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PHYSICAL AND HYDRATION PROPERTIES OF CHICKPEA AND LENTIL SEEDS:

Characterization of different varieties grown under diverse environmental conditions

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Student:
SARA BONELLI

Supervisor:
PROF. ELENA BITOCCHI

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ABSTRACT

Legumes species can be used for multiple purposes such as forage, grain, bloom, pharmaceutical, industrial and green manure. Pulses, such as lentils, beans, peas and chickpeas, play a crucial role in fulfilling the nutritional requirements of the growing population and can be processed in different ways. The use of genetic resources in breeding programs aims to improve the quality of food products from both a nutritional and technological point of view.

We analyzed seed quality of 24 and 41 different genotypes of chickpea and lentil, respectively; seeds used for the phenotypic characterization were obtained in designed experimental field trials (with three replicates), to allow estimation of the variance components and trait heritabilities. The following qualitative traits were considered: seed weight, size, volume, seed coat, hydration, shape and color. The characterization of such materials is essential to provide useful information on the traits of different accessions to allow their exploitation in breeding to develop novel varieties carrying interesting features such as improved seed quality.

High variability among the analyzed accessions was highlighted for all traits indicating the possibility to use the most interesting genotypes as sources for breeding. At the same time, high heritability estimates were observed, indicating that the environmental effect is minimal on characters and the possibility for breeding to make progress in selection. Correlations among traits were also found reflecting important trade-off that producers need to take into consideration during processing. Moreover, we test the potential genotype-by-environment interaction (GEI) which is another important pre-requisite for recommendation of selection for large-scale production.

Performing qualitative analyses, we obtained data that can be exploited for a wide range of sectors such as canning industry, pasta processing, baked good industry or for the preparation of ready to eat products. In particular, the use of legume flours for the production of pasta is an innovative and practical solution to increase the consumption of proteins of vegetable origins. The methods used in this work can be exploited for larger and different legume populations and can be extended to several aspects. The use of the multispectral imaging technique has been proven to be useful in providing important information about the seed quality traits and phenotyping parameters in the study of various morphological traits of different varietal seeds.

RIASSUNTO

I legumi possono essere utilizzati per molteplici funzioni come foraggio, grano, fioritura, concime verde, a livello industriale e farmaceutico. Legumi quali lenticchie, fagioli, piselli e ceci, svolgono un ruolo cruciale nel soddisfare le esigenze nutrizionali della popolazione in crescita e possono essere lavorati in diversi modi. L'uso di risorse genetiche in programmi di allevamento mira a migliorare la qualità dei prodotti alimentari dal punto di vista nutrizionale e tecnologico.

In questo studio, abbiamo analizzato la qualità dei semi di 24 e 41 diversi genotipi di cece e lenticchia, rispettivamente; i semi utilizzati per la caratterizzazione fenotipica sono stati ottenuti da prove sperimentali progettate su campo (con tre repliche), per stimare le componenti della varianza e dell'ereditarietà dei tratti. Sono stati considerati i seguenti tratti qualitativi: peso, dimensione, volume, mantello, idratazione, forma e colore. La caratterizzazione di tali materiali è essenziale per fornire informazioni utili sui tratti delle diverse accessioni per lo sviluppo in allevamento di nuove varietà con caratteristiche interessanti come una migliore qualità del seme.

È stata evidenziata un'elevata variabilità tra le accessioni analizzate per tutti i tratti che indicando la possibilità di utilizzare i genotipi più interessanti come fonti di riproduzione. Allo stesso tempo, sono state osservate stime di elevata ereditabilità che indicano che l'effetto ambientale è minimo sui caratteri e la possibilità per l'allevamento di progredire nella selezione. Sono state trovate anche correlazioni tra i caratteri che riflettono un importante compromesso che i diversi produttori devono tenere in considerazione durante la produzione. Inoltre, abbiamo testato la potenziale interazione genotipo-ambiente (GEI), un altro importante prerequisito per la raccomandazione di selezione per la produzione su larga scala.

I dati ottenuti dalle analisi qualitative possono essere sfruttati per una vasta gamma di settori come l'industria conserviera, dei prodotti da forno, la lavorazione della pasta o per la preparazione di prodotti pronti. In particolare, l'utilizzo di farine di legumi per la produzione della pasta è una soluzione innovativa e pratica per aumentare il consumo di proteine vegetali. I metodi utilizzati in questo lavoro possono essere sfruttati per popolazioni più grandi e differenti e possono essere estesi a diversi aspetti. La tecnica di immagine multispettrale si è dimostrata utile nel fornire informazioni importanti sui tratti di qualità del seme e sui parametri di fenotipizzazione nello studio di vari tratti morfologici di diversi semi varietali comuni.

CHAPTER 1
INTRODUCTION

1.1 **Legumes**

Legumes are plants belonging to the Fabaceae (or Leguminosae) family and represent one of the most extensively consumed food in the world. With close to 770 genera and over 19,500 species, the family is the third-largest land plant family for number of species, behind only the Orchidaceae and Asteraceae (Lewis et al. 2005; LPWG 2013). Economically, Leguminosae is second in importance only to Poaceae.

Recently, The Legume Phylogeny Working Group (LPWG) proposed a new subfamily classification of Leguminosae based on a taxonomically comprehensive phylogeny, with six subfamilies: a recircumscribed Caesalpinioideae DC., Cercidoideae Legume Phylogeny Working Group (stat. nov.), Detarioideae Burmeist., Dialioideae Legume Phylogeny Working Group (stat. nov.), Duparquetioideae Legume Phylogeny Working Group (stat. nov.), and Papilionoideae DC (**Figure 1.1**).

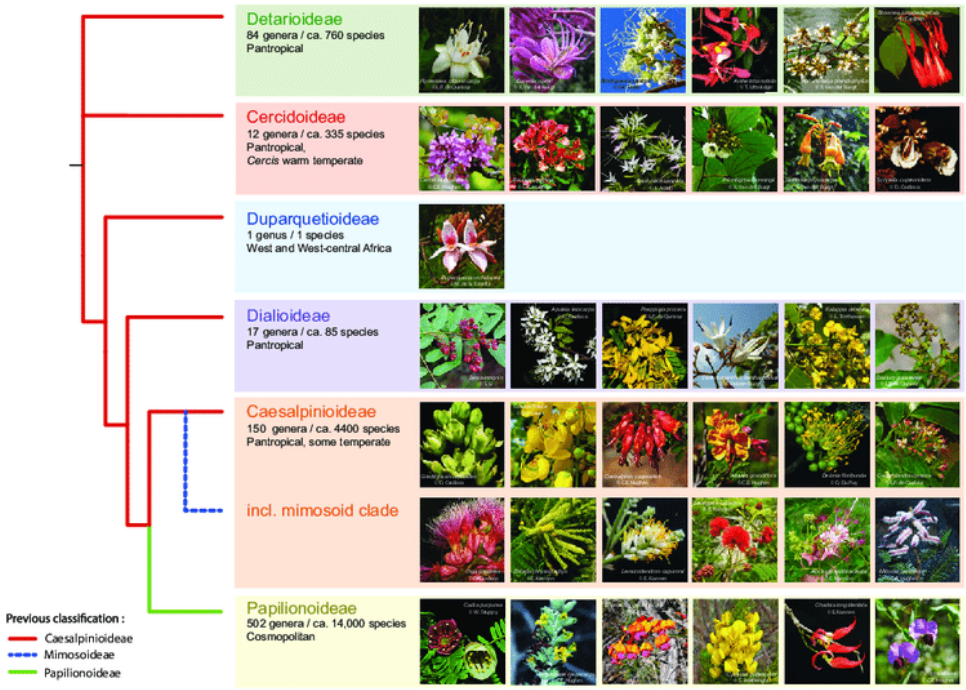


Figure 1.1 – New subfamily classification of the Leguminosae (source LPWG 2017).

Several Leguminosae species were domesticated by humans, independently in different parts of the world: mung bean (*Vigna radiata*), soybean (*Glycine max*), pigeon pea (*Cajanus cajan*) were domesticated in Asia, while cowpea (*Vigna unguiculata*) and Bambara groundnut (*Vigna subterraneanea*) in Africa. *Phaseolus* species, such as common bean (*Phaseolus vulgaris*), lima bean (Phaseolus lunatus), runner bean (Phaseolus coccineus), tepary bean (Phaseolus acutifolius) and year bean (*Phaseolus dumosus*), as well as peanut (*Arachis hypogea*) and Andean lupin (*Lupinus mutabilis*) were domesticated in the Americas. Finally, the cradle of cultivated lentil (*Lens culinaris*), faba bean (*Vicia faba*), chickpea (*Cicer arietinum*), pea (*Pisum sativum*) and white lupin (*Lupinus albus*) was the Fertile Crescent. Almost all the domesticated species belong to the Papilionoideae group.

Legumes play multiple roles, they can be used as forage, grain, bloom, pharmaceutical, industrial, fallow/green manure, and timber species. Most legume crops fill two or more roles simultaneously, depending upon their degree of maturity when harvested. Grain legumes are cultivated for their seeds, which are used for human and animal consumption or to produce oils for industrial uses. The term "pulse" refers to legume species which are cultivated to produce dried seeds. Pulses, such as lentils, beans, peas and chickpeas are a critical part of the general food basket and play crucial role in fulfilling the nutritional requirements of the growing population in a cost-effective manner, especially for developing or underdeveloped countries where animal protein consumption is either limited or expensive (Aguilera et al. 2013). Providing highly nutritious source of protein and bioactive compounds, legumes can greatly benefit the human health. They are recommended by the World Health Organization (WHO) for the management of chronic non-communicable diseases such as diabetes and heart diseases (Hosseinpour-Niazi et al. 2015). The low-fat content and interaction of their sterols have proven effective in maintaining low LDL cholesterols levels and reducing blood pressure (Hosseinpour-Niazi et al. 2015).

At the same time, pulses can play a key role in sustainable crop systems, not only for their role in improving soil fertility, such as by nitrogen fixation through symbiosis with rhizobia (Nulik et al. 2013), but also because, from a nutritional point of view, grain legumes represent a valuable protein source that can substitute animal-based proteins. In this regard, the 2019 report of the International Panel for Climate Change (IPCC) indicated that the plant-based diet is an important opportunity for mitigating climate change, while generating significant co-benefits in terms of human health. In this scenario, food legume crops represent valuable resources for diet change and reduction of environmental impact of protein production (Stagnari et al., 2017).

Legumes are consumed all over the world, especially south Asia and Sub-Saharan Africa, where they provide dietary proteins and are therefore vitally important to the population of less developed countries (Navarro et al. 2014). Pulses are a very good source of proteins for human consumption (Graham and Vance, 2003). Seeds of grain legumes contain at least 20% to 40% of protein. In developing countries of the world, legumes complement cereals or root crops, the primary source of carbohydrates, in terms of amino-acid composition, indeed cereal seed proteins are deficient in Lysin, while legume seed proteins are deficient in sulfur-containing amino acids and Tryptophan (Wang et al. 2003). Legumes contain complex carbohydrates, such as dietary fiber and resistant starch, which, unlike simple sugars, are slowly digested and absorbed by the intestine, favoring their consumption by diabetic individuals (Maria Angeles Martin-Cabrejas, 2019). Dietary fiber is recognized as a very important food component of good daily nutrition. According to Kaczmarczyk et. al 2012, the health benefits of dietary fibers include the prevention and mitigation of type 2 diabetes, cardiovascular diseases and colon cancer.

Legumes are important source of minerals such as Fe, Zn and Ca, and vitamins such as folate; however, the bioavailability is poor due to the presence of some antinutritional factors, such as proteinase inhibitors, lectin, raffinose oligosaccharides, saponins, polyphenols and phytate, that interfere with the absorption of some nutritional compounds (Ann-Sofie Sandberg, 2002). For this reason, legumes seeds are usually processed before being consumed in order to reduce the presence of these antinutritional factors and improve the sensorial characteristics and nutrient availability of the final product. Once inactivated, protein inhibitors may even play a positive nutritional role, due to their high content of sulphur-containing amino acids (Sparvoli et al. 2015). Legumes can be processed in a wide variety of products, depending on the requirements. Processing implies all the operations carried out after harvesting to convert them into primary products. If properly stored, grain legumes can remain in edible condition for several years.

Legumes also contain phenolic compounds, mainly represented by tannins and flavonoids, mostly accumulated in the seed coats where they contribute to the determination of the color. Phenolic compounds are resistant to oxidation and protect cells damage to prevent risk of degenerative diseases (Do Tan Khang et al. 2016).

Representing a basic pillar of human nutrition due to their nutritional and health associated benefits, together with economic and environmental factors crucial in our sustainable future, the Food and Agricultural Organization (FAO) of the United Nations (UN) declared 2016 the International Year of Pulses (IYP) (A/RES/68/231). The aims were to increase public awareness

of the nutritional benefit of pulses as part of sustainable food production aimed towards food security and nutrition, and to promote their importance in mitigating biodiversity loss and climate change. The consumption of vegetable proteins (mainly represented by dried legumes) should be preferred to proteins of animal origin since the global livestock sector accounts for 14,5% of anthropogenic greenhouse gas emission, playing an important role in the climate change (Folloni et al. 2017). The Year created a unique opportunity to encourage connections throughout the food chain that would better utilize pulse-based proteins, further global production of pulses, better utilize crop rotations and address the challenges in the trade of pulses. Because legumes provide numerous benefits, they have gained attention for incorporation in the formulation of functional foods and may act as an affordable ally against malnutrition; they may be considered as a superfood for the future.

1.1.1 Chickpea

Chickpea (*C. arietinum*) is an herbaceous annual plant belonging to the order of Fabales, family Fabaceae, subfamily Papilionoindeae, tribe Ciceraceae, genus *Cicer*. It is the unique cultivated species of genus *Cicer*. It is a self-pollinated and diploid ($2n = 2x = 16$) crop, with a genome size of ~740 Mbp (Varshney et al 2013).

Chickpea is the world's second most grown food legume after common bean (Rawal et al., 2019). In 2018, the global chickpea cultivated area was about 17.81 million ha, with a production of 17.19 million metric tons (FAOSTAT, 2018). Ninty-six% of all production takes place in developing countries, with Asian farmers contributing about 81% of the global production. India is the largest producer, and also the largest importer. The statistics shows that chickpea production has increased compared to the previous years (**Figure 1.2**).

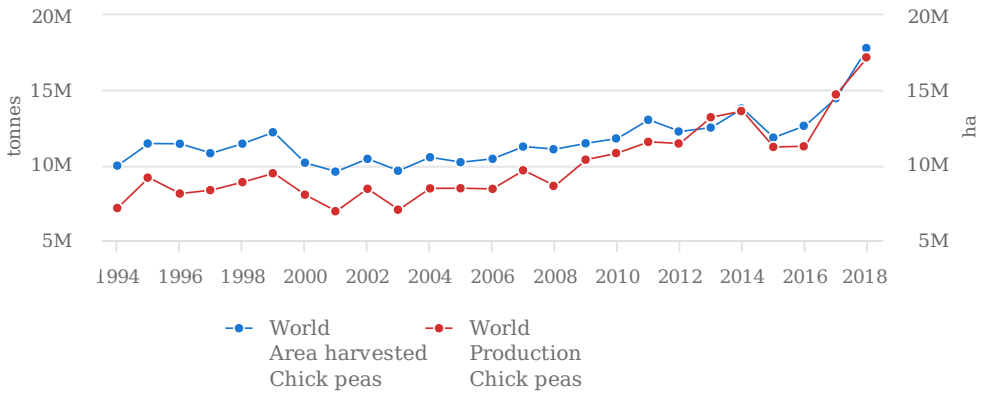


Figure 1.2 – Production/Yield quantities of Chickpea in World 1994-2018 (FAOSTAT).

It was domesticated in the Fertile Crescent, in particular in South-Eastern Turkey (Abbo et al., 2003; Ladizinsky et al., 1976). Since the Bronze Age, chickpea cultivation has spread throughout the Mediterranean Basin, Central Asia, and Africa (Varshney et al., 2019)

An interesting study showed as domestication has an effect in the free tryptophan level present in the seeds, with an increase of it in the domesticated species (*C. arietinum*) compared to the wild forms (*Cicer reticulatum*) (Kerem et al 2007). Dietary tryptophan determines brain serotonin synthesis (Fernstrom and Fernstrom, 1995), which in turn affects certain brain functions and human behavior. According to the authors, this nutritive factor may explain the choice of the early Neolithic farmers of harvesting and domesticate this species.

Two distinct types of chickpea are recognized (**Figure 1.3**): i) *desi* type, characterized by purple flower and colored seed coat. The common seed colors include various shades and combinations of brown, yellow, green and black. Seeds are generally small and angular with a rough surface. Desi types account for 80-85% of chickpea area; ii) *kabuli* type, characterized by white flower and white or beige-colored seed. Compared to desi types, the kabuli types have a thinner seed coat (J. A. Wood et. al 2011), making them more tender and higher levels of sucrose and lower levels of fibers (Serrano et al. 2017). Kabuli types generally have large sized seeds and receive higher market price than desi types. The price premium in kabuli types generally increases as the seed size increases (Gaur *et al*. 2010).

Figure 1.3 – Desi and kabuli type chickpeas.

Chickpea seeds are consumed in a variety of ways with or without seed coat. The nutritional quality can vary depending on the environment, climate, soil nutrition and biology, agronomic practice, stress factors (biotic and abiotic) and industrial processing.

Chickpeas are a good source of carbohydrates and protein, whom quality is considered to be better than other pulses, mineral and vitamins, with a wide range of essential amino acids, making it one of the best nutritionally balanced pulses for human consumption (Jukanti, 2012).

Analyzing a panel of 79 genotypes chickpea, Serrano et al. (2017) found high concentration of tocopherols, mainly γ tocopherol, in particular for desi accessions, that showed also higher carotenoids concentration than kabuli accessions. At the same time the protein and fiber contents of desi types (20.3% and 8.40%, respectively) were higher than those of kabuli accessions (18.2% and 4.56%, respectively).

The physical proprieties of seeds like shape, color, weight and hydration capacity are important attribute of quality influencing consumers acceptance and consequently market premium (DVSSR Sastry *et al.* 2014).

1.1.2 Lentil

Lentil (*Lens culinaris*) is the common name for a small annual plant belonging to the order of Fabales, family Fabaceae, subfamily Papilionoindeae, tribe Vicieae, genus *Lens*. It is a self-pollinated and diploid ($2n = 2x = 14$) species, with a genome size of ~4Gb. It grows as a bushy leguminous plant typically 20-45 cm tall, producing many small purse-shapes pods containing one to two seeds each. Lentils exist as a spectrum of colors, which includes yellow, orange, red, green, brown and black depending on the cultivar, the composition of the seed coat and cotyledon (**Figure 1.5**).

According to the Food and Agriculture Organization statistics report in 2018, the global lentil cultivated area was about 6,1 million ha, with a production of 6,3 million metric tons. Canada and India are the main producers of lentil, followed by Turkey, Bangladesh, Iran, China, Nepal and Syria. As well as for chickpeas, the production has increased in the last years as showed in **Figure 1.4**.

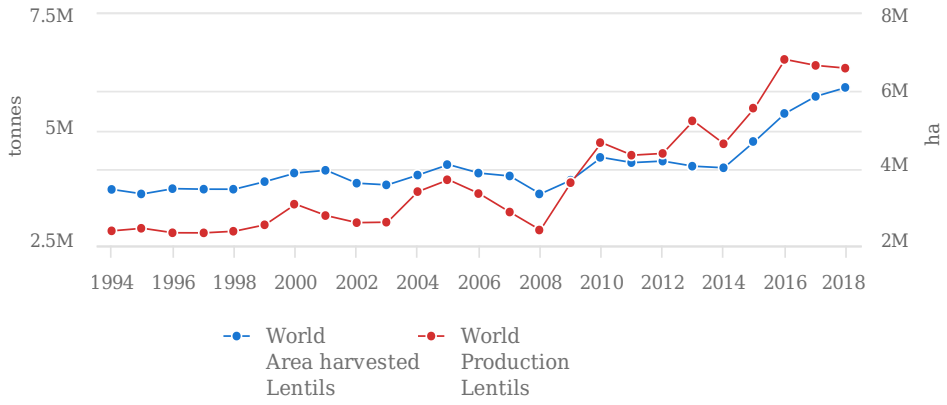


Figure 1.4 – Production/Yield quantities of Lentils in World 1994-2018 (FAOSTAT).

