stressed environments where climate change is expected to increase yield uncertainty. Studies conducted under Mediterranean environment are limited and the intent of the present study is to elucidate the usefulness of such tools under these harsh environmental conditions.

Genotypic Effects

Grain yields were similar (Aparicio et al., 2000; Gutierrez et al., 2010; Lobos et al., 2014) or lower from average yields reported in experiments under stressed conditions for rainfed cereal crops (Babar et al., 2006c; Lopes and Reynolds, 2012; Bowman et al., 2015; Gizaw et al., 2016a) indicating the severe stress that plants experience during their growing cycle.

The significant differences between genotypes for SVIs are in line with previous reports (i.e., Aparicio et al., 2000; Babar et al., 2006c; Prasad et al., 2007b; Gutierrez et al., 2010). The differences in the present study were more profound during the 1st year. Regarding the 2nd year, the discriminating ability of SVIs was affected by the higher experimental error due to the unusual drought conditions during the vegetative stage. Soil heterogeneity becomes more apparent under drought conditions (Masuka et al., 2012) increasing the experimental error and undermining field screenings, including phenotyping with SVIs (Zaman-Allah et al., 2015).

During the 1st year, there was less variation among genotypes for NDVI and SR at booting. This is in agreement with the

TABLE 7 | Pearson correlations between NDVI at different stages, SPAD at milk and dough stages and chlorophyll b with grain yield from the same set of genotypes grown at different years and locations (DR12, Dromolaxia 2011/12; AX12, Achelia 2011/12; ATH13, Athalassa 2012/2013; DR13, Dromolaxia 2012/13; AX13, Achelia 2012/13; ATH14, Athalassa 2013/2014).

	DR 12	AX12	ATH13	DR13	AX13	ATH14
NDVI booting ATH13	ns	0.557*	–	ns	ns	ns
NDVI milk ATH13	ns	ns	–	–0.450*	–0.478*	ns
Chlorophyll b ATH13	ns	ns	–	ns	–0.491*	ns
NDVI heading ATH14	–0.615**	ns	ns	–0.536*	ns	–
NDVI anthesis ATH14	–0.547*	ns	ns	–0.574**	ns	–
NDVI milk ATH14	–0.537*	ns	ns	–0.653**	ns	–
NDVI dough ATH14	–0454*	ns	ns	–0.660**	–0.449*	–
SPAD milk ATH14	ns	ns	ns	ns	–0.613**	–
SPAD dough ATH14	ns	ns	ns	–0.543*	–0.641**	–
Chlorophyll b ATH14	ns	ns	ns	–0.569**	–0.566**	–

*ns (not significant), *$p < 0.05$, **$p < 0.01$, $n = 20$.*

findings of Royo et al. (2003), Babar et al. (2006b), and Prasad et al. (2007a). The maximum Leaf Area Index (LAI) for wheat grown under Mediterranean conditions occurs at booting. The usefulness of SR and NDVI for estimating grain yield and other important agronomic traits is limited to LAI values lower than 3 to 4 (Aparicio et al., 2000, 2002). Contrary, GNDVI explained more variation at booting during the 1st year indicating that it is less affected by high LAI values. Gitelson et al. (2002) reported that R_{red} sensitivity was at least three times lower than R_{green} when vegetation fraction was more than 60%, thus vegetation indices using green wavelength are likely to perform better at high LAI values.

The variability explained by the growth stage was much higher than the variation explained by genotypes for SVIs and SPAD values, as deduced in **Table 4**. These findings are in agreement with the results of Aparicio et al. (2002), Bort et al. (2005), Babar et al. (2006b,c), and Prasad et al. (2007b). SVI mean values progressively reduced from booting to dough stage as was shown in previous studies (i.e., Babar et al., 2006a; Prasad et al., 2007a; Gizaw et al., 2016b). The non-significant reduction from milk to dough stage in this study is justified by the fact that severe leaf senescence was present when plants were at milk stage.

Previous studies reported significant interactions between genotypes and growth stages under irrigated and stressed

TABLE 8 | Pattern matrix of the PCA analysis.