Lens culinaris ssp. *orientalis* is considered to be the progenitor of the cultivated lentil. There is archeological evidence of lentil, dated back to 7.500 – 6.500 BC (Alihan Cokkizgin, 2013). Lentils were among the earliest plant species that have been domesticated by humans and are associated with the start of the 'agricultural revolution' in the Near East. The crop was part of the assemblage of near eastern grains that spread across the Old World until the American continent. Canada is the leading lentil producer and exporter, growing around 40% of world production (FAO 2019).; lentil is commonly used for human nutrition, animal feed and soil fertility. Because of its high average protein content and fast cooking characteristics, lentil is the most desired legume in many regions (Abraham Reda, 2015). Lentils have been classified among soft-coated pulses that require shorter cooking time, and thus have smaller losses in nutrients as compared to those with hard seed coat. For this reason, lentils are considered very convenient for human consumption (Faris et al. 2013). Lentils can be prepared in several methods including soaking, boiling, sprouting/germination, fermentation, frying and dry-heat methods. Other ways to benefit from it, are processed lentil, lentil snacks and medicinal uses. is well known for its lens-shaped edible seed, which has the most significant dietary compositions, containing macro- and micro-nutrients. Seed shape, color, and pattern of lentil are important quality traits as they determine market class and possible end uses (Fedoruk et al. 2013). On the other hand, hydration kinetics studies allow to go deepen on the seed soaking dynamics that is an important aspect for germination, cooking, fermentation and processed products (Kumar et al. 2016)

Figure 1.5 – Variation in seed color of different lentil seeds (Source: Singh Punia et al. 2014).

1.2 Genetic resources and breeding

Biodiversity is the results of four billion years of evolution, in which about 30 million living species, still not all classified today, have been developed on Earth. The word "biodiversity" is thought to have first been coined as a contraction of the term "biological diversity" in 1985 and then popularized by a number of authors. The concept is broad and complex. The Convention on Biological Diversity (CBD), proposed during the Earth Summit in Rio de Janeiro on 5 June 1992, defines biodiversity as "the variability of living organisms of all origins and ecological systems of which they are part: this includes diversity within species, between species and of ecosystems". The CBD provides a universal legal framework to work on biological diversity with the main goal to develop national strategies for the management and sustainable use of biological variability (Jeyabalan et al. 2019). Thus, the genetic diversity and its conservation are of great importance because contributes to food security, intended as the interconnection of food availability, accessibility and utilization and to food nutritional and functional quality. Genetic resources (crops, fishery, livestocks and forestry) have been exploited and eroded due to the ever increasing world population, and nowadays their maintenance takes the breeders' attention for a sustainable food production.

Plant Genetic Resources (PGR) are the set of heritable materials, among and within plant species, resulted from the species evolution (Ogwu et al. 2014). Different plant genetic resources exist: i) *landraces* or *local varieties* that are domesticated local plant varieties that did not undergo an intensive selection during a formal breeding program. They are grown on farm by farmers who reproduce their seeds year after year; this aspect allows them to present a high level of genetic

variation due to the different pressure given by human/natural selection, that make them highly resistant to adverse environmental conditions, although the production yield is not so high; thus, landraces are variable populations where variation can be seen between and within populations (Zeven 1998); ii) *Cultivars* that are (more or less) homogeneous populations derived by a formal breeding program; they are characterized by a one-dimensional genetic structure, where the genetic diversity is between different cultivars, often constituted by a single genotype (pure lines, clones, single-cross hybrids); iii) *Crop Wild Relatives* (CWR) that can be defined as wild plant species that are more or less genetically related to crops, but unlike them, have not been domesticated; iv) *wild species*, that were not domesticated and are used by humans as they are, without undergoing the domestication process; v) *genetic stocks* that are plants or populations generated and/or selected for genetic studies; v) *genomic DNA or DNA libraries* that are used for scientific researches.

1.2.1 Chickpea and lentil genetic resources

Considering the current challenges posed by climate, agriculture and food production, the importance of conservation of plant genetic resources (PGRs) is now becoming imperative, along with their characterisation and use (Mousavi-Derazmahalleh et al., 2019; Mc Couch et al., 2020). The conservation of genetic resources gives to breeders the raw materials needed for the development of new varieties and allows also the modification of their crops in response to environmental changes or new market requests (Ogwu et al. 2014). Generally, the major portion of the genetic diversity of a crop is contributed by the wild relatives, as they did not experience domestication (Diamond, 2002; Glémin and Bataillon, 2009; Gepts, 2010). Landraces are also important repositories of genetic diversity of a crop, as these represent local varieties that have evolved through natural and artificial selection over millennia, without undergoing genetic bottlenecks due to modern breeding (Zeven, 1998), and they have adapted to specific and diversified agro-environmental conditions (Zhu et al., 2000; Bellucci et al., 2013; Bitocchi et al., 2009, 2015; Dwivedi et al., 2016). Thus, landraces and wild relatives harbour functional and adaptive genetic variation that needs to be more easily managed and used.

Such PGRs and their wide diversity therefore need to be maintained as the first step. Germplasm banks can guarantee the conservation *ex-situ* of such biodiversity. Genesys PGR is a free online global portal that allows exploration of plant species diversity through a single website (accessible at www.genesys-pgr.org).

Current lentil cultivars have a narrow genetic base and are challenged with many biotic and abiotic stresses. Diversity of germplasm stored in genebanks is a vital source for discovering

useful genes which serve as a resource for lentil breeding programs. There are currently over 58,000 lentil accessions held in various genebanks worldwide. Genesys displays information for about 70% of these (https://www.genesys-pgr.org/c/lentil). ICARDA, with 12,463 accessions, is the centre with the largest lentil collection

Based on Genesys data (https://www.genesys-pgr.org/welcome) the largest collections of chickpea seeds are maintained at ICARDA and ICRISAT, centers of CGIAR with unique accessions estimated at more than 15,000 and 20,000, respectively. The largest collection of wild materials and derived introgression lines is maintained at UC Davis in California.

1.2.2 Breeding to improve legume varieties

Genetic improvement is one of the most viable strategies to obtain varieties that in addition to having improved agronomic traits, such as yield and resistance to biotic and abiotic stress, also have the best nutritional characteristics to satisfying consumer needs (Bailey-Serres et al., 2019). Exploiting the diversity in plant genetic resources represents a very efficient strategy to increase the nutritional quality of elite varieties. However, to do this, characterization of the plant resources is needed.

Many studies focused on the characterization of the quality of seeds, do not take carefully into consideration the wide variability present within the germplasm of a crop. This is demonstrated by the fact that the majority of literature focused on analysis of morphological and chemical composition of seeds are based on the analysis of very few genotypes (often less than 10 genotypes). Moreover, most of these studies were not based on materials grown in one or more specific field trials that involve replicates. This aspect is crucial, because quality traits are highly influenced by environmental conditions. Indeed, environment strongly influences the quality traits, such as morphological traits, and chemical composition. Li et al. (2018) showed as environmental factors affected head rice ratio, grain length, alkali consumption, and amylose and protein content of rice seeds.

Sehgal et al. (2017) showed that heat and drought resulted in marked reduction in the rate and duration of seed filling to decrease the final seed size of lentil; drought resulted in more damage than heat stress. Combined stresses increased the damage of seed starch, storage proteins and their fractions, minerals, and several amino acids.

Food grain legume can be grown in different array of environments and agronomic techniques that can potentially influence seed quality traits. Further studies are needed in order to identify traits variability in different environments and their related heritability.

1.3 Physical properties of legumes

Among physical properties of leguminous plant seeds, 1000 seed mass, size, color and shape are very important quality parameters. They allow to distinguish between particular species, as well as between varieties within a species.

Seed size and shape determine, in part, the ways in which pulses are prepared as foods, their cooking times and the perceived consumer preferences. It also influences the composition and nutritive value of the seed, because larger seeds will generally have a lower proportion of seed coat (assuming the seeds coat thickness is the same), which is high in fiber-type components. In similar sized seeds, a higher proportion of seed coat implies a lesser portion of cotyledon, which means a reduced protein and starch contents in the whole seeds. Seed size is an important contributor to evolution and ecology of plant species and can also affect agronomic management, because larger seeds are generally more prone to mechanical damages. In addition, a negative relationship between seed size and seed yield exists for most pulse crops (Wood and Harden, 2017). The increase of seed size of diverse legume species was and still is pursued in breeding programs.

Analyzing the physical properties of legume seeds is essential to facilitate and improve the design of the equipment for their harvesting, processing and storage. This analysis gives important information on how crops behave during processing (Ojo Moses and Ade-Omowaye, 2015).

Soaking is the initial stage of most technological processes in the group of leguminous plant seeds. Grains are harvested dry, as dryness is a big advantage to extend shelf-life during storage; therefore, before being consumed or processed, they need to be soaked into water to increase the moisture content. This provides several beneficial effects on their physicochemical and nutritional quality. The amount of water absorbed by seeds during soaking determine the protein denaturation and the degree of starch granule gelatinization during cooking. For this reason, it is considered one of the most important physical characteristics determining good seed texture (Giczewska and Borowska, 2003). The hydration capacity of seeds depends on cell wall structure, composition of seed and compactness of the cells into the seed (Kaur and Singh, 2006) and is directly correlated to seed weight and size, as showed in **Figure 1.6.** Soaking is often, but not always, a prelude to cooking pulse seeds. Hydration and cooking are two separated, but related processes: hydration needs to occur before or during cooking for seed to soften and starch to gelatinize. Softening of seed structure reduces the cooking time, which is an important factor that define the whole cooking quality of pulses (Wood and Harden, 2006).

Figure 1.6 – Dried (left) and hydrated (right) chickpea seed.

Grain legumes are most commonly cooked before being eaten and those that require long cooking times are considered less convenient, more energy consuming and, therefore, less desirable for consumers and processors. Pulse breeding programs are interested in quicker-cooking pulse varieties, however there is no standards method for the evaluation of cooking time (Wood, 2016). Physical properties influence pulse cooking quality. According to Wani et al. (2014), cooking time has significant positive correlation with swelling capacity, porosity and fat content. However, it has significant negative correlations with cooked seed adhesiveness and cooked seed hardness. Cooking time is a heritable characteristic that differs widely among genotypes; long cooking time represents one of the main drawbacks that limit the utilization of legumes (Kaur and Singh, 2006).

CHAPTER 2
MATERIALS AND METHODS

2.1 **Plant materials**

In the present study a total of 65 accessions were analyzed. The set of plant materials includes 24 and 41 genotypes of chickpea (**Table 2.1**) and lentil (**Table 2.2**), respectively. Chickpea accessions consist in 9 landraces, 8 cultivars, and 7 breeding materials. 20 kabuli types and 4 desi types were analyzed. Lentil accessions are mainly landraces (24 landraces, 8 cultivated materials, 7 cultivars and 2 breeding materials).

Row	**Varieties/accession/lines**	**Donor**	**Type**	**Biological status**
1	Ares	Sais	Kabuli	Cultivar
2	Principe	Sais	Kabuli	Cultivar
3	Vittoria	Sais	Kabuli	Cultivar
4	Sultano	Isea	Kabuli	Cultivar
5	Pascia	Isea	Kabuli	Cultivar
6	Reale	Isea	Kabuli	Cultivar
7	Maragia	Isea	Kabuli	Cultivar
8	Ottava	Univpm	Kabuli	Landrace
9	Ituchi	Suba	Kabuli	Cultivar
10	Quercia Appignano	Nazzareno Medei	Kabuli	Landrace
11	Nero Appignano	Nazzareno Medei	Desi	Landrace
12	Palazzo San Gervasio	Unibas	Kabuli	Landrace
13	Nero Tolve	Unibas	Desi	Landrace

14	Tricarico	Unibas	Kabuli	Landrace
15	Filiano bianco	Unibas	Kabuli	Landrace
16	Filiano nero	Unibas	Desi	Landrace
17	Bianco (scalo)	Unibas	Kabuli	Landrace
18	IS-CE-1	Isea	Kabuli	Breeding material
19	IS-CE-2	Isea	Kabuli	Breeding material
20	IS-CE-3	Isea	Kabuli	Breeding material
21	IS-CE-5	Isea	Kabuli	Breeding material
22	IS-CE-6	Isea	Kabuli	Breeding material
23	IS-CE-7	Isea	Kabuli	Breeding material
24	IS-CE-BRUNO	Isea	Desi	Breeding material

Table 2.1 – List of chickpea genotypes used in this study.

Varieties/accession/lines	**Donor**	**Origin**	**Biological status**
Anicia	Agro obtention	France	Cultivar
Crimson	Unibas	Egypt	Cultivar
Elsa	Isea	Italy	Cultivar
Flora	Agro obtention	France	Cultivar
Gaia	Isea	Italy	Cultivar
IG 1959	Unibas	Ethiopia	Landrace
IGP Altamura	Unibas	Italy	Landrace
ILL 11557	Unibas	India	Cultivated material
ILL 213	Unibas	Afghanistan	Cultivated material
ILL 4605	Unibas	United Arab Emirates	Cultivated material
ILL 624	Unibas	Macedonia	Landrace

Itaca	Isea	Italy	Cultivar
Onano	Suba	Italy	Landrace
Palazzo San Gervasio	Unibas	Italy	Landrace
PI 178971 LSP	Unibas	Turkey	Landrace
PI 181771 LSP	Unibas	Lebanon	Landrace
PI 298122 LSP	Unibas	France	Cultivated material
PI 298120 LSP	Unibas	Mexico	Cultivated material
PI 299121 LSP	Unibas	Mexico	Cultivated material
PI 299351 LSP	Unibas	Chile	Cultivated material
PI 426778 LSP	Unibas	Pakistan	Landrace
PI 431622 LSP	Unibas	Iran	Landrace
PI 431663 LSP	Unibas	Iran	Landrace
PI 431710 LSP	Unibas	Iran	Landrace
PI 431714 LSP	Unibas	Iran	Landrace
PI 431717 LSP	Unibas	Iran	Landrace
PI 431728 LSP	Unibas	Iran	Landrace
PI 431731 LSP	Unibas	Iran	Landrace
PI 431739 LSP	Unibas	Iran	Landrace
PI 431753 LSP	Unibas	Iran	Landrace
PI 432002 LSP	Unibas	Iran	Landrace
PI 432033 LSP	Unibas	Iran	Landrace
PI 432145 LSP	Unibas	Iran	Landrace
PI 432245 LSP	Unibas	Lebanon	Landrace
PI 432588 LSP	Unibas	Egypt	Cultivated material
PI 533693 LSP	Unibas	Spain	Cultivar

PI 612875	Unibas	India/Ethiopia	Breeding material
Rossa Tricarico	Unibas	Italy	Landrace
Santo Stefano	Ettore Ciarrocca	Italy	Landrace
Val di Nevola	Sais	Italy	Landrace
W6 27760 LSP	Unibas	USDA	Breeding material

Table 2.2 – List of lentil genotypes used in this study.

2.2 Methods

2.2.1 Field trials

The chickpea accessions were evaluated in two trials carried out in 2019 at Centro di Ricerca Cerealicoltura e Culture Industriali (CREA-CI) of Osimo (Ancona, Italy), in autumn and spring sowing seasons, while the lentil accessions were evaluated in two trials carried out in autumn sowing season in two different localities: Osimo (CREA-CI) and Metaponto (Matera, Italy) (**Table 2.3**). Each field trial was carried out in plots of 10 m^2 with three replicates using a randomized complete block design (**Figure 2.1a, b, c** and **d**). As previously mentioned, the experiments were conducted in two sowing seasons (autumn and spring) for the chickpea genotypes and in two different localities for lentil genotypes (Osimo and Metaponto).

Locality	**Experiment**
Osimo (AN)	Lentil - 10 m^2 plots –Sowing date (6 December 2018)
Osimo (AN)	Chickpea - 10 m^2 plots –Sowing date (6 December 2018)
Osimo (AN)	Chickpea - 10 m^2 plots –Sowing date (18 March 2019)
Metaponto (PZ)	Lentil - 10 m^2 plots –Sowing date (11 December 2018)

Table 2.3 – Field experiments carried out for the set of materials used in the present study and relative sowing date.

	Replicates		
	I	II	III
Anicia	6	64	89
Flora	29	78	94
S. Stefano	11	69	121
Val di Nevola	24	74	99
Onano	15	66	102
Elsa	20	44	112
Gaia	39	62	87
Itaca	32	49	97
PI 533693	3	58	116
PI 431622 LSP	36	53	119
PI 178971 LSP	26	82	110
IG 1959	22	71	108
PI 432245 LSP	18	76	96
PI 432033 LSP	41	80	92
PI 432145 LSP	34	60	90
PI 431739 LSP	8	56	84
PI 431717 LSP	13	51	100
PI 612875	30	47	113
PI 431710	37	42	98
ILL 213	2	70	86
PI 299120 LSP	28	79	123
PI 431731 LSP	19	63	88
PI 472588 LSP	16	50	104
PI 298122 LSP	25	57	106
PI 431714 LSP	31	45	91
PI 299351 LSP	5	73	115
W6 27760 LSP	10	67	122
ILL 11557	33	55	117
ILL 4605	38	46	85
PI 432002 LSP	21	54	101
PI 431753 LSP	7	68	95
PI 431728 LSP	1	65	114
ILL 624	40	59	120
PI 431663 LSP	23	52	107
PI 181771 LSP	27	75	103
Crimson	12	72	83
PI 426778 LSP	17	43	109
PI 299121 LSP	9	48	93
Rossa di Tricarico	35	77	105
Palazzo San Gervasio	4	81	118
IGP Altamura	14	61	111

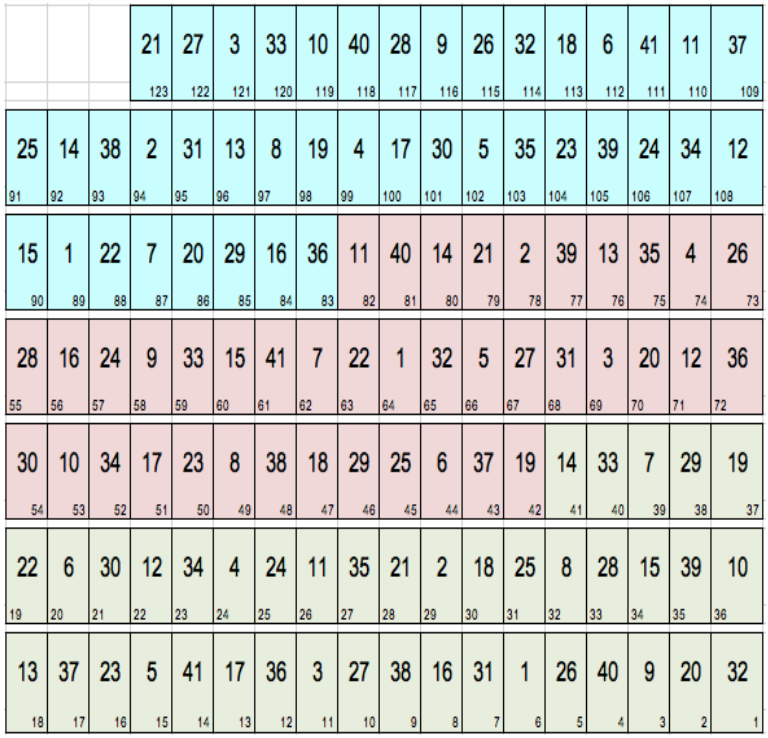


Figure 2.1a – Randomized Complete Block Design used for lentil autumn sowing in Osimo (AN): 41 accessions x 3 replicates for a total of 123 plots.

	Replicates		
	I	II	III
Ares	20	32	68
Principe	6	37	63
Vittoria	15	33	57
Sultano	10	41	52
Pascia	24	36	71
Reale	22	30	54
Maragia	3	40	65
Ottava	17	43	53
Ituchi	13	48	60
Quercia Appignano	8	44	50
Nero Appignano	21	38	58
Palazzo San Gervaso	4	34	62
Nero Tolve	23	42	59
Tricarico	12	26	49
Filiano bianco	18	47	55
Filiano nero	9	29	67
Bianco (scalo)	14	45	70
IS-CE-1	1	39	51
IS-CE-2	16	31	66
IS-CE-3	11	27	61
IS-CE-5	5	25	56
IS-CE-6	2	28	64
IS-CE-7	19	46	72
IS-CE-BRUNO	7	35	69

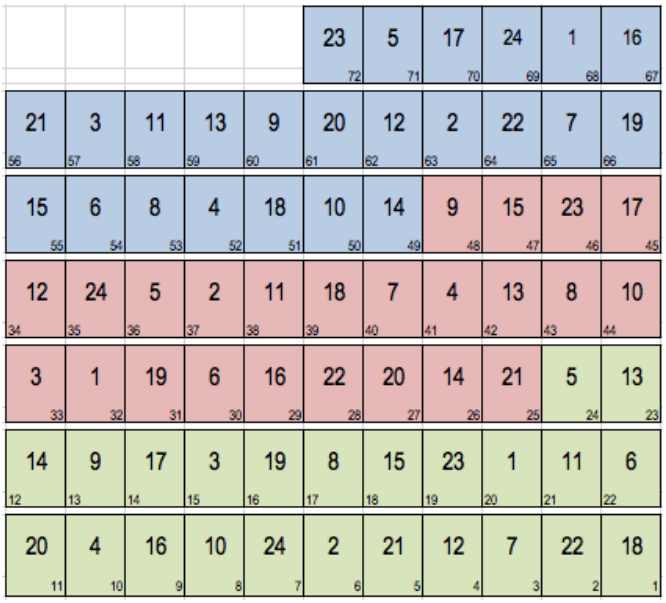


Figure 2.1b – Randomized Complete Block Design used for chickpea autumn sowing in Osimo (AN): 24 accessions x 3 replicates for a total of 72 plots.