Pattern matrix							
	1st year				2nd year		
	PC1	PC2	PC3		PC1	PC2	PC3
Chlorophyll b	0.963			Carotenoids	0.938		
Carotenoids	0.952			Chlorophyll b	0.933		
NDVI milk	0.921			Anthocyanin	0.887		
GNDVI milk	0.895			VW	0.837		
Anthocyanin	0.861		0.313	SPAD milk	0.784		
GDDHD	0.781	–0.389		NDVI milk	0.625	0.360	–0.356
VW	0.778		0.383	NTLSM		0.924	
GNDVI booting	0.716	0.370	–0.382	GRYLD		0.849	
NDVI booting	0.657	0.478		PH		0.764	
NTLSM		0.917	0.322	GDDPM	0.560	0.679	
GRYLD	0.417	0.645		GNDVI heading		0.332	–0.784
PH	–0.377	0.452		TKW	0.520		0.765
SPS			–0.888	GDDHD	0.561		–0.699
TKW	0.491		0.682	NDVI heading	0.336	0.416	–0.623
				GNDVI anthesis	0.361	0.473	–0.586
				SPS			–0.584
Cumulative variance (%)	50.01	66.52	78.46	Cumulative variance (%)	46.56	66.33	79.97

PCA was based on agronomic traits, anthocyanin, carotenoids, chlorophyll b, NDVI and GNDVI at booting and milk stage for the 1st year and on the agronomic traits, anthocyanin, carotenoids, chlorophyll b, SPAD at milk stage, NDVI at heading and milk stage and GNDVI at heading and anthesis for the 2nd year. GRYLD, Grain yield; NTLSM, number of tillers per m^2; SPS, seeds per spike; VW, volume weight; TKW, thousand kernel weight; PH, plant height; GDDHD, growing degree days to heading; GDDPM, growing degree days from heading to physiological maturity.

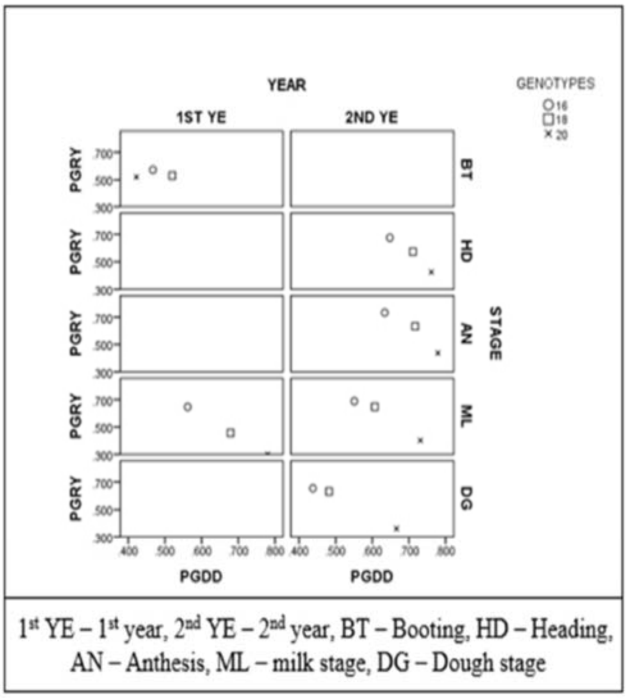

FIGURE 3 | Correlation coefficients between growing degree days to heading with NDVI (PGDD) plotted against coefficients between grain yield and NDVI (PGRY) for each year and recording stage. Each point represents the correlations when all genotypes were taken into account (*n* = 20), when the two late heading genotypes were excluded (*n* = 18) and when the four late heading genotypes were excluded (*n* = 16).

conditions (Babar et al., 2006b,c; Prasad et al., 2007b; Gutierrez et al., 2010; Gizaw et al., 2016a). Those authors pointed out that the interactions of growth stages and indices indicate that care must be taken to identify a suitable growth stage at which the indices will be applied to discriminate most effectively among genotypes in breeding trials. In the present study, the high correlation between SVIs recorded at different stages in the 2nd year is consistent with the non-significant interactions between growth stage and genotypes. During the 1st year, the correlations between SVIs recorded at booting and milk stage were weaker, justifying the existence of significant interactions. The interactions observed during the 1st year can be attributed to the noise induced to the data from the 1st recording stage at booting, when LAI values were at maximum. SR is less affected by the saturation effect of LAI greater than 3 compared with NDVI (Serrano et al., 2000; Aparicio et al., 2002) which might explain the lack of significant interactions for SR. Aparicio et al. (2002) reported significant interactions between genotypes and recording stage for NDVI but not for SR. Montazeaud et al. (2016) stated that NDVI saturation is not easily attained in the rainfed conditions of low yielding environments. During the 2nd year, measurements were taken at heading and onward, when NDVI saturation effect becomes less significant, reducing the noise in the data. These results indicate that, under severe stress, and for SVI measurements taken after heading, the interactions between growth stage and genotypes are likely to be low or non-significant.

Associations between SVI Indices and Photosynthetic Pigments

Several authors stressed the strong relationship between SVIs (i.e., Bort et al., 2005; Gizaw et al., 2016b). Previous studies reported associations between SVIs recorded at different growth stages in the same environment and between SVIs recorded at different environments under more favorable (Babar et al., 2006b; Prasad et al., 2007b) and stressed conditions (Babar et al., 2006c). The results of this study are in agreement with the previously mentioned observations.