	Replicates		
	I	II	III
Ares	15	26	69
Principe	20	34	55
Vittoria	11	46	49
Sultano	24	30	71
Pascia	6	48	66
Reale	13	40	52
Maragia	3	38	60
Ottava	18	36	67
Ituchi	16	42	50
Quercia Appignano	9	44	61
Nero Appignano	4	31	64
Palazzo S. Gervaso	1	37	59
Nero Tolve	22	32	53
Tricarico	8	35	63
Filiano bianco	12	39	57
Filiano nero	19	28	65
Bianco (scalo)	23	33	58
IS-CE-1	7	27	68
IS-CE-2	14	25	54
IS-CE-3	17	29	51
IS-CE-5	10	41	70
IS-CE-6	5	43	56
IS-CE-7	2	45	72
IS-CE-BRUNO	21	47	62

Figure 2.1c – Randomized Complete Block Design used for chickpea spring sowing in Osimo (AN): 24 accessions x 3 replicates for a total of 72 plots.

	Replicates		
	I	II	III
Anicia	36	54	81
Crimson	1	79	86
Elsa	8	45	87
Flora	22	53	109
Gaia	16	59	84
IG 1959	39	47	100
IGP Altamura	25	62	112
ILL 11557	35	56	85
ILL 213	20	80	114
ILL 4605	2	64	113
ILL 624	30	57	91
Itaca	33	63	95
Palazzo San Gervasio	32	75	104
Rossa Tricarico	34	74	101
Onano	13	73	120
PI 178971 LSP	26	41	108
PI 181771 LSP	15	58	102
PI 298122 LSP	11	66	106
PI 299120 LSP	38	50	82
PI 299121 LSP	31	67	111
PI 299351 LSP	21	49	88
PI 426778 LSP	7	77	115
PI 431622 LSP	23	48	99
PI 431663 LSP	12	65	90
PI 431710	17	46	93
PI 431714 LSP	24	72	92
PI 431717 LSP	6	70	110
PI 431728 LSP	18	61	89
PI 431731 LSP	28	68	98
PI 431739 LSP	37	44	94
PI 431753 LSP	5	42	116
PI 432002 LSP	29	43	117
PI 432033 LSP	3	51	107
PI 432145 LSP	27	60	103
PI 432245 LSP	4	76	83
PI 472588 LSP	19	69	96
PI 533693	40	78	119
PI 612875	14	71	105
S. Stefano	9	52	97
W6 27760 LSP	10	55	118

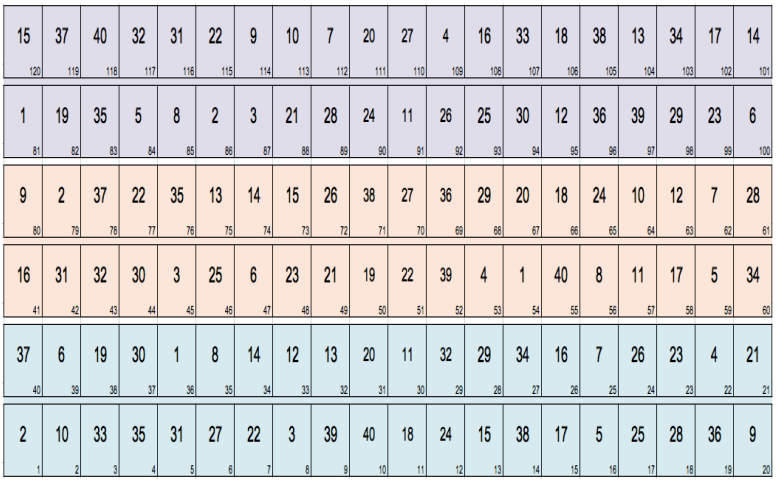


Figure 2.1d – Randomized Complete Block Design used for lentil autumn sowing in Metaponto (MT): 40 accessions x 3 replicates for a total of 120 plots.

The seeds obtained from these field trials were used for seed traits characterization.

2.2.2 Phenotyping

The seed quality was assessed by evaluating the set of different traits listed in **Table 2.4**.

Seed-nutritional quality analyses
1000 Seed Weight
Seed Size Index (SSI)
Seed shape (mm)
Seed volume (ml)
Seed coat weight (testa content) (% s.s.)
Seed coat thickness (mg/mm)
Seed color
Seed hydration (%)
Seed hydration rate

Table 2.4 - Set of evaluated traits.

The analyses have been carried out sampling approximately 200 grams and 100/150 grams of chickpea and lentil seeds, respectively from each plot. The seeds have been cleaned before the analyses.

2.2.2.1 1000-seed weight (g)

Replicated samples of clean seed (broken grain and foreign material removed) were sampled randomly and 1000-seed were counted and weighed.

2.2.2.2 Seed Sieve method to calculate seed size index (SSI)

The standard method for measurement of seed size was applied by using a nested set of sieves (**Figure 2.2**) of different diameter classes (5.0, 5.6, 6.3, 6.7, 7.1, 8.0, 9.0, 9.5 and 10 mm for chickpea; 3.15, 3.35, 3.55, 4.00, 4.5, 5.0, 5.6, 6.3 mm for lentil). A defined quantity of seeds has been sieved for 10 minutes through the sieve column. The SSI was calculated from the weighed mean of seed retained on each sieve, following the method APQ 103, Seed Size Distribution of Pulses Seeds, in the Australian Pulse Quality Laboratory Manual (Burridge et al. 2001)

$$SSI = \sum_{i=1}^{n} P(i) \times S(i)$$

Where *S(i)* is the size (in millimeters) of sieve *i* and *P(i)* is the proportion by weight of seeds on sieve *i*.

Figure 2.2 – Mechanical sieve shaker used to calculate SSI.

A normal probability curve was used to fit the distribution of frequencies of the different diameter classes obtained for each genotype and the average and standard deviation were calculated. The standard deviation gives information about the uniformity of the accessions for seed size: higher is the standard deviation, the greater is the uniformity. yields.

2.2.2.3 Seed volume (ml)

The seed volume has been estimated by randomly selecting and weighting 10 seeds in chickpea and 20 seeds in lentil for each genotype and putting them in a graduated cylinder filled with a known volume of distilled water (10 ml). Volume was recorded in ml from the difference between the initial volume of water and the final volume with seeds.

2.2.2.4 Seed coat

Seed coat analyses were carried out only for chickpea genotypes on 10 seeds for each line. Seeds were dehulled after soaking in distilled water for few minutes at room temperature; the seed coat was then removed by using a scalpel and tweezers and dried in silica gel (**Figure 2.3a and 2.3b)**. Seed coat weight (g) was calculated by subtracting the seed weight without the seed coat from the initial seed weight, while the seed coat content (%) was calculated as the ratio between the seed coat weight and the initial seed weight. The seed coat thickness (mm) was measured with a digital caliper (**Figure 2.3c**).

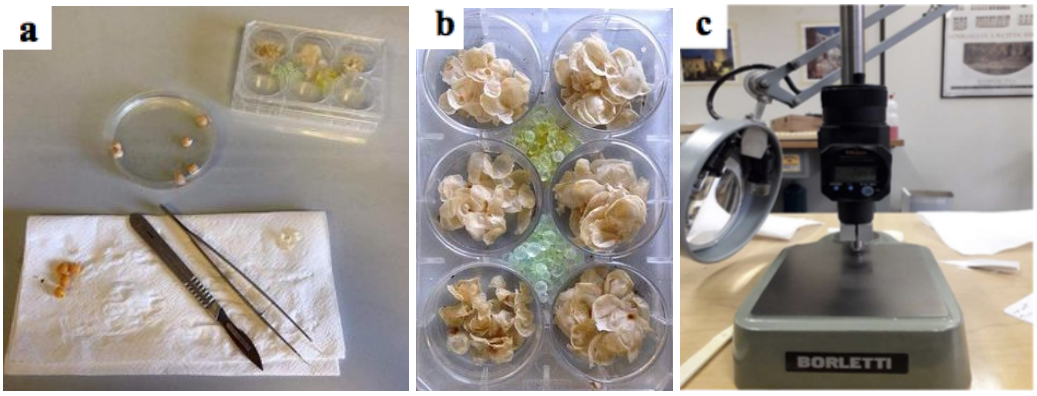


Figure 2.3 – Seed coat analysis, (**a**) seed dehulling; (**b**) dried seed coat in silica gel; (**c**) digital caliper.

2.2.2.5 Seed hydration

The seed hydration capacity has been measured at room temperature. For each accession of chickpeas and lentil genotype, 50 seeds were weighted and placed in a plastic backer containing distilled water as showed in **Figure 2.4**. Different soaking time were applied. At each time, seeds were weighted by removing the water. Weight gain was calculated as follow:

$$Weight\ gain = \frac{Wt - Wo}{Wo}$$

Where: *Wt* is the weight gain recorded at time *t* and *Wo* is the initial weight.

t = time (2h; 4.5h; 7h; 24h for chickpea; 1.5h; 3h; 5h; 24h for lentil).

According to Wood and Harden (2006) the Mitscherlich model with two parameters has been used:

$$WG_t = \alpha(1 - \beta^t)$$

Where *WGt* is the weight gain at time *t*, α is the asymptote of the curve and estimate the max hydration (*Hmax*) and ($1 - \beta$) is the estimation of the rate of hydration (*Hrate*). The larger is the value of ($1-\beta$), the quicker the chickpeas hydrate.

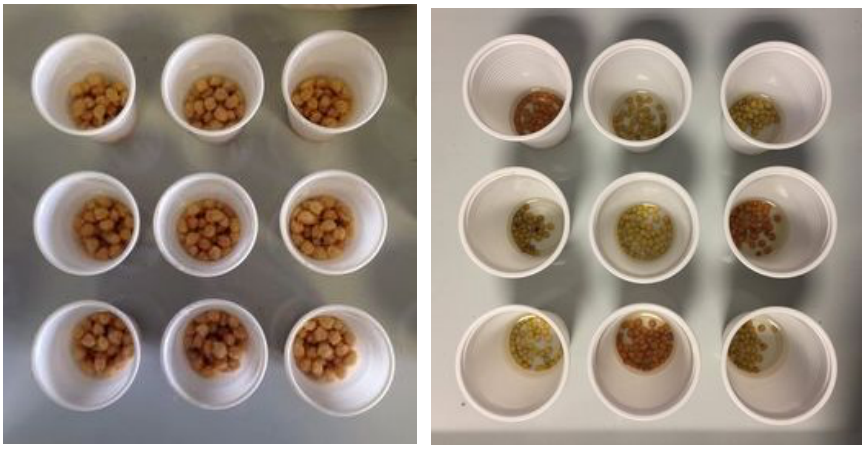


Figure 2.4 – Soaked seed samples for the calculation of the hydration capacity.

The parameters of the mathematical model used were estimated using a nonlinear regression procedure using the JMP8 software. Since its relative simplicity, this model is considered the most appropriate for testing a large number of samples.

2.2.2.7 Image analysis

The image analysis has been conducted with the SpectroCam multispectral camera (**Figure 2.5a**). The camera allows to perform size/shape analysis and multispectral analysis. Images suitable to measure seed size and shape were obtained by using different plastic holders, characterized by wells of different depth and thickness which allow the proper positioning of the seeds (**Figure 2.5b** and **Figure 2.6**). The sample holders were in two different colors, black and white, in order to provide an appropriate contrasting background to highlight the color variation of the seeds. This analysis was carried out for both chickpea and lentil genotypes.

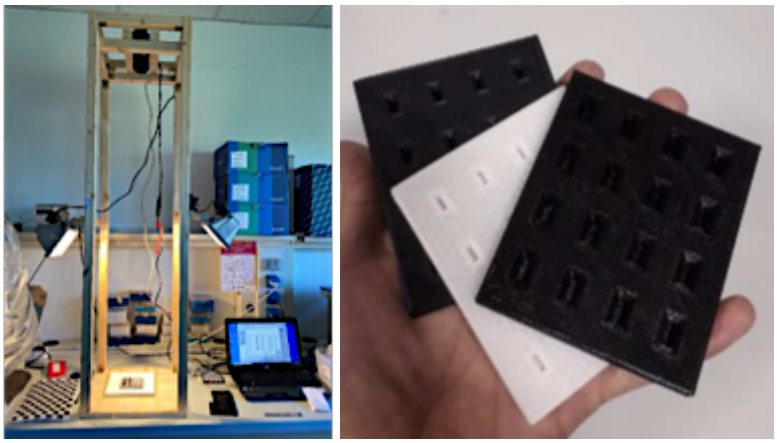


Figure 2.5 – (**a**) Multispectral image system; (**b**) sample plastic holders.

For all the replicates of each accession/variety, eight seeds were used for the images. Pictures were captured with eight different filters for different wavelength (452, 532, 667, 715, 840, 918 and 970 nm). The camera was firstly calibrated by examining the histograms of each filter and setting them between 500 and 600, adjusting the exposure time. The focus of the camera was then adjusted to ensure that sharp images were acquired. Calibration pictures of the white board (on which the sample trays were placed), the black tray and the black tray with the chess were taken as references (**Figure 2.7**). Two pictures were acquired for the horizontal and lateral side of each replica of each genotype.

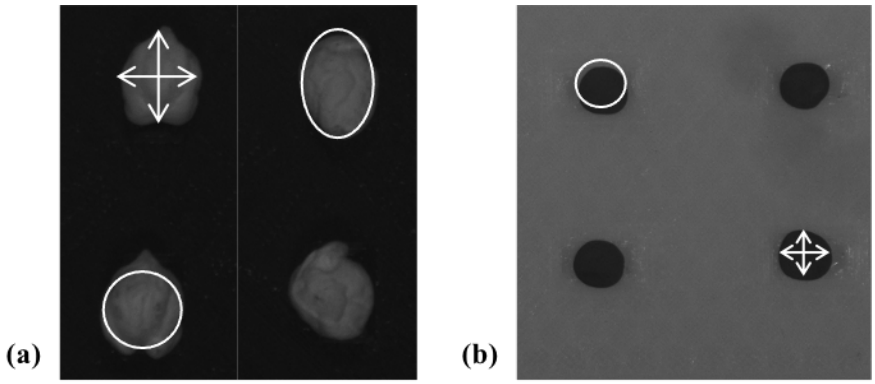


Figure 2.6 – Imaging analysis of chickpea (**a**) and lentil (**b**) seeds and considered parameters.

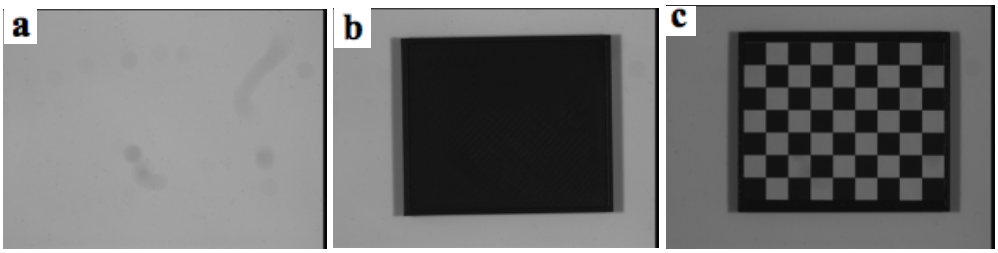


Figure 2.7 – Calibration pictures; (**a**) white board; (**b**) black tray; (**c**) black tray with chess.

The following seed shape and color parameters were measured for both horizontal and lateral side:

- Minor axis (mm);
- Major axis (mm);
- Seed area (mm^2);
- r_0 = minor axis / major axis;
- r_1 = circle area / seed area;
- r_2 = ellipse area / seed area;
- Avg Reflectance.

2.3 Data analysis

All the statistical analyses were performed with JMP software version 8 (SAS Institute Inc., Cary, NC, USA). One-way analysis of variance (ANOVA) was used to test differences among accessions for some of the considered quality traits (**Table 2.3**). Mean discrimination was performed applying a T Student test and statistically significant differences were determined at the probability level of $p < 0,05$. From the variance component estimates from the ANOVA analyses, we calculated the broad-sense heritability of the different traits (with 95% confidence limits).

The broad sense heritability (h_B^2) has been defined as the ratio between genotypic variance and phenotypic variance (Hanson, 1963).

$$h_B^2 = \sigma_G^2 / \sigma_P^2 = \sigma_G^2 / (\sigma_G^2 + \sigma e^2)$$

Where, σ_G^2 is genotypic variance and σ_P^2 is phenotypic variance and σe^2 is error variance as an estimation of environmental variance.

For the image analysis, all the data analysis was performed using custom-made scripts written in MATLAB (The Mathworks, Natick, MA, USA). A PCA was performed in order to identify traits at the basis of differentiation among accessions.

GEI (Genotypes by Environment Interaction) was assessed for yield, 1000 seed weight, SSI and Volume for chickpea and lentil and seed coat weight and seed coat content for chickpea only using a two-way ANOVA model as follow:

$$Yij = \mu + Gi + Ej + (GEI)ij$$

where Yij is the observation of the ith genotype in the jth environment, μ is the general mean, G is the genotypic main effect, E the environmental main effect and GEI the effect of interaction between the ith genotype and the jth environment. For our purpose we considered different environments the two growing seasons (autumn and spring) for chickpea and the two localities (Osimo and Metaponto) for lentil. We regarded the two factors (genotypes and environment) and their interaction as random effects.

CHAPTER 3
RESULTS

CHICKPEA

Seeds of each replicate and accession obtained in the two field experiments (autumn and spring sowing) were used to determine 1000 seed weight (g), SSI, volume (ml), seed coat weight (g), seed coat content (‰), hydration (%), shape (mm) and color (nm).

Genotype-by-environment interaction (GEI) plot and analysis of variance components were carried out for 1000 seed weight as showed in **Figure 3.1.** A significant higher 1000 seed weight was found for seeds obtained in the autumn sowing compared to spring sowing field (P = 0.017*). The GEI plot showed no relevant cross interaction, with higher 1000 seed weight obtained in autumn sowing experiment for almost all the accessions; IS-CE-6 and Principe showed a slightly higher 1000 seed weight in spring compared to autumn sowing. High heritability was found for such trait, with 73.1% of total variance explained by the genotype, while 6.6% of the total variance was due to GEI and 7.9% to season effect factors.

Distribution of the trait and broad sense heritability (h^2) were computed for SSI, volume, seed coat weight, and seed coat content (**Figure 3.2**). Wide variability was detected for all the traits. Very high h^2 estimates were obtained for such traits, with the highest being for seed coat content (h^2 = 99.1% for the autumn sowing and h^2 = 98.8% for the spring sowing), followed by seed size index (h^2 = 95.6% for the autumn sowing and h^2 = 97.8% for the spring sowing), seed coat weight (h^2 = 96.8% both the sowings) and volume (h^2 = 77.3% for the autumn sowing and h^2 = 87.5% for the spring sowing).

For the same traits, one-way ANOVA highlighted significant differences among the genotypes grown in autumn (**Figure 3.3**) and spring (**Figure 3.4**) sowing season fields. SSI ranged from 7.05 (Palazzo San Gervasio) to 8.83 (Ituchi) for the autumn season (**Figure 3.3a**) and from 6.97 (Palazzo San Gervasio) to 9.65 (Ituchi) for the spring season (**Figure 3.4a**). Genotypes showing the highest values of SSI were kabuli types (Ituchi, Bianco (scalo), IS-CE-1 and Tricarico genotypes) for both the seasons.

Volume values ranged from 1.50 (Filiano nero) to 3.83 (Ituchi) ml for the autumn season (**Figure 3.3b**) and 1.50 (Palazzo San Gervasio) to 4.33 (Ituchi) ml for the spring season (**Figure 3.4b**). Accessions Ituchi, IS-CE-1, Bianco (scalo), and Tricarico showed also the highest values of volume in both seasons.

Seed coat weight and seed coat content traits resulted strongly different among Desi and Kabuli types, indeed the Desi genotypes Filiano nero, Nero Tolve, Nero Appignano and IS-CE-BRUNO showed the highest values, while the Kabuli types were characterized by lowest values for both the seasons (**Figure 3.3c, d; Figure 3.4c, d**).

Interaction plots and analysis of the variance components for SSI, volume, seed coat weight and seed coat content for the two growing seasons are showed in **Figure 3.5.** The seed size did not show significant difference between the growing seasons and the interaction plot shows no cross interactions, with the exception for Ituchi, where the SSI is higher in spring compared to autumn sowing (**Figure 3.5a**). Variance components show high heritability for SSI with 88.5% of the total variance explained by the genotype, confirming the importance of genetic component for such trait. Similarly, no significant difference, as well as no significant cross interactions, were detected for volume, seed coat weight and seed coat content between the growing seasons (**Figure 3.5b, c** and **d**). Variance components show high heritability for volume, seed coat weight and seed coat content, with 82.4%, 88.4% and 96.7% of the total variance explained by the genotype, respectively.

The parameters of Hydration max (Hmax) and Hydration rate (Hrate) obtained from the model are not replicated. **Figure 3.6** and **Figure 3.7** show the parameters for all the genotypes analyzed in both growing seasons. For both the sowing seasons, the Kabuli Ituchi and Bianco (scalo) genotypes showed the highest values of Hydration max, while the Desi genotypes, Filiano nero and Nero Tolve the lowest (**Figure 3.6**). Concerning the hydration rate, Desi Filiano nero and Nero Tolve genotypes the results showed the highest values, while the lower values were detected for Reale, Ituchi and IS-CE-BRUNO genotypes (**Figure 3.7**). The correlated response between autumn and spring is significant for both hydration traits, with a correlation coefficient of 0.92 and 0.67 for the Hydration max and Hydration rate (0.67), respectively.

Component	Percent of Total Variance
Genotype	73.1
Season	7.9
Genotype*season	6.6
Residual	12.2
Total	100.0

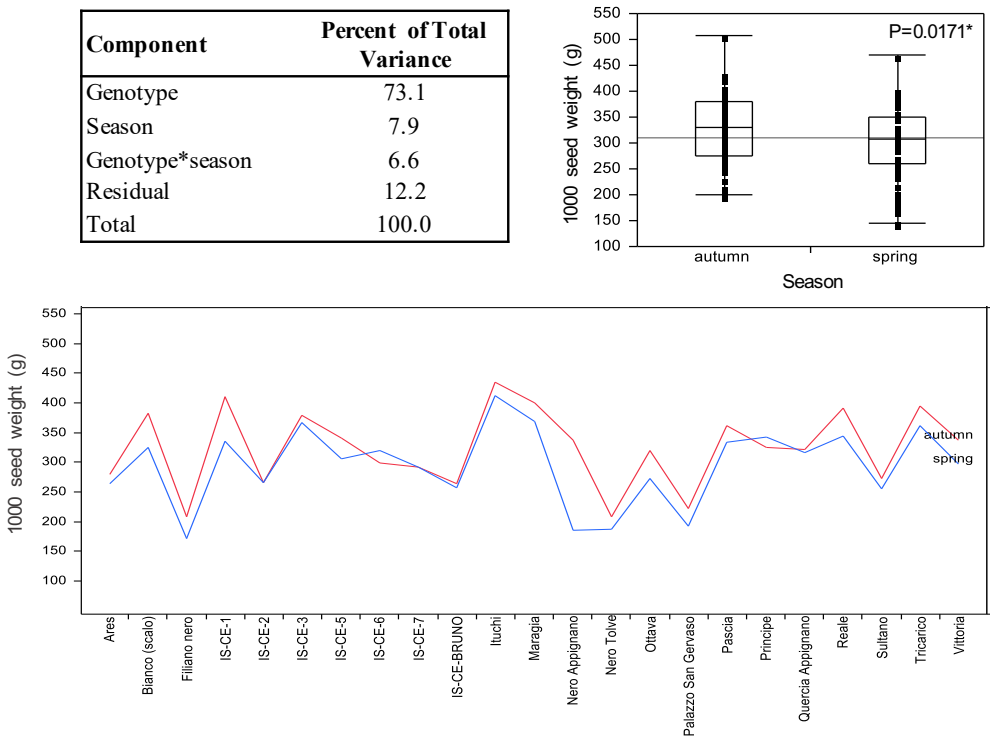


Figure 3.1: GEI plot and variance components for 1000 seed weight.

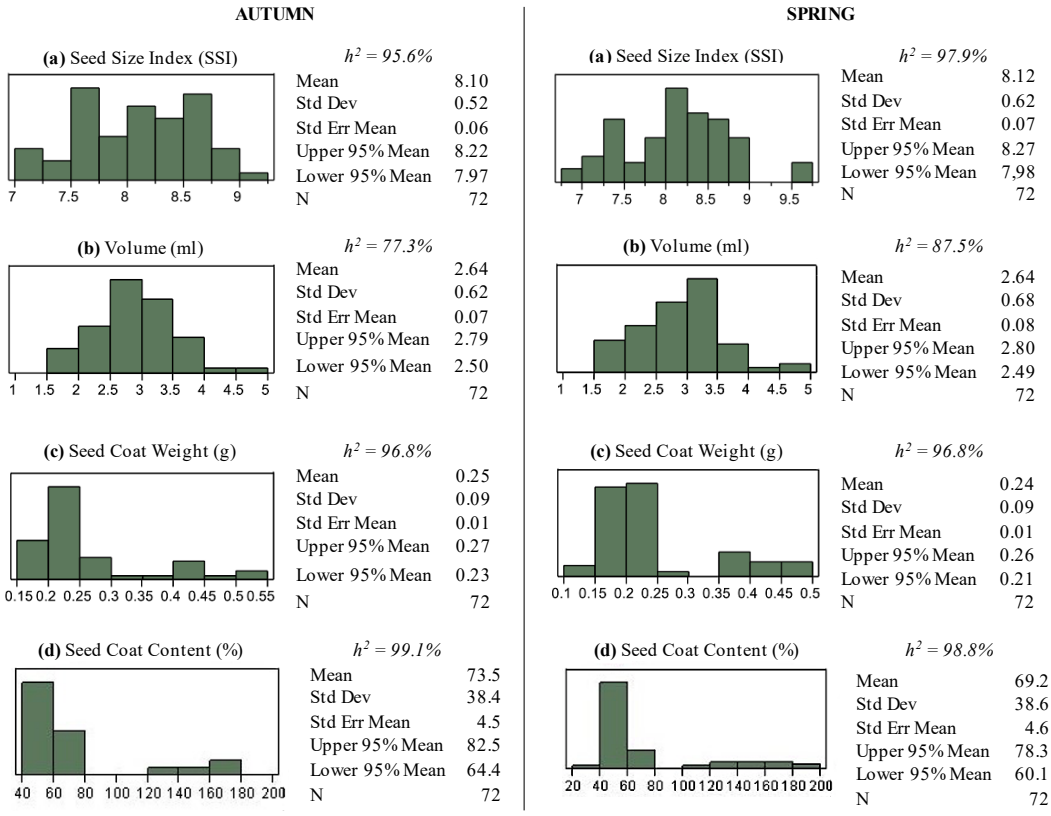


Figure 3.2: SSI (**a**), volume (**b**), seed coat weight (**c**), seed coat content (**d**) distribution and heritability estimation of autumn (left) and spring sowing (right).

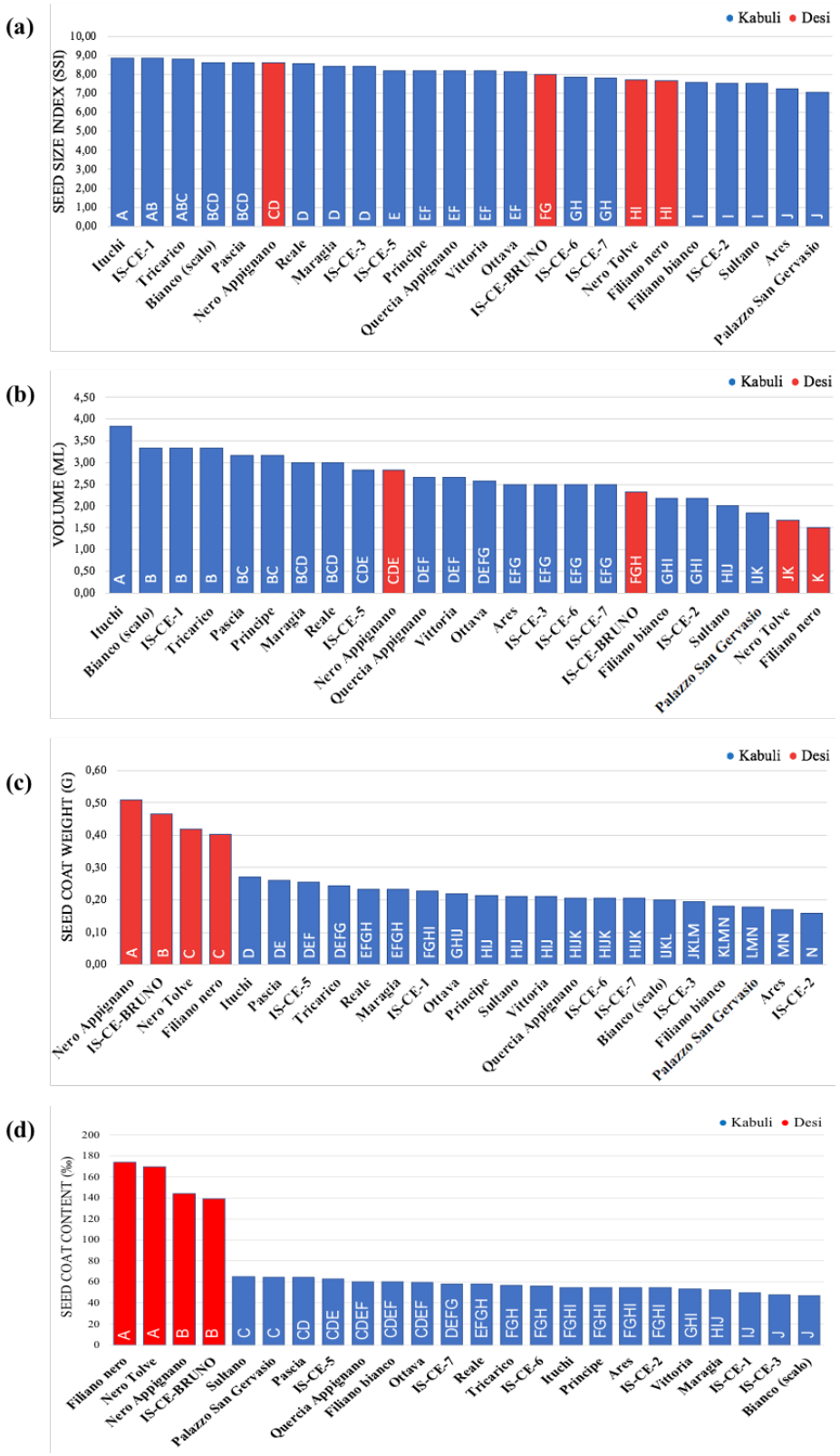


Figure 3.3 - Average seed size **(a)**, volume **(b)**, seed coat weight **(c)**, and seed coat content **(d)** for chickpea genotypes of autumn sowing trial; levels not connected by same letter are significantly different ($P < 0.05$; T student test).

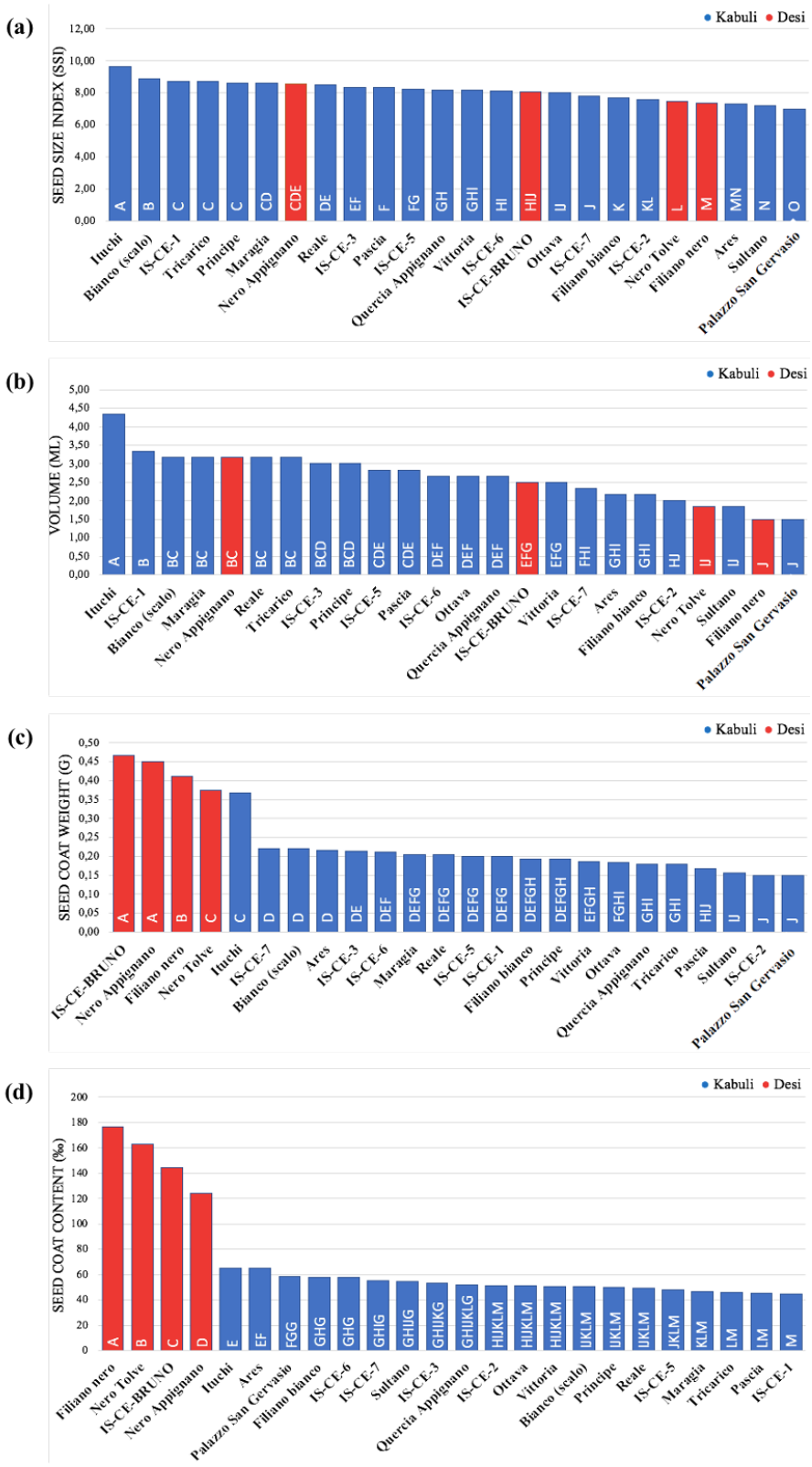
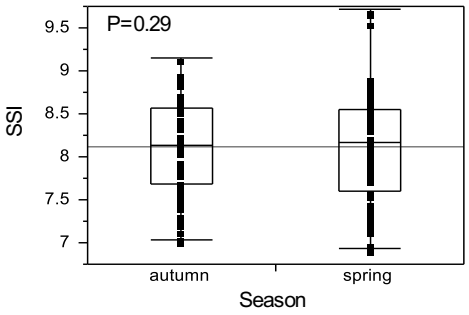
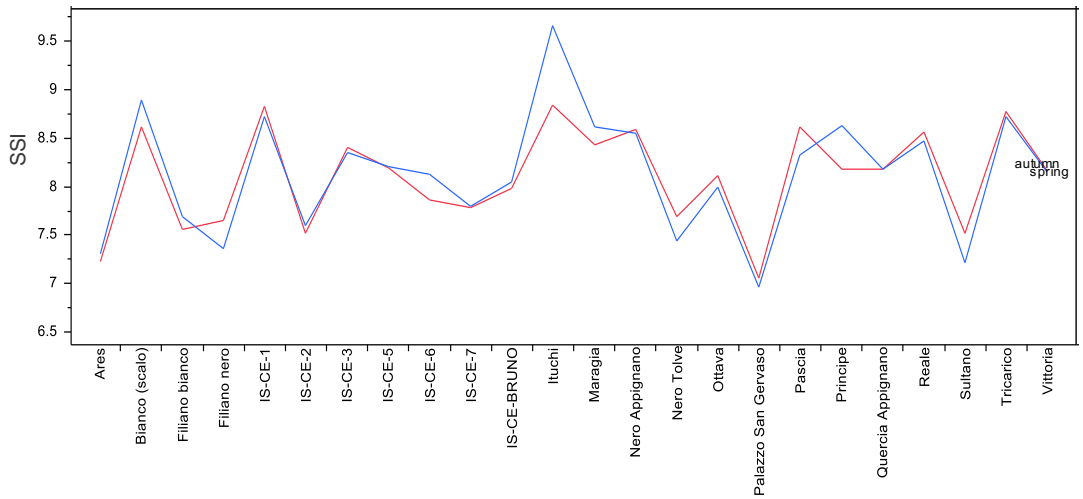


Figure 3.4: Average seed size **(a)**, volume **(b)**, seed coat weight **(c)**, and seed coat content **(d)** for chickpea genotypes of spring sowing trial; levels not connected by same letter are significantly different ($P < 0.05$; T student test).

(a)

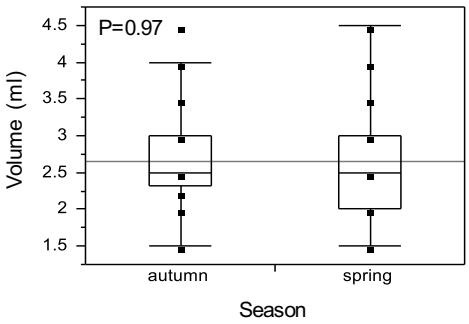
Component	Percent of Total Variance
Genotype	88.5
Season	-0.3
Genotype*season	8.1
Residual	3.6
Total	100.0

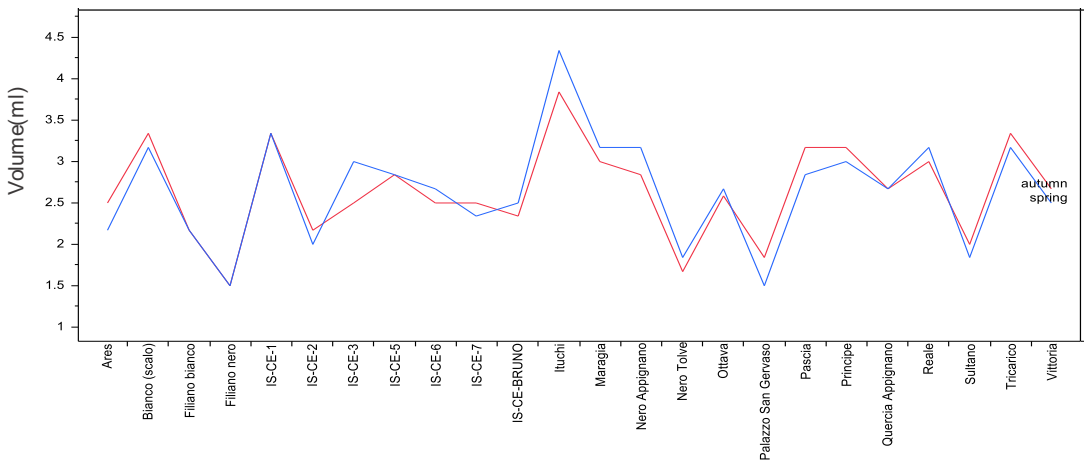




(b)

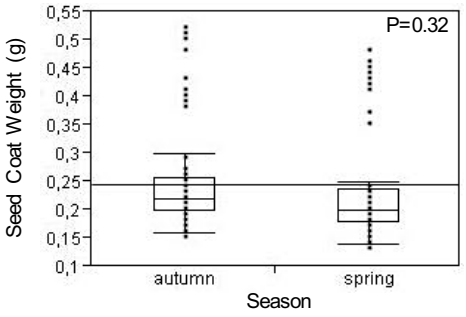
Component	Percent of Total Variance
Genotype	82.4
Season	-0.3
Genotype*season	0.7
Residual	17.2
Total	100.0