The strong positive correlations between SVIs with SPAD values and photosynthetic pigments confirm the close associations between SVIs and canopy greenness. Serrano et al. (2000) also observed significant correlations between NDVI/SR and chlorophyll a. Non-significant or very weak correlations were observed between SVIs and SPAD values at heading and anthesis, contrary to the significant positive correlations at milk and dough stages, due to SPAD values above

50, which are less reliable (Minolta SPAD502 plus manual[1]). This is further justified by the weak or non-significant correlations between SPAD values at heading and anthesis with the values recorded at milk and dough stages. Previous studies found non-significant or very weak correlations between SPAD and NDVI measurements for bread and durum wheat under Mediterranean conditions (Yousfi et al., 2016) or negative correlations for maize (Liebisch et al., 2015). Contrary, in the present study, positive significant correlations were found between SPAD values with SVIs at both milk and dough stages. This is in line with the significant positive correlations between SPAD values and photosynthetic pigments. Similarly, Babar et al. (2006a) found positive correlations between reflectance spectral indices (RARS), which are associated with photosynthetic pigments and SPAD measurements.

Associations with Agronomic Traits

The significant correlations between grain yield and SVIs are in agreement with previous studies proposing SVIs as a mean for estimating important traits such as grain yield under heat and/or drought conditions (Aparicio et al., 2000; Bort et al., 2005; Gutierrez et al., 2010; Lobos et al., 2014; Tattaris et al., 2014, 2016; Bowman et al., 2015; Zaman-Allah et al., 2015; Gizaw et al., 2016a,b). The weak or non-significant correlations of grain yield with SPAD values and photosynthetic pigments confirm the superiority of SVIs compared to SPAD measurements as predictors of grain yield under stressed conditions (Lopes and Reynolds, 2012; Yousfi et al., 2016).

Other authors postulated that SVIs are likely to be more successful under moderate rather than under severe stressed conditions (Babar et al., 2006c), where genotypes are able to express their yield potential (Royo et al., 2003). These studies were conducted with hand-held sensors. Gonzalez-Dugo et al. (2015) reported non-significant correlations between grain yield and vegetation indices under Mediterranean conditions, derived by hyper spectral camera mounted on manned aircraft. In their study, there was only one sampling date during the critical period of grain filling. Our results indicate that SVIs derived by UAV imagery are likely to be useful in severe stressed Mediterranean conditions, with average grain yield as low as 1700 Kg/ha. In drought stressed conditions, small variability in soil depth and texture have increasingly large effects on variability (Ceccareli and Grando, 1996), thus whole plot measurements derived by UAV imagery are likely to be more representative than hand-held measurements. This also justifies the higher correlations between SVIs and grain yield compared with SPAD values and photosynthetic pigments. Multiple sampling dates are necessary from booting to physiological maturity since significant correlations with grain yield might be obtained only in one growth stage that can vary with experiment. The need for multiple measurements during the crop cycle was already stressed by several authors. For example, repeated measurements on the same genotypes over different growth stages accumulate information on the respective health of genotypes through time, thus average values across growth stages can give better predictions of yield (i.e., Babar et al., 2006c; Prasad et al., 2007b; Gizaw et al., 2016b).

An association between indices measured in one site and the yield of the same genotypes in another site, would mean that the indices could be used to predict yield in diverse environments. Significant correlations between indices measured in one environment and yield measured in a different environment were previously reported (Bort et al., 2005; Gutierrez et al., 2010; Gizaw et al., 2016a). In the present study, both positive and negative significant correlations were observed between NDVI and grain yield for the same set of genotypes grown at different environments (**Table 7**). Negative correlations between NDVI and grain yield were observed when negative correlations between grain yield and growing degree days to heading were recorded. Positive correlations were observed when the correlations between grain yield and growing degree days to heading were non-significant (data not shown). The majority of the previous studies reported positive relations between grain yield and vegetation indices. For example, Lobos et al. (2014) and Gizaw et al. (2016b) reported positive correlations between NDVI and grain yield under sever water stress, and non-significant correlations between grain yield and days to heading. However, negative correlations were reported under severe stress conditions, where negative correlations between SVIs and grain yield coexisted with negative correlations between days to heading and grain yield (Lopes et al., 2014; Rutkoski et al., 2016). Early maturing genotypes are likely to be more productive in stressed environments (Bort et al., 2005). The superiority of early maturing genotypes in their study justified the negative associations between NDVI and SR at the latest recording stage. They concluded that the changes in the values and the signs of the correlations between grain yield and reflectance indices reflect genotypic differences in response to high temperature and drought during late grain filling. The results of the present study are in agreement with their findings.