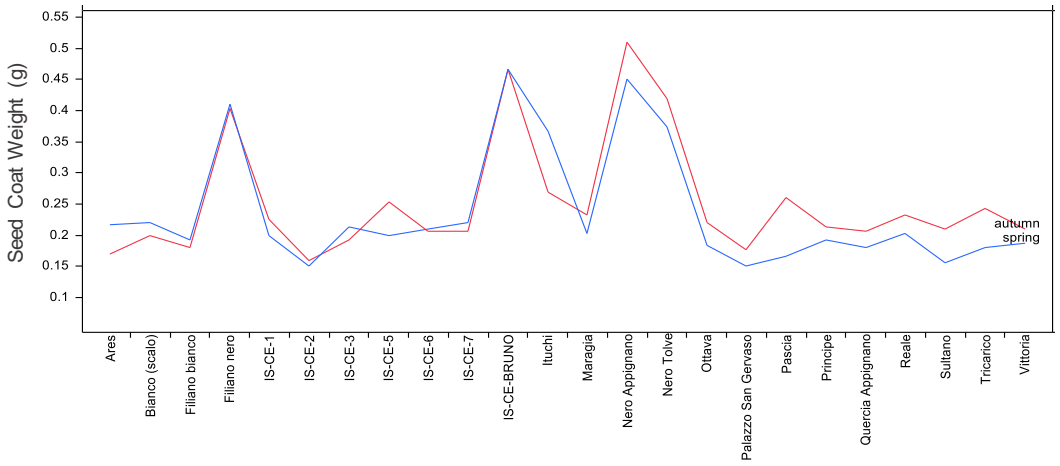




(c)

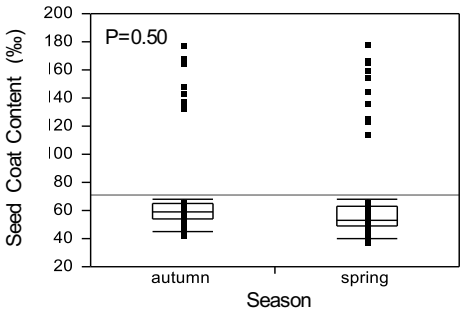
Component	Percent of Total Variance
Genotype	88.4
Season	1.0
Genotype*season	7.5
Residual	3.2
Total	100.0





(d)

Component	Percent of Total Variance
Genotype	96.7
Season	0.5
Genotype*season	1.7
Residual	1.0
Total	100.0



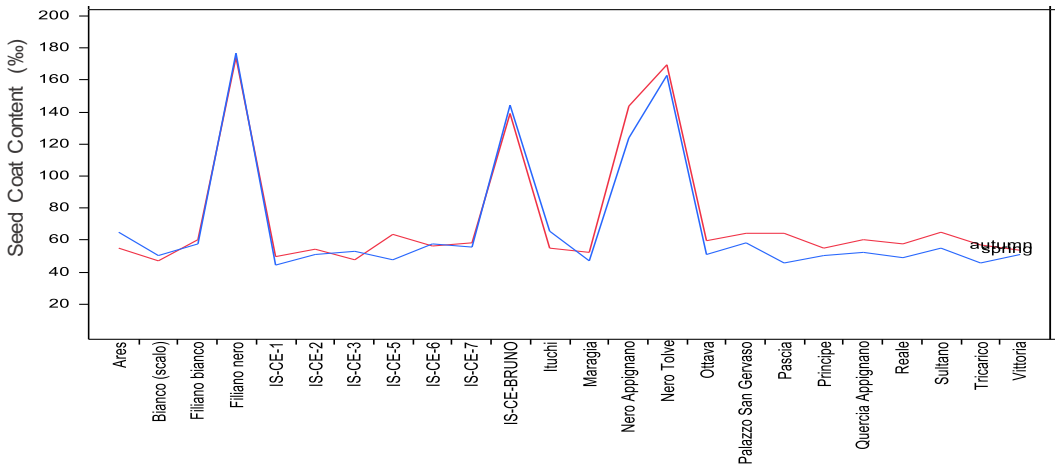


Figure 3.5 - Interaction plot GEI analysis and variance components for SSI (**a**), Volume (**b**), Seed Coat Weight (**c**) and Seed Coat Content (**d**).

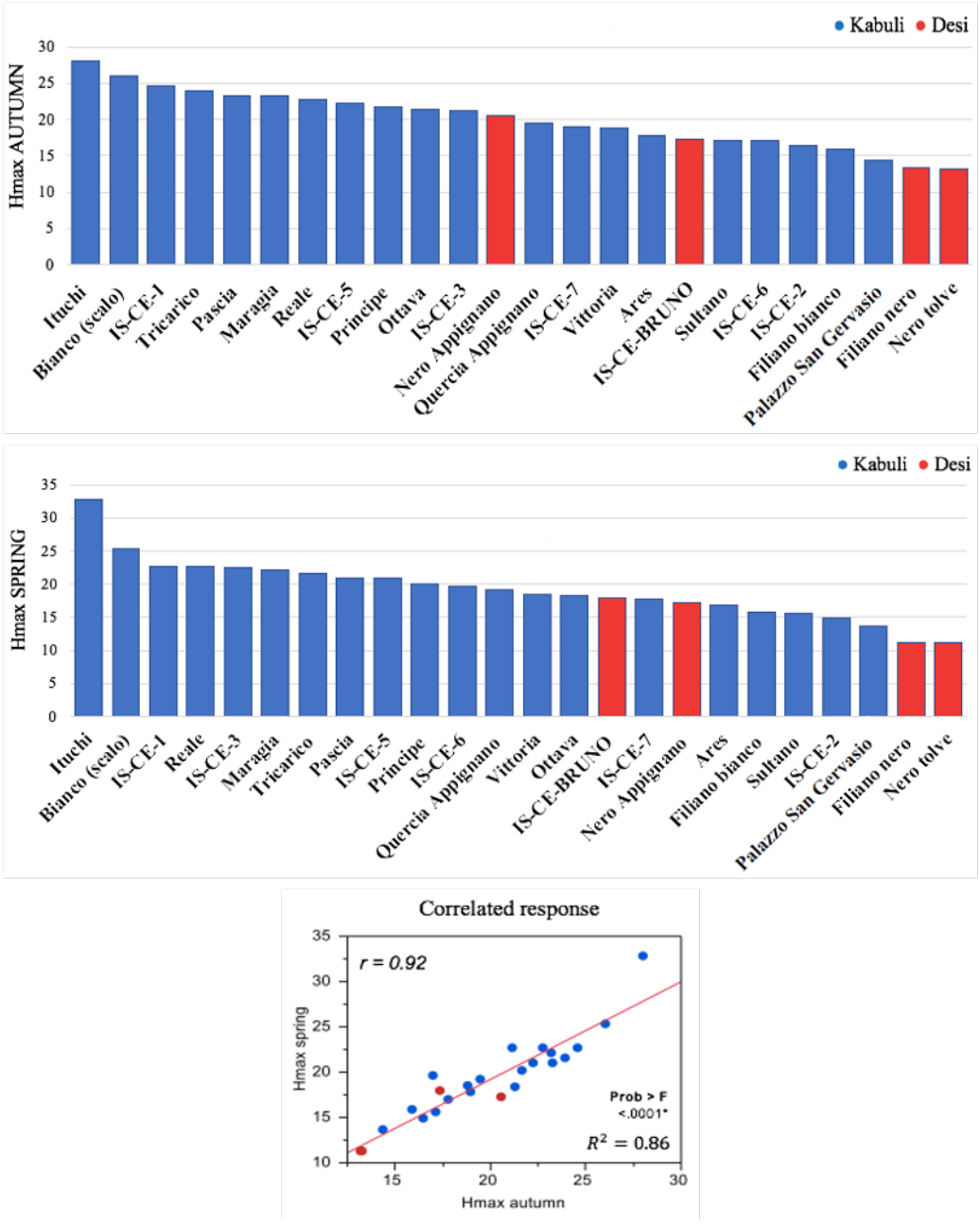
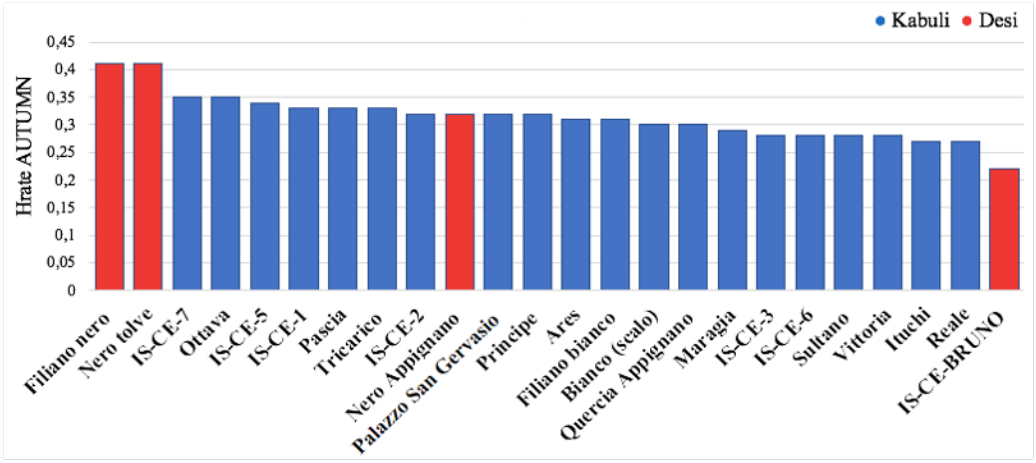
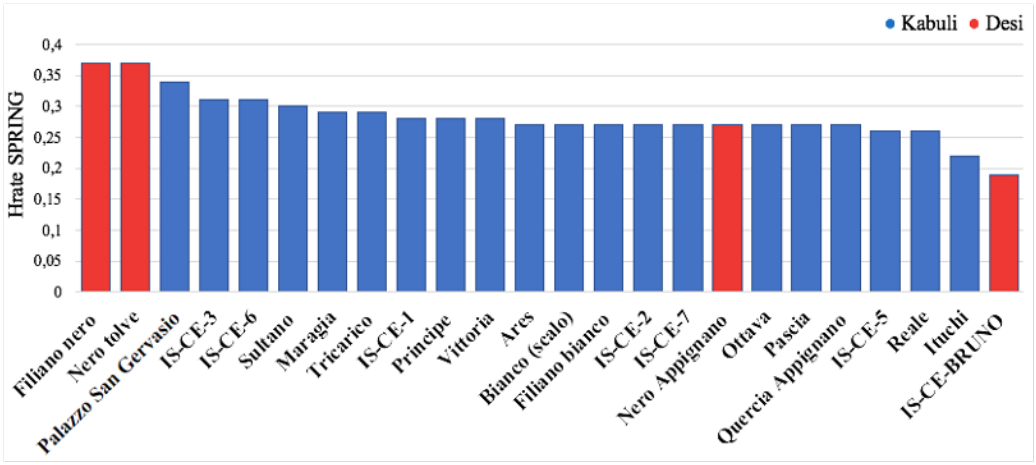


Figure 3.6: Hydration max for all genotypes in autumn and spring sowing.





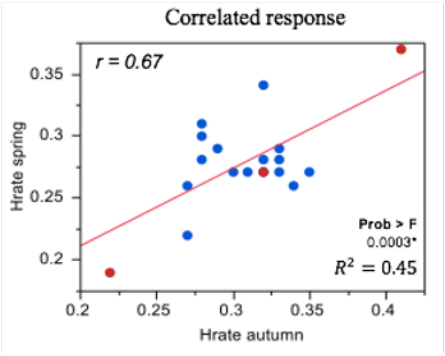
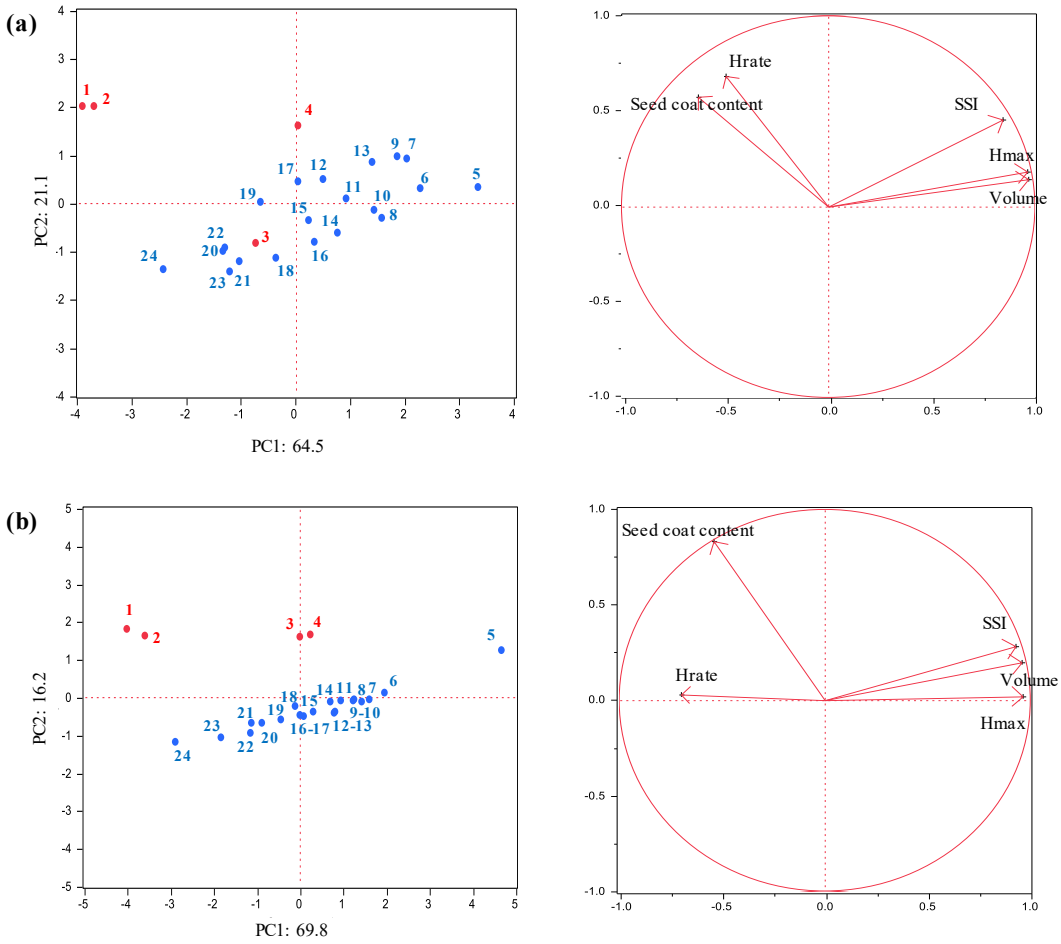


Figure 3.7: Hydration rate for all genotypes in autumn and spring sowing.

Figure 3.8 shows the PCA carried out for SSI, volume, seed coat content, hydration max and hydration rate traits of autumn (**Figure 3.8a**) and spring sowing season (**Figure 3.8b**). The Principal component 1 (PC1) and Principal component 2 (PC2) explain 64.5% and 21.1% and 69.8% and 16.2% of the total variance for the autumn and spring sowing season, respectively. The PC1 is positively correlated to SSI, volume and hydration max and negatively correlated to hydration rate and seed coat content, independently by the season. The PC2 is positively correlated with all the considered traits for both autumn and spring sowing season. The PCA confirmed the results obtained for the one-way Anova analysis with desi type, Nero Tolve and Filiano nero, characterized bu the highest values for seed coat content and hydration rate and with kabuli type, Ituchi, showing bigger size.

Correlations between SSI, volume, seed coat content, hydration max and hydration rate of the two sowing seasons were also investigated (**Figure 3.9**). Seed size index of autumn season showed positive correlation with volume ($r = 0.87$, $P < 0.05$) and hydration max ($r = 0.87$, $P < 0.05$) of the autumn season and volume ($r = 0.89$, $P < 0.05$) and hydration rate ($r = 0.89$, $P < 0.05$) of the spring season. A significant positive correlation was also found between volume and hydration max, independently by the season. Seed coat content and hydration rate showed significant positive correlation between the two sowing seasons ($r = 0.98$ and $r = 0.67$, $P < 0.05$). Hydration max of the autumn season showed significant negative correlation with seed coat content ($r = -0.53$, $P < 0.05$) and hydration rate of spring season ($r = -0,54$, $P < 0.05$).



1- Filiano nero	9- Tricarico	17- Ottava
2- Nero Tolve	10- Maragià	18- IS-CE-6
3- IS-CE-Bruno	11- Principe	19- IS-CE-7
4- Nero Appignano	12- IS-CE-5	20- Filiano bianco
5- Ituchi	13- Pascià	21- Ares
6- Bianco (scalo)	14- IS-CE-3	22- IS-CE-2
7- IS-CE-1	15- Quercia Appignano	23- Sultano
8- Reale	16- Vittoria	24- Palazzo San Gervasio

Figure 3.8 - PCA and loading plot for SSI, volume, seed coat content, hydration max and hydration rate traits of chickpea seeds of autumn (**a**) and spring sowing season (**b**). Red, Desi type, blue Kabuli type.

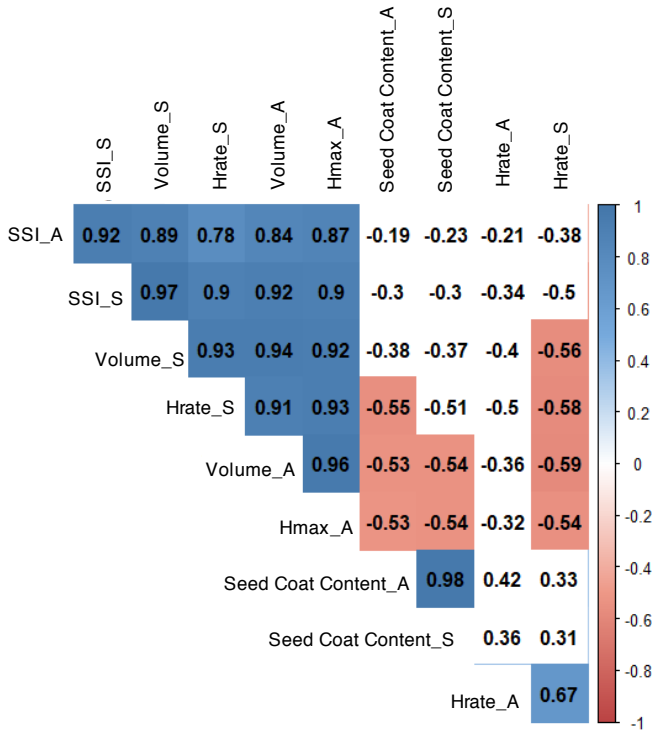


Figure 3.9 – Correlation (Pearson's coefficient, r) between SSI, Volume, seed coat content, hydration max and hydration rate of autumn and spring sowing season.

Distribution and broad sense heritability (h^2) were also calculated for both the seasons for the following shape traits: area (mm^2), major axis (mm), minor axis (mm), r0 (mm), r1 (mm) and r2 (mm) (**Figure 3.10**). The analyses were performed on both horizontal and lateral side of the seeds. For the horizontal side autumn sowed seeds, the highest h^2 estimates were obtained for r2 (h^2 = 87.4%), r1 (h^2 = 85.5%), major axis (h^2 = 82.6%), area (h^2 = 74.3%) and r0 (h^2 = 74.1%). Slightly lower heritability was found for minor axis (h^2 = 62.6%). For the seeds obtained in the spring season high h^2 estimates for all the analyzed shape parameters were found, with values that resulted higher compared to those of seeds obtained in the autumn season for area, major axis, minor axis and r0 and lower for r1 and r2. For the lateral side, the seeds obtained in the autumn sowing season trial showed highest estimates for major axis (h^2 = 82.4%), area (h^2 = 80.9%) and minor axis (h^2 = 76.6%). Lower heritabilities were found for r0 (h^2 = 64.4%), r1 (62.1%) and r2 (46.5%). Seeds for spring sowing season trials, showed higher h^2 estimates compared to those of spring sowing trials for major axis (h^2 = 92.2%), area (h^2 = 87.7%), minor axis (h^2 = 80.6%) and r0 (72.7 %), while lower heritabilities were observed for r1 (h^2 = 52.1%) and r2 (h^2 = 45.6%).

For the same traits, one-way ANOVA (**Figure 3.11** and **Figure 3.12**) and PCA (**Figure 3.13** and **Figure 3.14**) were performed. One-way ANOVA highlighted significant differences among

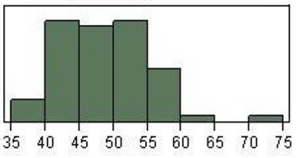
the different accessions of autumn and spring sowing season (P = <0.0001*) for all the dimensional traits analyzed, independently by the side. Area showed a range of variability from 37.9 mm^2 to 62.9 mm^2 and from 34 mm^2 to 59.7 mm^2 in the seeds from autumn and spring sowing season, respectively. Major and minor axis range from 7.68 mm to 10.4 mm and from 6.2 mm to 7.9 mm in the seeds from autumn sowing season, respectively; it ranged from 7.2 mm to 10.3 mm and from 5.9 mm to 10.1 mm for the seeds obtained in the spring sowing season, respectively. For the above mentioned dimensional traits, the highest and lowest values were found for Ituchi and Palazzo San Gervasio genotypes, respectively, independently by the sowing season and side. Highest values of r1 and r2 were obtained for Desi genotypes for the horizontal side in both the seasons.

A PCA analysis was carried out for dimensional traits obtained by image analysis. The Principal component 1 (PC1) and Principal component 2 (PC2) obtained with data from autumn sowing trial explain 63.7% and 30.1% and 74.8% and 17.0% of the total variance for the horizontal side and lateral side analyses, respectively (**Figure 3.13**). For the spring sowing season, the PC1 and PC2 explain 60.2% and 31.9% of the total variance for the horizontal side, respectively, while they explain the 66.2% and 18.9% of the total variance for the lateral side analyses, respectively (**Figure 3.14**). The PC1 is positively correlated with minor and major axis, area, r1 and r2, while it is negatively correlated with r0, independently by the side for both autumn and spring sowing season. The PC2 is positively correlated with minor and major axis and area and negatively correlated to r1 and r2 in the horizontal side, and it is positively correlated to r0, minor and major axis and area in the lateral side, independently by the sowing season. The main outcome for both autumn and spring sowing seasons and sides, was the clear separation of the Desi genotypes Nero Tolve and Filiano nero; they are characterized by the highest values of r1 and r2 reflecting a more flattened and less rounded shape. Moreover, lower values of minor and major axis and area means smaller dimensions of the seeds. Among Kabuli types, the highest dimension values were found for Ituchi genotype, lowest value for Palazzo San Gervasio genotype, for both the sowing seasons and sides (**Figure 3.13** and **3.14**).

(a) Seed shape parameters measured for the horizontal side

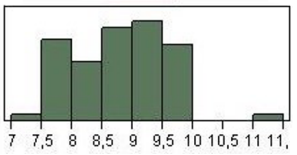
AUTUMN

Area (mm^2) — $h^2 = 74.3\%$



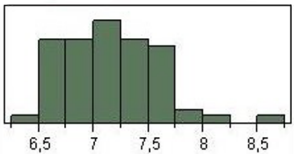
Mean	49.14
Std Dev	6.68
Std Err Mean	0.79
Upper 95% Mean	50.71
Lower 95% Mean	47.57
N	72

Major axis (mm) — $h^2 = 82.6\%$



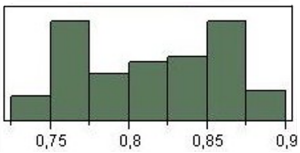
Mean	8.83
Std Dev	0.73
Std Err Mean	0.09
Upper 95% Mean	9.01
Lower 95% Mean	8.66
N	72

Minor axis (mm) — $h^2 = 62.6\%$



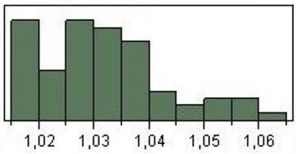
Mean	7.17
Std Dev	0.42
Std Err Mean	0.05
Upper 95% Mean	7.27
Lower 95% Mean	7.07
N	72

r0 (mm) — $h^2 = 74.1\%$



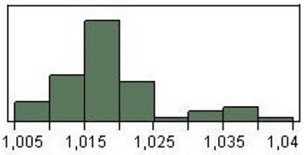
Mean	0.82
Std Dev	0.04
Std Err Mean	0.05
Upper 95% Mean	0.83
Lower 95% Mean	0.81
N	72

r1 (mm) — $h^2 = 85.5\%$



Mean	1.03
Std Dev	0.01
Std Err Mean	0.01
Upper 95% Mean	1.03
Lower 95% Mean	1.03
N	72

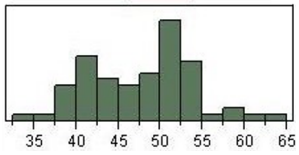
r2 (mm) — $h^2 = 87.4\%$



Mean	1.02
Std Dev	0.01
Std Err Mean	0.01
Upper 95% Mean	1.02
Lower 95% Mean	1.02
N	72

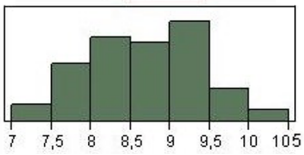
SPRING

Area (mm^2) — $h^2 = 85.2\%$



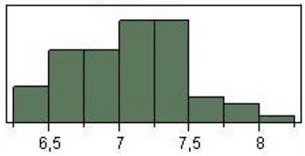
Mean	47.73
Std Dev	6.12
Std Err Mean	0.72
Upper 95% Mean	49.17
Lower 95% Mean	46.29
N	72

Major axis (mm) — $h^2 = 91.9\%$



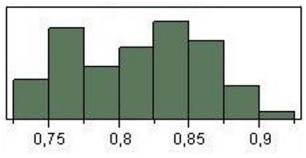
Mean	8.72
Std Dev	0.71
Std Err Mean	0.08
Upper 95% Mean	8.89
Lower 95% Mean	8.55
N	72

Minor axis (mm) — $h^2 = 74.4\%$



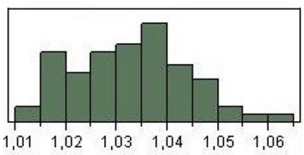
Mean	7.07
Std Dev	0.41
Std Err Mean	0.05
Upper 95% Mean	7.16
Lower 95% Mean	6.97
N	72

r0 (mm) — $h^2 = 86.6\%$



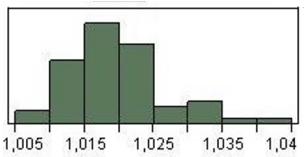
Mean	0.81
Std Dev	0.05
Std Err Mean	0.01
Upper 95% Mean	0.83
Lower 95% Mean	0.81
N	72

r1 (mm) — $h^2 = 83.7\%$



Mean	1.03
Std Dev	0.01
Std Err Mean	0.01
Upper 95% Mean	1.04
Lower 95% Mean	1.03
N	72

r2 (mm) — $h^2 = 77.8\%$



Mean	1.02
Std Dev	0.01
Std Err Mean	0.01
Upper 95% Mean	1.02
Lower 95% Mean	1.02
N	72

(b) Seed shape parameters measured for the lateral side

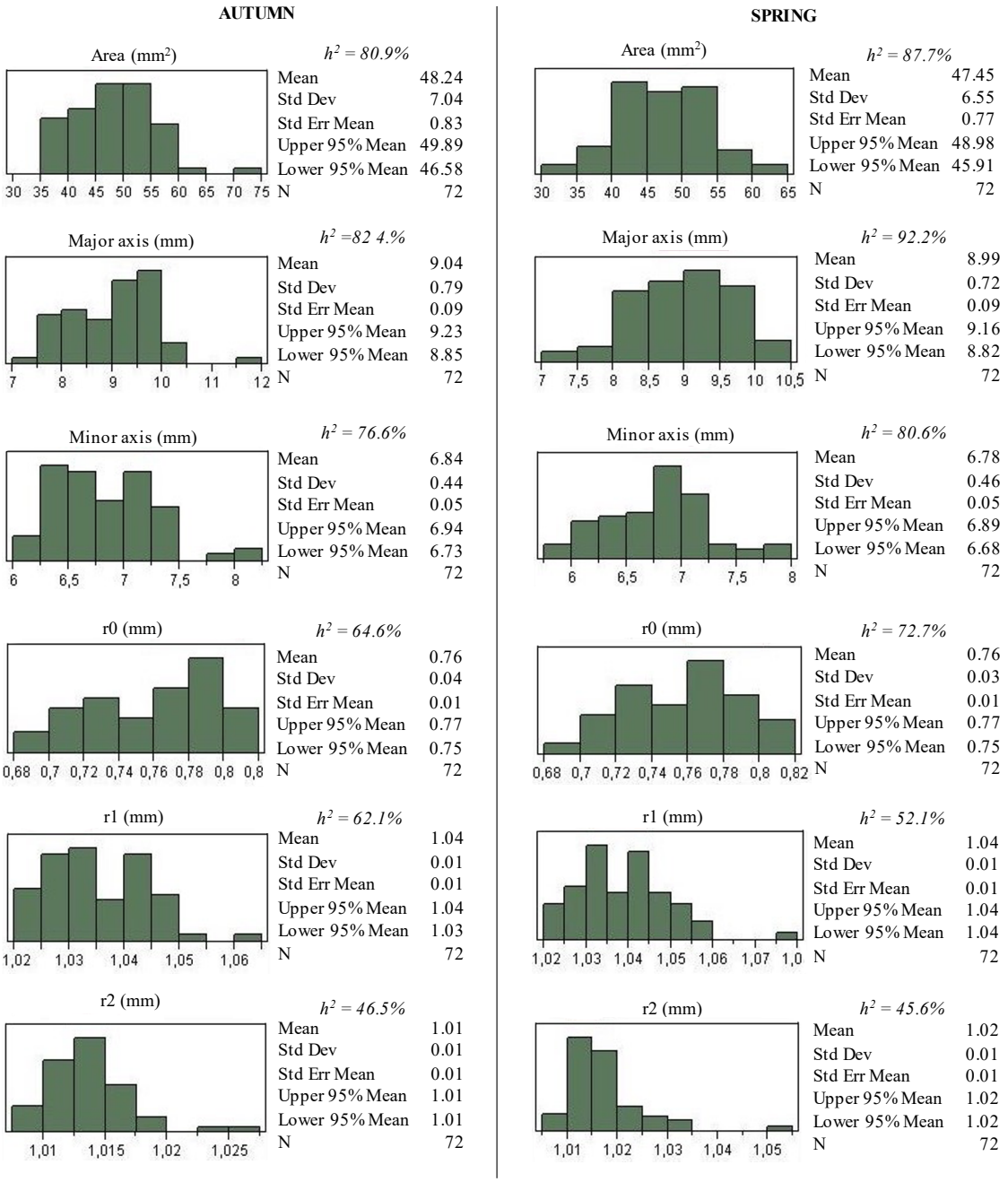
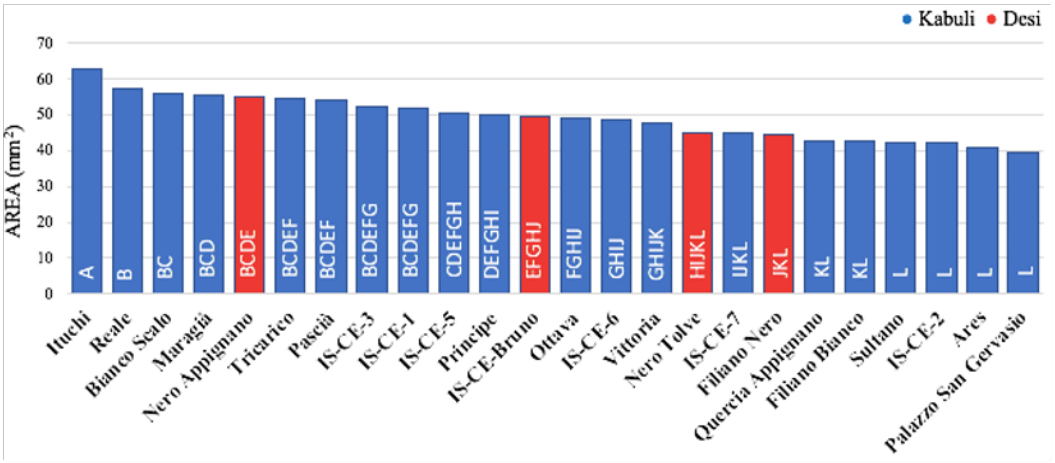
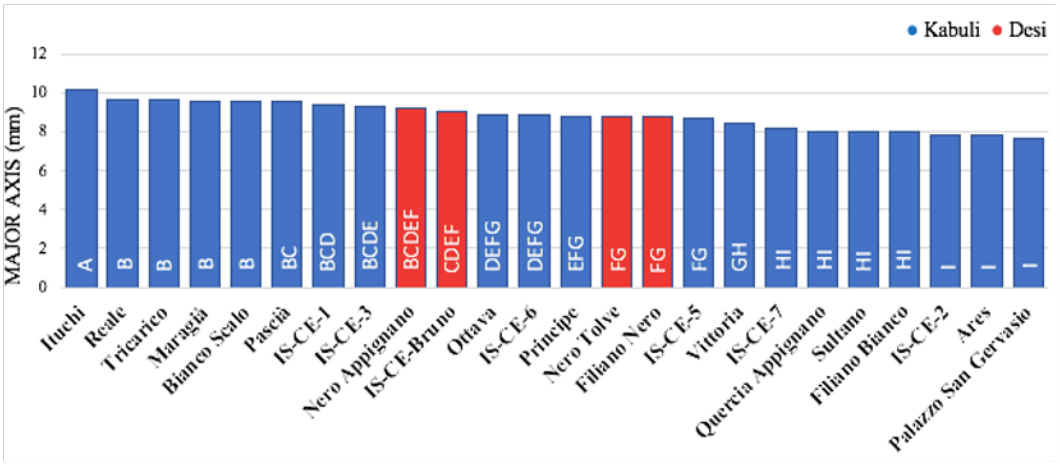
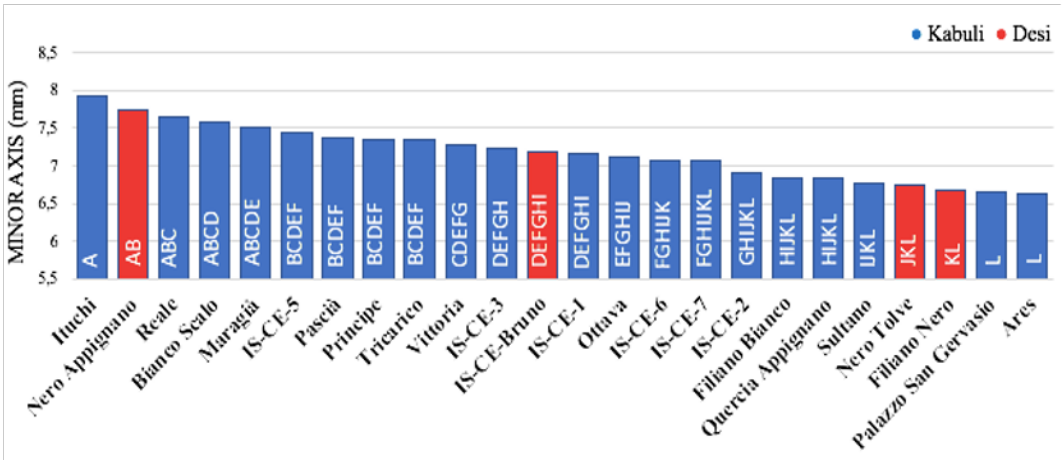


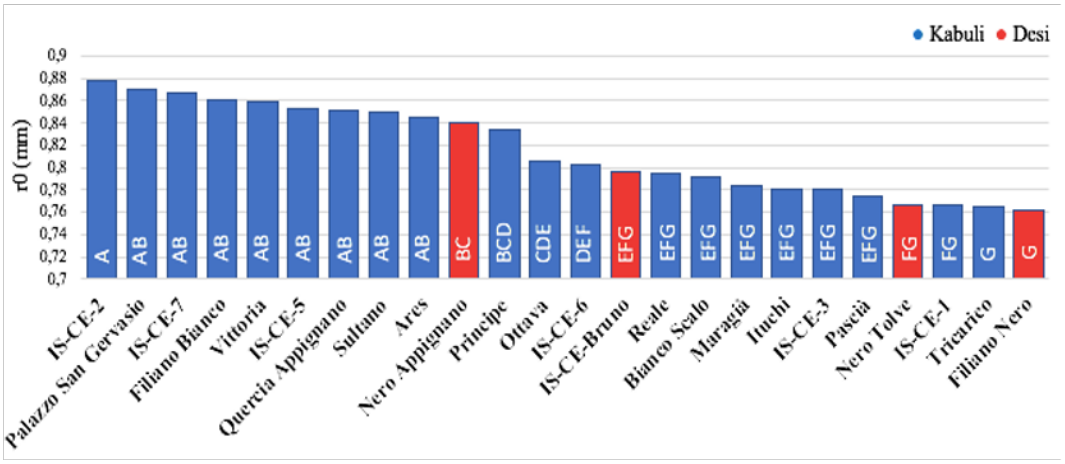
Figure 3.10 - Area, major axis, minor axis, r0, r1 and r2 distribution and heritability estimation for autumn (left) and spring (right) sowing seasons. Seed shape parameters were measured for both horizontal (**a**) and lateral side (**b**).

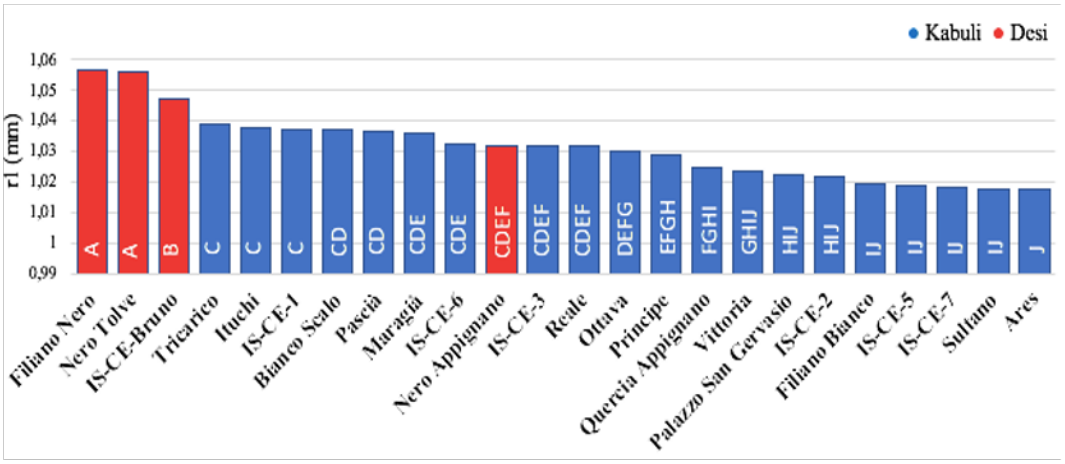
(a) Seed shape parameters measured for the horizontal side