The variation explained by the first three PCA components was similar to the variation recently reported by Gizaw et al. (2016b). The first component was highly correlated with SVIs and volume weight for both years. Other studies showed less consistent correlations between SVIs and volume weight. For example, Arguello et al. (2016) reported volume weight and NDVI in the same clustering of a PCA analysis conducted under water logged conditions, but not under normal conditions. Gizaw et al. (2016b) did not find any close association between volume weight and vegetation indices. In the present study, the consistent correlations between SVIs and volume weight can be attributed to the fact that late heading genotypes had higher volume and higher SVI values. Number of tillers per m^2, plant height, thousand kernel weight and number of seeds per spike, were less correlated to SVIs. Previous studies also reported lower and inconsistent relations between spectral reflectance indices and the above mentioned agronomic traits (Aparicio et al., 2002; Babar et al., 2006b; Lobos et al., 2014; Gizaw et al., 2016b).

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[1] https://www.konicaminolta.com/instruments/download/catalog/color/pdf/spad502plus_catalog_eng.pdf

Implications with Phenology

The implications between plant phenology and SVIs can affect the correlations between SVIs and agronomic traits, particularly grain yield (Lopes and Reynolds, 2012; Tattaris et al., 2016). Principal component analysis showed a consistent strong correlation between SVIs and growing degree days to heading, as has been previously reported (Lopes et al., 2014; Lobos et al., 2014; Elazab et al., 2015; Gizaw et al., 2016b). Plant phenology affected the ability of SVI to predict yield, as it is deduced by the negative trend between correlations of NDVI with grain yield and correlations between NDVI with growing degree days to heading.

Rate of senesce, estimated as the slope of the NDVI decay against thermal time, and stay green, as an estimation of NDVI at physiological maturity, can give an independent measurement of stay green without the confounding effect of phenology (Lopes and Reynolds, 2012). In the present study, the rate of senescence and stay green were not related with grain yield (data not shown). On the contrary, NDVI values at the intercept of the slope with the *Y* axis, which estimates NDVI values at the end of booting-beginning of heading, were significantly and positively correlated with grain yield ($r = 0.583$, $p < 0.01$). The correlations were similar when the four late heading genotypes were excluded ($r = 0.545$, $p < 0.05$). The intercept NDVI was not correlated with growing degree days to heading implying that it is not related with phenology. Montazeaud et al. (2016) reported positive correlations between grain yield and maximum greenness, as estimated by NDVI measurements. Maximum greenness coexists with booting, when water is relatively available and the temperatures are still not high.

In stressed environments, biomass accumulation before heading is associated with grain yield as it is related to carbohydrate remobilization to grain during the grain filling stage (Villegas et al., 2001). Significant correlations between SVIs and biomass have been reported (Aparicio et al., 2002; Babar et al., 2006a). The positive correlation with grain yield that was observed for GNDVI recorded at booting the 1st year, and for intercept NDVI at the 2nd year might be associated with genotypes that manage to accumulate high biomass before heading. The SVI measurements at this stage were independent of phenology as it is shown by the non-significant correlations with days to heading. SVI measurements at later stages were depended from phenology and they were not associated with grain yield because late heading genotypes had higher SVI values, but they were less productive. Lopes et al. (2014) confirmed that NDVI measurements after booting are related to plant greenness and selecting for high NDVI after booting, late flowering genotypes will be selected which are low yielding. When late heading genotypes were excluded, high positive correlations were obtained implying that within a narrower range of heading, stay-green genotypes were more productive. In environments where days to heading were negatively associated with grain yield, significant negative correlations were obtained between NDVIs and grain yield. SVIs are predictors of canopy greenness (Aparicio et al., 2000), thus early maturing genotypes were associated with low SVI values.

The results of the present work highlight the potential use of SVIs derived by UAV imagery for durum wheat phenotyping under low yielding Mediterranean conditions. The optimum recording stage varied with experiment. The ability of SVIs as yield predictors was affected by plant phenology. The implications between plant phenology and SVIs derived by UAV imagery should be investigated in future studies, employing parameters that are less related to plant phenology. Other indices, such as water indices (Babar et al., 2006c; Gutierrez et al., 2010) and/or RGB indices (Elazab et al., 2015; Vergara-Diaz et al., 2016) were found to be superior compared to SVIs in field phenotyping. Additional research should be conducted in the future, addressing the performance of these indices derived from UAV imagery.

AUTHOR CONTRIBUTIONS

ACK and AK carried out the design of the experiment. ACK conducted the field and laboratory measurements. DS and VV carried out the UAV flights and processed the aerial imageries. GM contributed to the data analysis. ACK analyzed the data and wrote the paper under the supervision of AK and with contributions from all the other authors.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fpls.2017.01114/full#supplementary-material

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