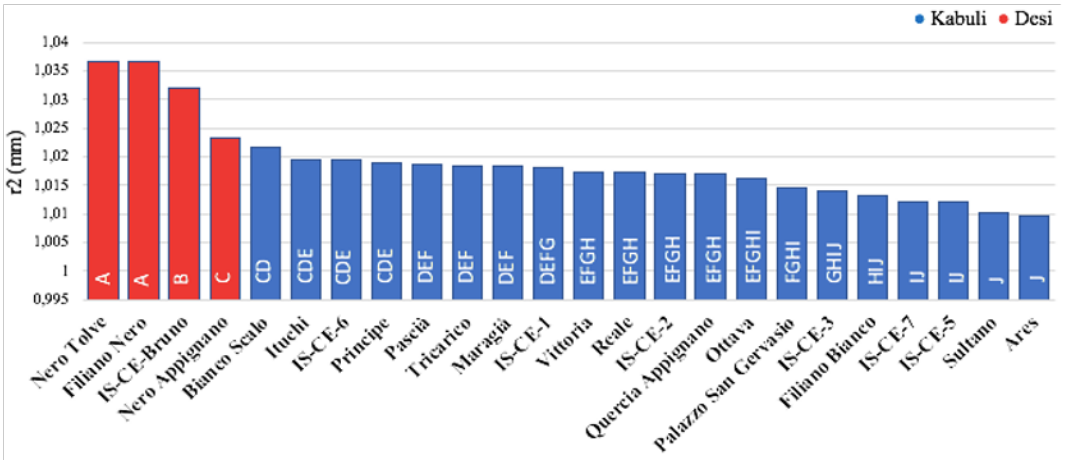




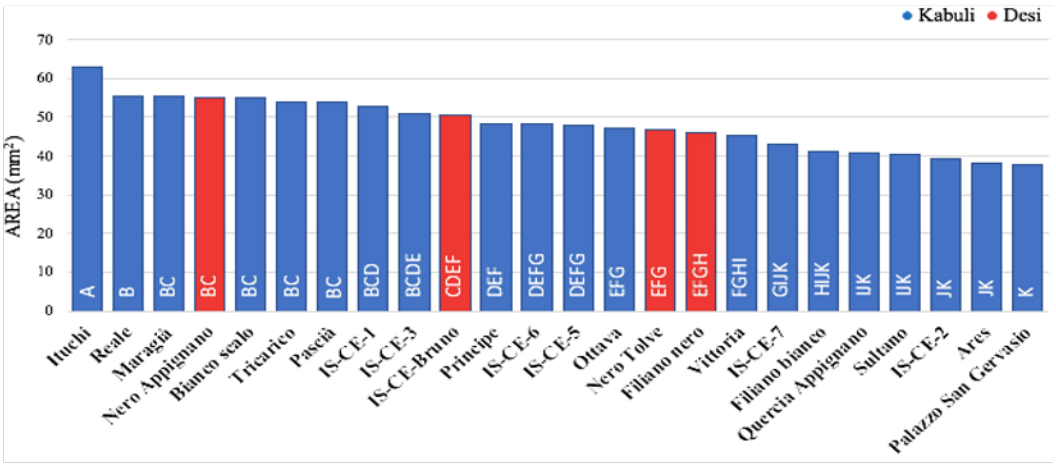


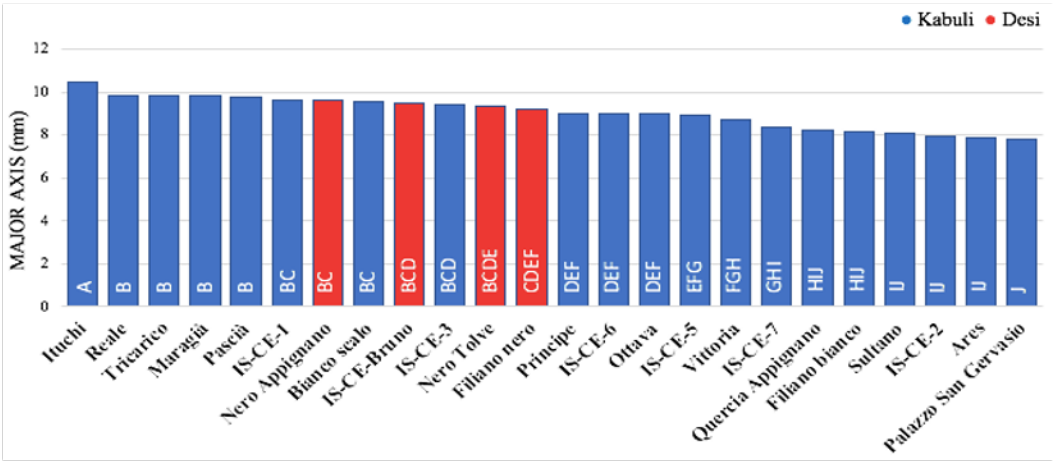

Kabuli
Desi
r0 (mm)
0,9
0,88
0,86
0,84
0,82
0,8
0,78
0,76
0,74
0,72
0,7
A
AB
AB
AB
AB
AB
AB
AB
AB
BC
BCD
CDE
DEF
EFG
EFG
EFG
EFG
EFG
EFG
EFG
FG
FG
G
G
IS-CE-2
Palazzo San Gervasio
IS-CE-7
Filiano Bianco
Vittoria
IS-CE-5
Quercia Appignano
Sultano
Ares
Nero Appignano
Principe
Ottava
IS-CE-6
IS-CE-Bruno
Reale
Bianco Scalo
Maragià
Ituchi
IS-CE-3
Pascià
Nero Tolve
IS-CE-1
Tricarico
Filiano Nero

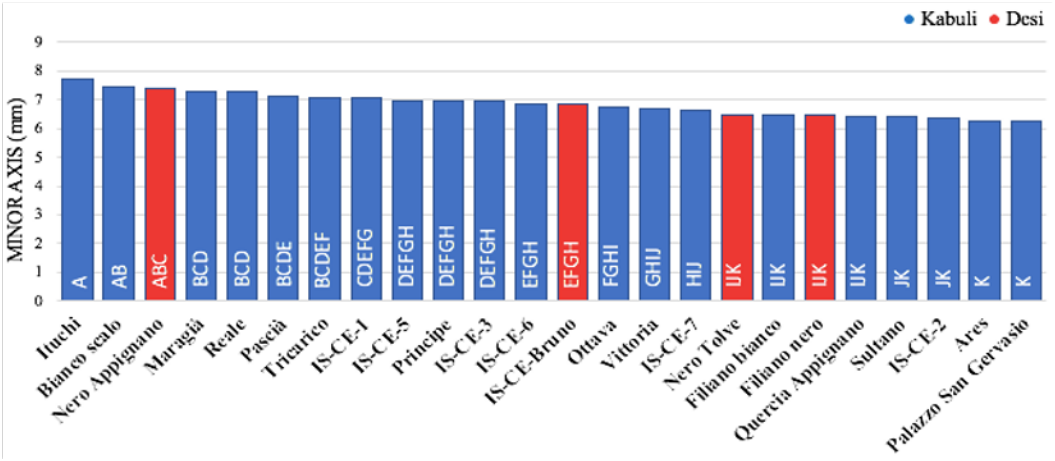

Kabuli
Desi
r1 (mm)
1,06
1,05
1,04
1,03
1,02
1,01
1
0,99
A
A
B
C
C
C
CD
CD
CDE
CDE
CDEF
CDEF
CDEF
DEFG
EFGH
FGHI
GHIJ
HIJ
HIJ
IJ
IJ
IJ
IJ
J
Filiano Nero
Nero Tolve
IS-CE-Bruno
Tricarico
Ituchi
IS-CE-1
Bianco Scalo
Pascià
Maragià
IS-CE-6
Nero Appignano
IS-CE-3
Reale
Ottava
Principe
Quercia Appignano
Vittoria
Palazzo San Gervasio
IS-CE-2
Filiano Bianco
IS-CE-5
IS-CE-7
Sultano
Ares


Kabuli
Desi
r2 (mm)
1,04
1,035
1,03
1,025
1,02
1,015
1,01
1,005
1
0,995
A
A
B
C
CD
CDE
CDE
CDE
DEF
DEF
DEF
DEFG
EFGH
EFGH
EFGH
EFGH
EFGHI
FGHI
GHIJ
HIJ
IJ
IJ
J
J
Nero Tolve
Filiano Nero
IS-CE-Bruno
Nero Appignano
Bianco Scalo
Ituchi
IS-CE-6
Principe
Pascià
Tricarico
Maragià
IS-CE-1
Vittoria
Reale
IS-CE-2
Quercia Appignano
Ottava
Palazzo San Gervasio
IS-CE-3
Filiano Bianco
IS-CE-7
IS-CE-5
Sultano
Ares

(b) Seed shape parameters measured for the lateral side







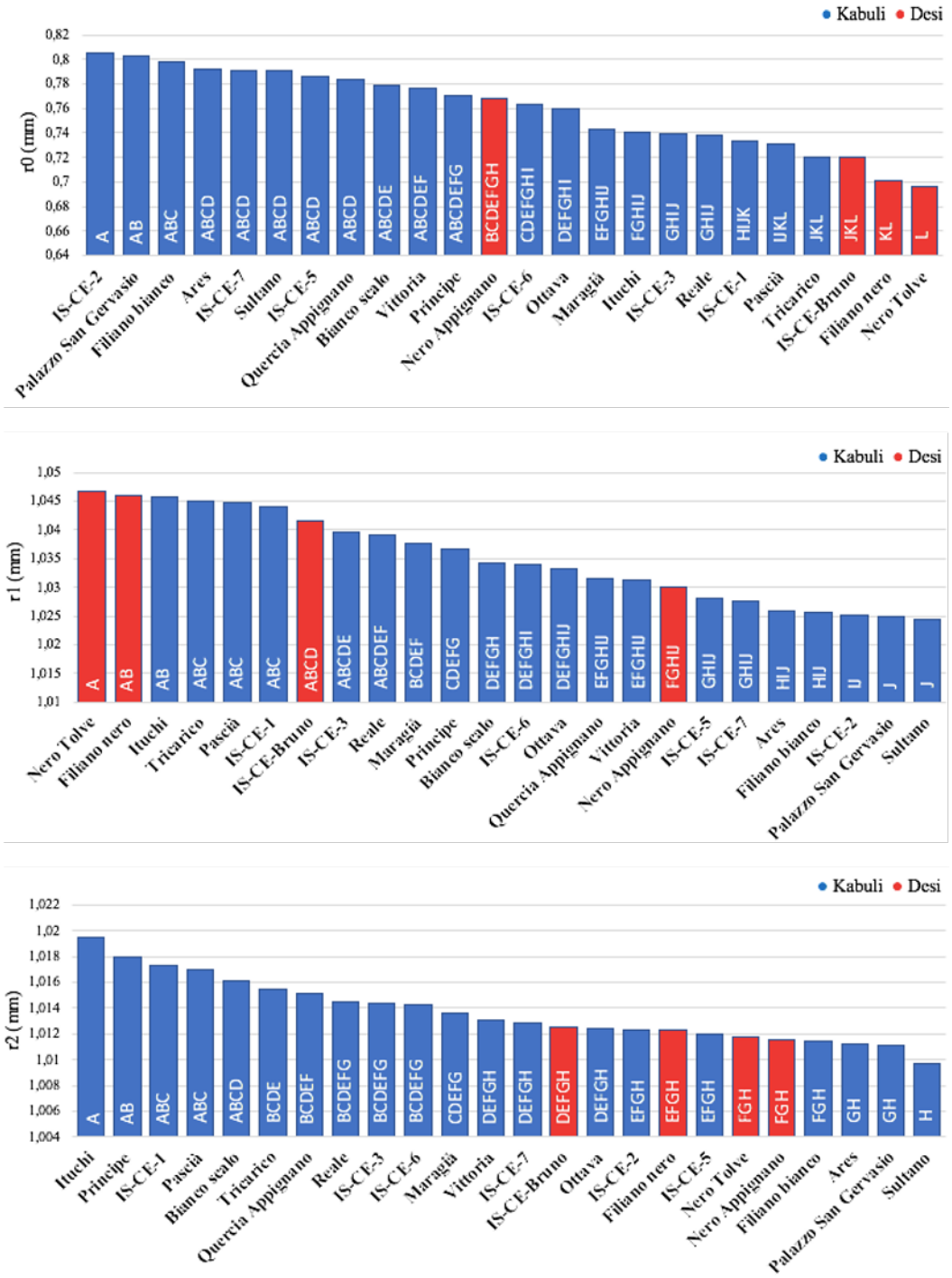
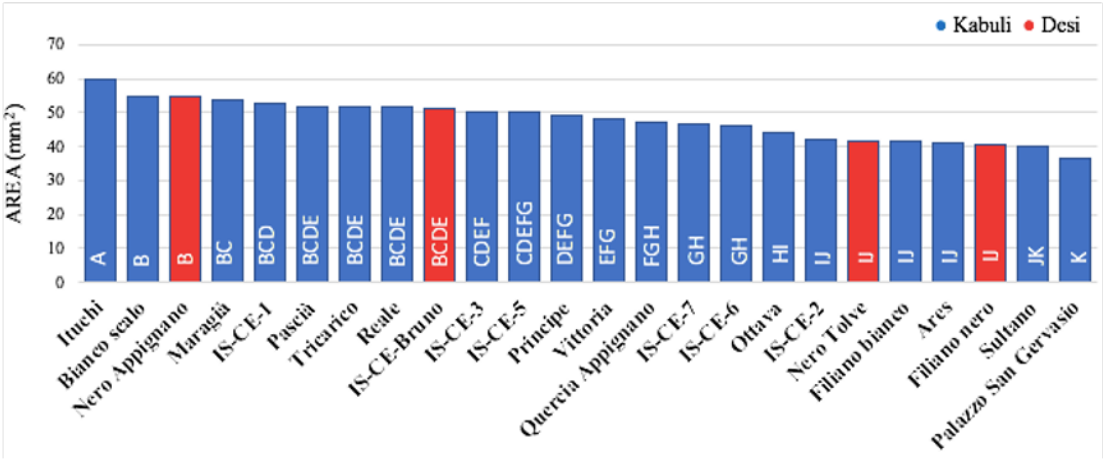
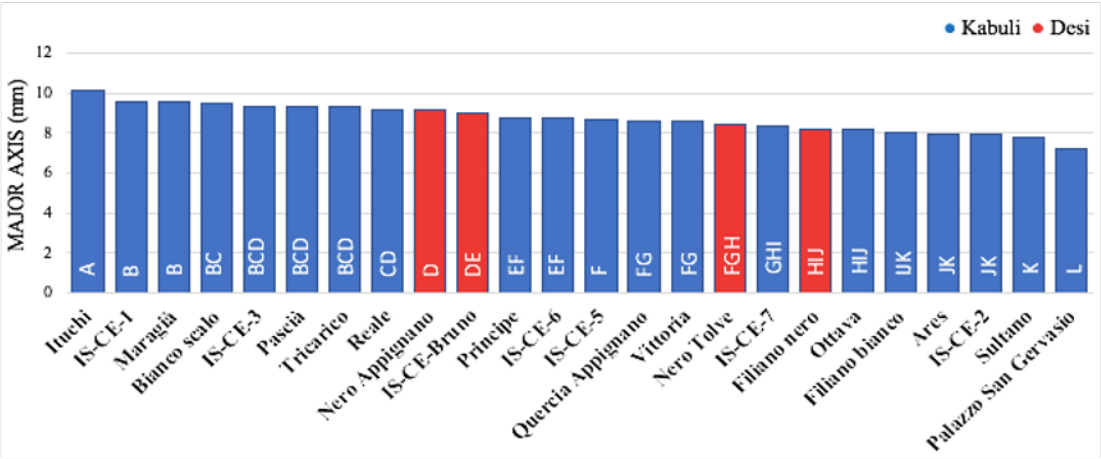
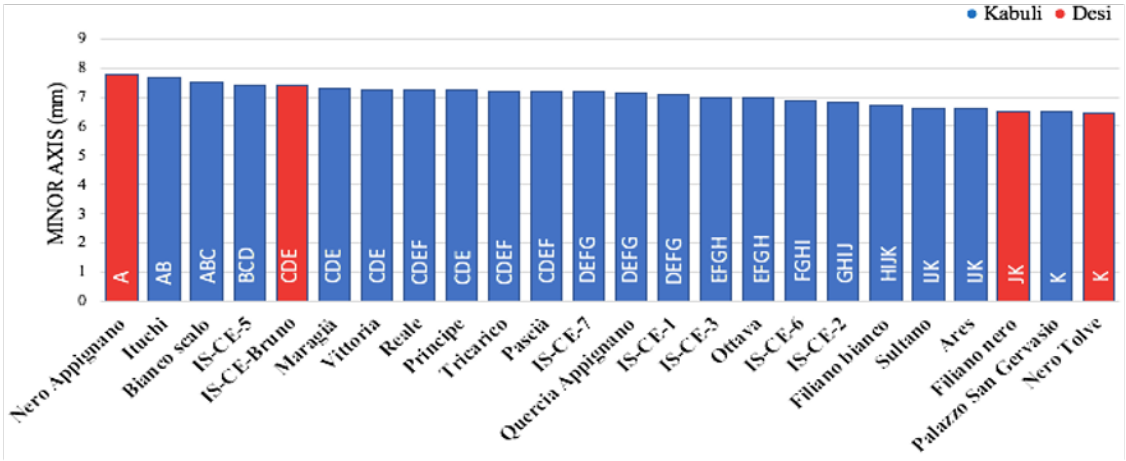


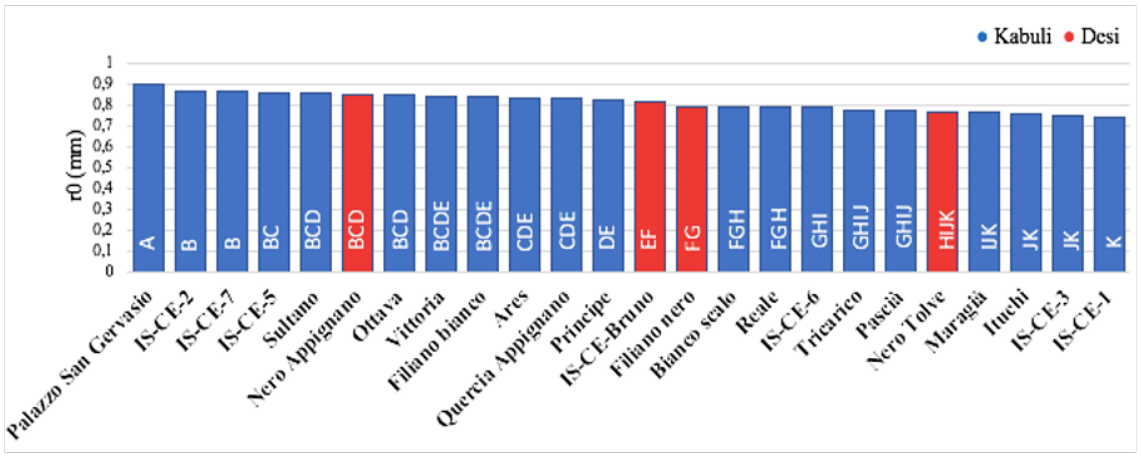
Figure 3.11 - Average area, major axis, minor axis, r0, r1 and r2 for autumn sowing season for horizontal (**a**) and lateral side (**b**); levels not connected by same letter are significantly different ($P < 0.05$; T student test).

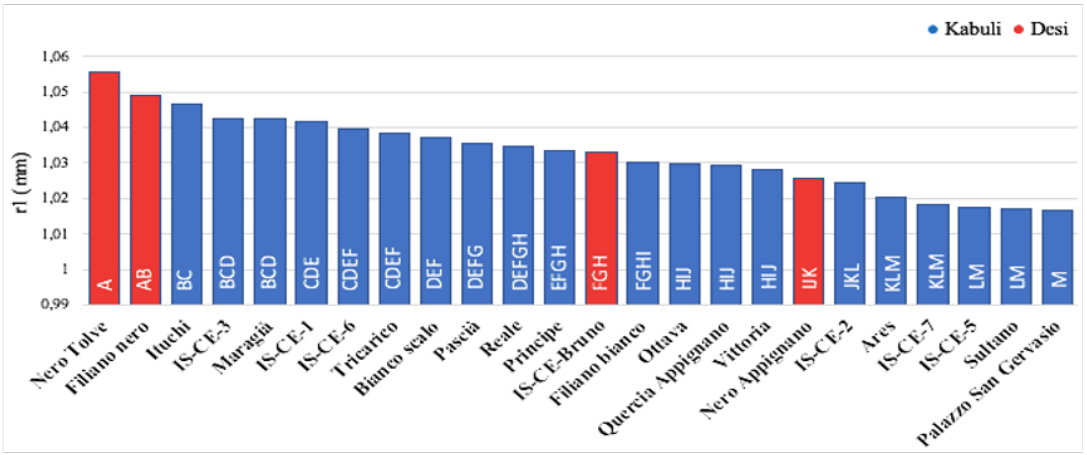
(a) Seed shape parameters measured for the horizontal side

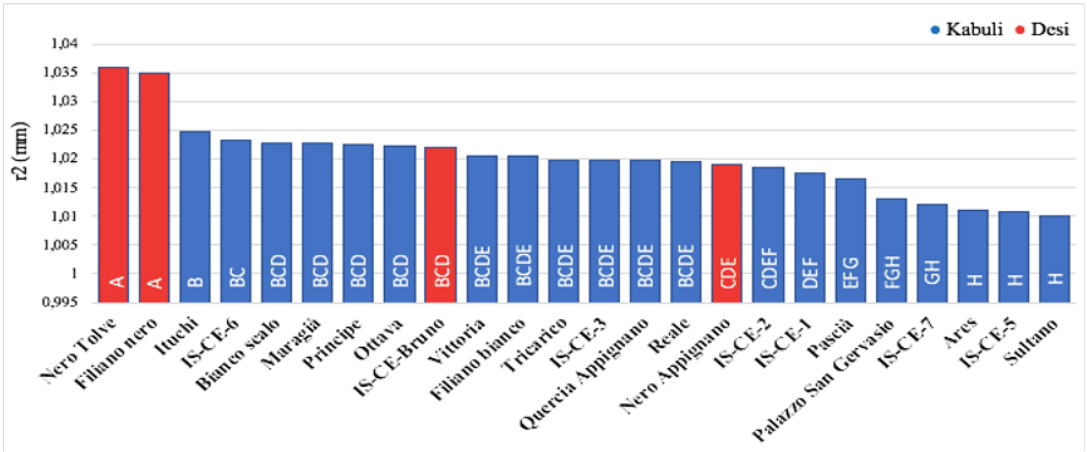




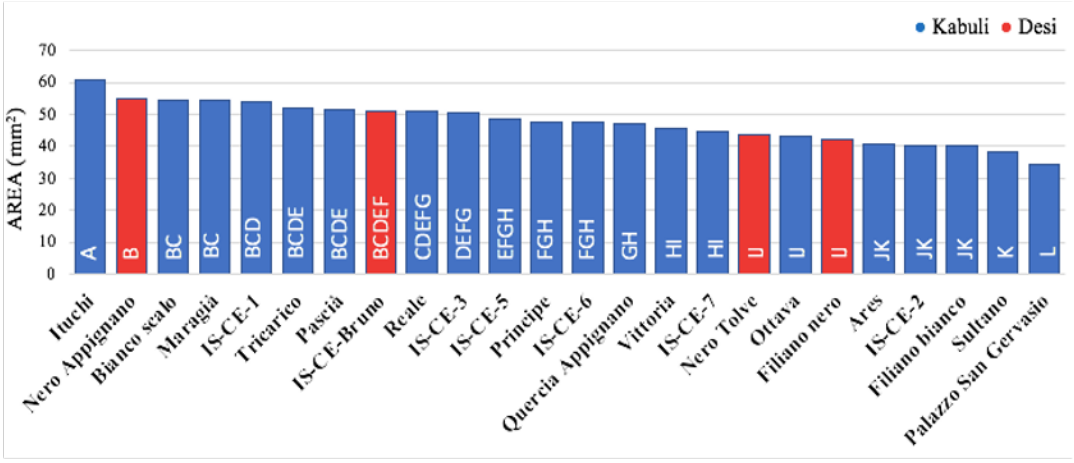


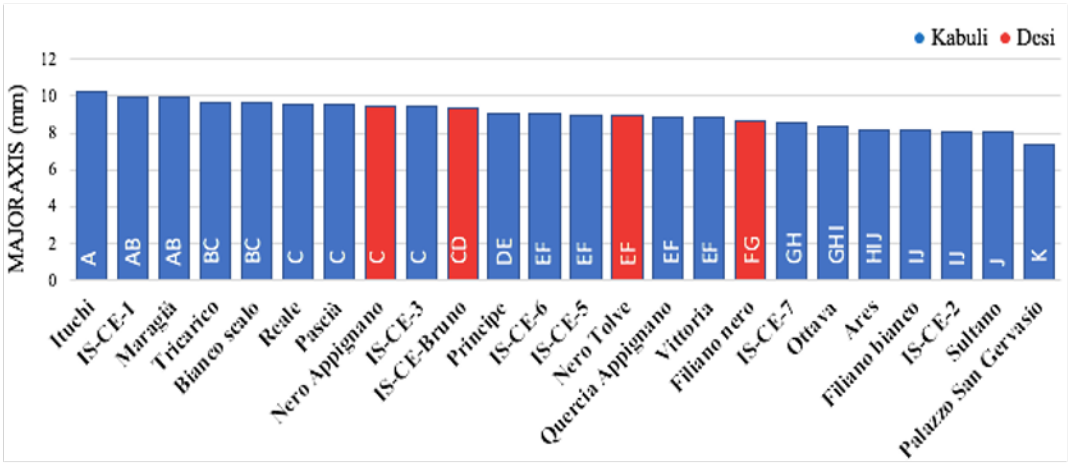

Kabuli
Desi
r0 (mm)
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
A
B
B
BC
BCD
BCD
BCD
BCDE
BCDE
CDE
CDE
DE
EF
FG
FGH
FGH
GHI
GHIJ
GHIJ
HIJK
IJK
JK
JK
K
Palazzo San Gervasio
IS-CE-2
IS-CE-7
IS-CE-5
Sultano
Nero Appignano
Ottava
Vittoria
Filiano bianco
Ares
Quercia Appignano
Principe
IS-CE-Bruno
Filiano nero
Bianco scalo
Reale
IS-CE-6
Tricarico
Pascià
Nero Tolve
Maragià
Ituchi
IS-CE-3
IS-CE-1

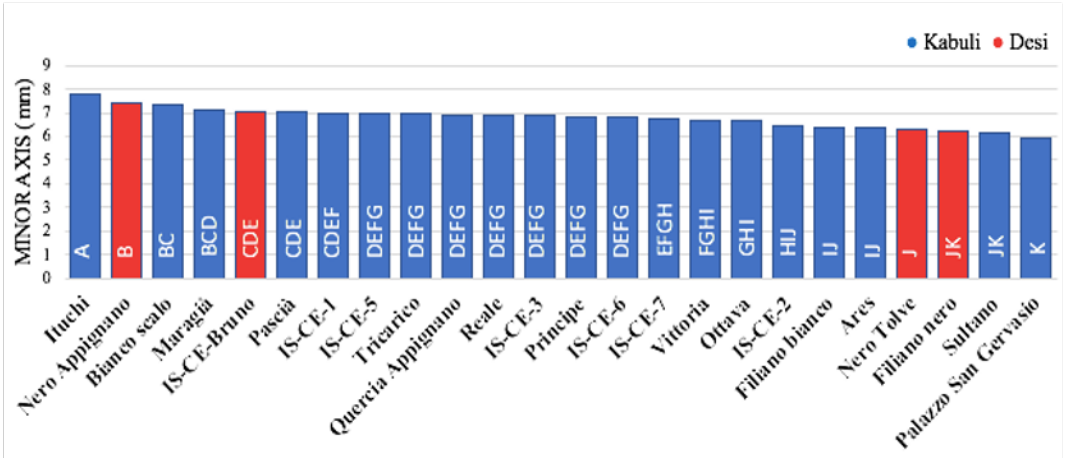

Kabuli
Desi
r1 (mm)
1,06
1,05
1,04
1,03
1,02
1,01
1
0,99
A
AB
BC
BCD
BCD
CDE
CDEF
CDEF
DEF
DEFG
DEFGH
EFGH
FGH
FGHI
HIJ
HIJ
HIJ
IJK
JKL
KLM
KLM
LM
LM
M
Nero Tolve
Filiano nero
Ituchi
IS-CE-3
Maragià
IS-CE-1
IS-CE-6
Tricarico
Bianco scalo
Pascià
Reale
Principe
IS-CE-Bruno
Filiano bianco
Ottava
Quercia Appignano
Vittoria
Nero Appignano
IS-CE-2
Ares
IS-CE-7
IS-CE-5
Sultano
Palazzo San Gervasio


Kabuli
Desi
r2 (mm)
1,04
1,035
1,03
1,025
1,02
1,015
1,01
1,005
1
0,995
A
A
B
BC
BCD
BCD
BCD
BCD
BCD
BCDE
BCDE
BCDE
BCDE
BCDE
BCDE
CDE
CDEF
DEF
EFG
FGH
GH
H
H
H
Nero Tolve
Filiano nero
Ituchi
IS-CE-6
Bianco scalo
Maragià
Principe
Ottava
IS-CE-Bruno
Vittoria
Filiano bianco
Tricarico
IS-CE-3
Quercia Appignano
Reale
Nero Appignano
IS-CE-2
IS-CE-1
Pascià
Palazzo San Gervasio
IS-CE-7
Ares
IS-CE-5
Sultano

(b) Seed shape parameters measured for the lateral side







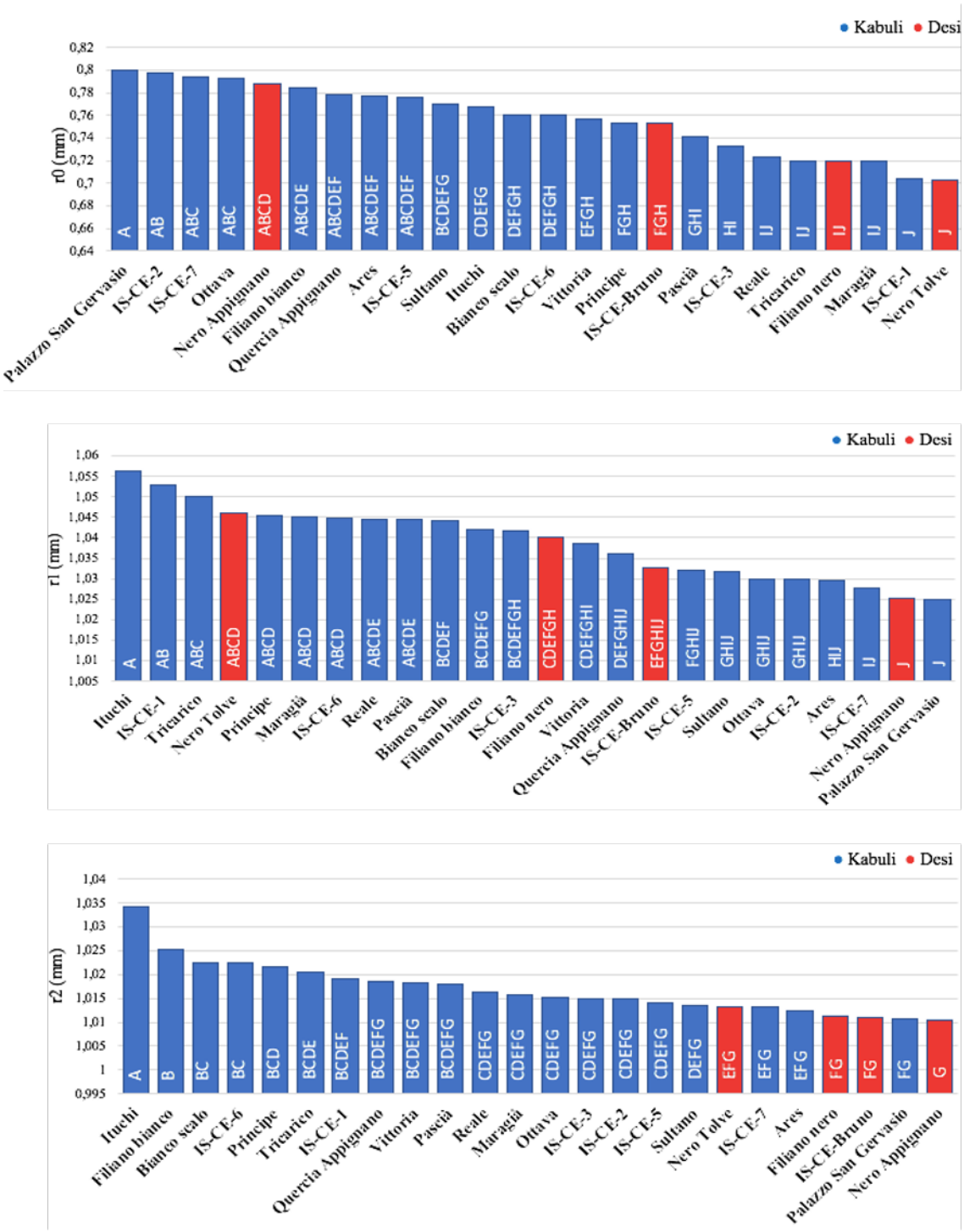
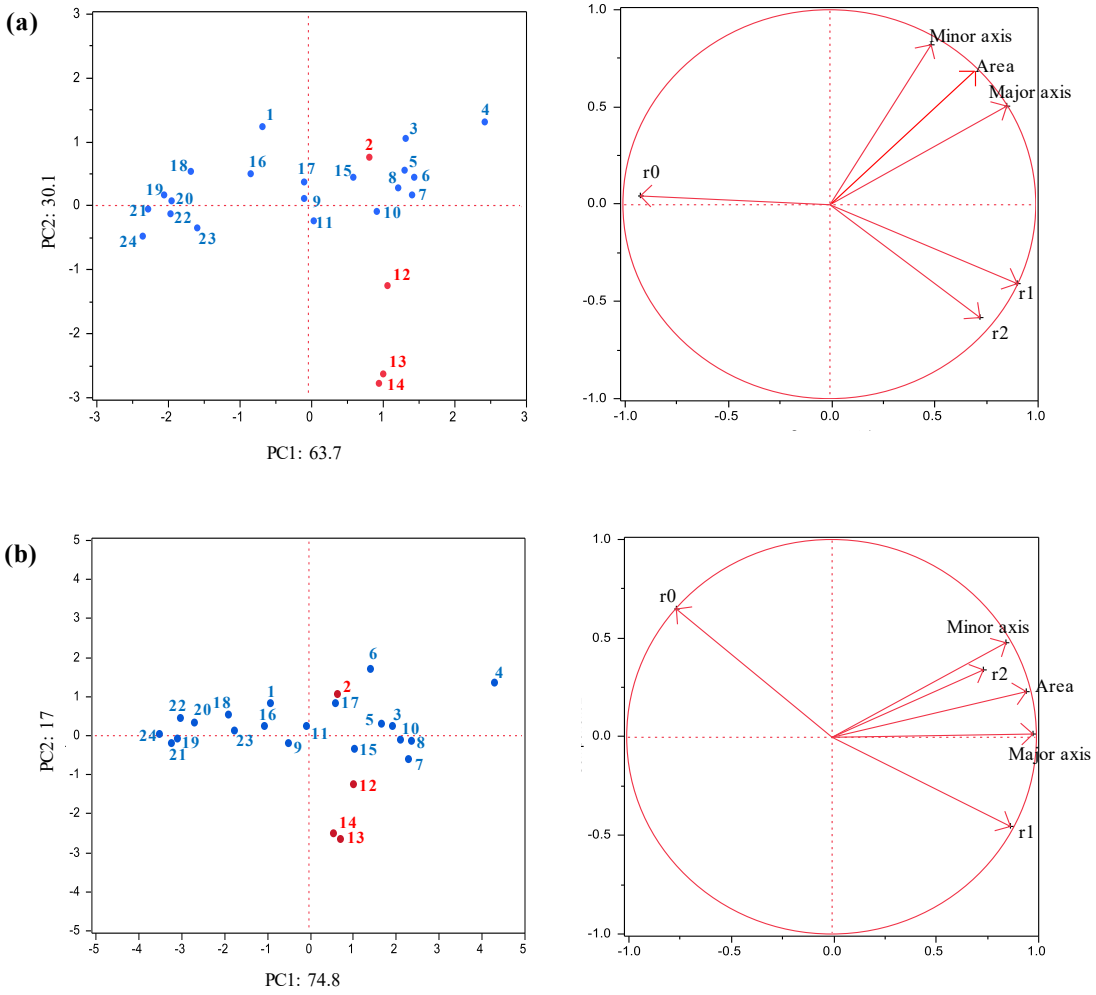
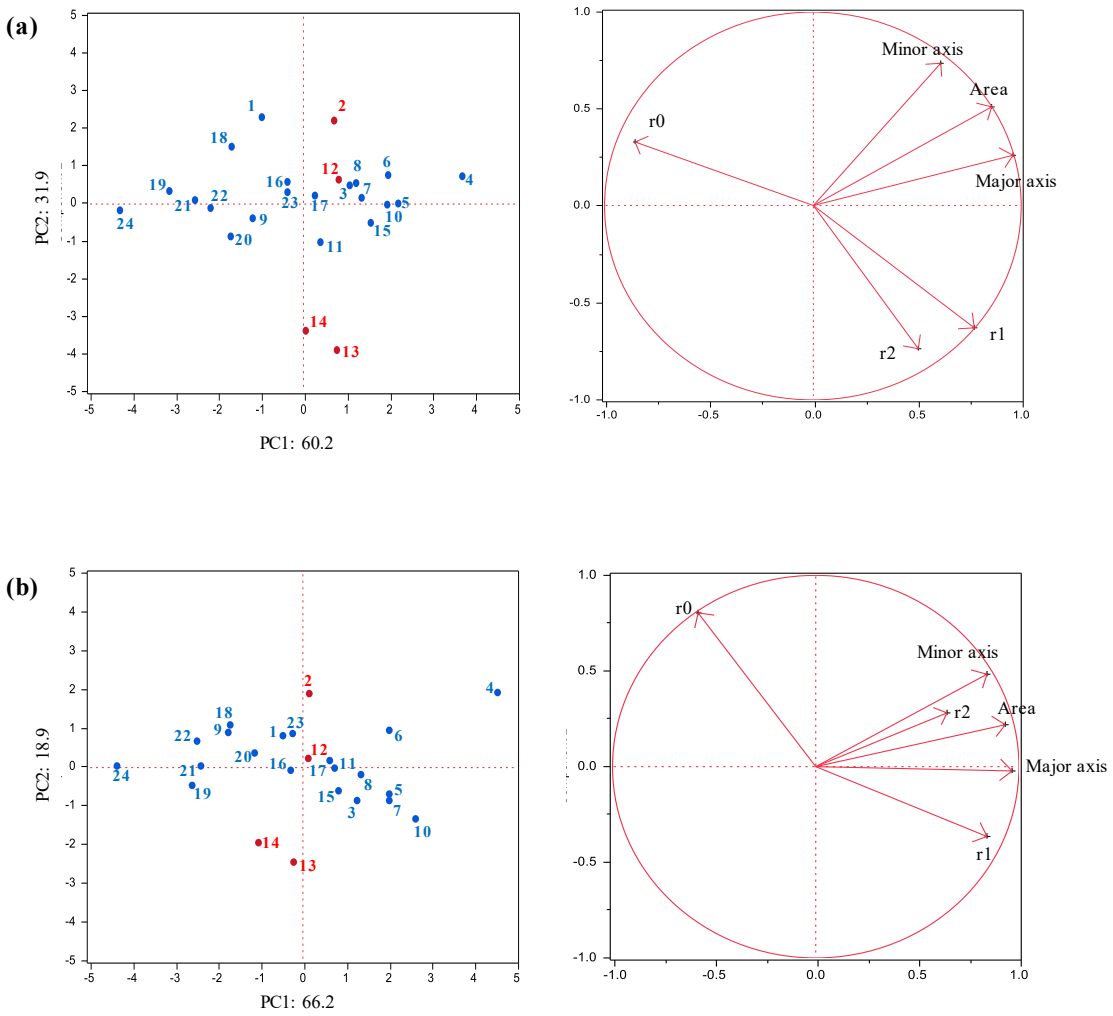


Figure 3.12 - Average area, major axis, minor axis, r0, r1 and r2 for horizontal (**a**) and lateral side (**b**) for spring sowing season; levels not connected by same letter are significantly different ($P < 0.05$; T student test).



1- IS-CE-5	9- Ottava	17- Principe
2- Nero Appignano	10- IS-CE-1	18- IS-CE-7
3- Reale	11- IS-CE-6	19- Sultano
4- Ituchi	12- IS-CE-Bruno	20- Filiano bianco
5- Maragià	13- Nero Tolve	21- Ares
6- Bianco (scalo)	14- Filiano nero	22- IS-CE-2
7- Tricarico	15- IS-CE-3	23- Quercia Appignano
8- Pascià	16- Vittoria	24- Palazzo San Gervasio

Figure 3.13 - PCA and loading plot for the dimensional traits of chickpea seeds of autumn season for horizontal (**a**) and lateral side (**b**). PCA and loading plot were performed considering Desi and Kabuli types together. Red, Desi type, blue Kabuli type.

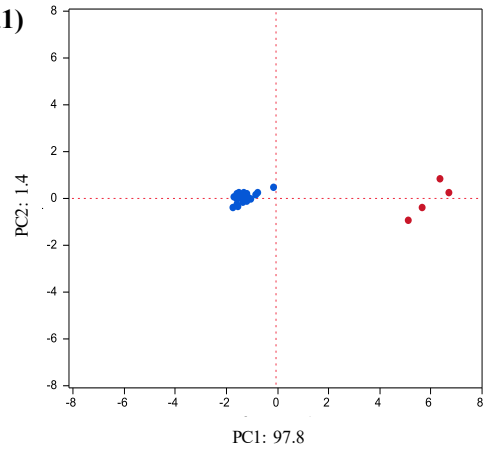


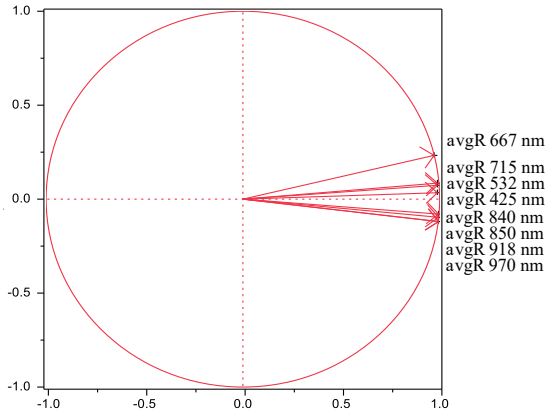
1- IS-CE-5	9- Ottava	17- Principe
2- Nero Appignano	10- IS-CE-1	18- IS-CE-7
3- Reale	11- IS-CE-6	19- Sultano
4- Ituchi	12- IS-CE-Bruno	20- Filiano bianco
5- Maragià	13- Nero Tolve	21- Ares
6- Bianco (scalo)	14- Filiano nero	22- IS-CE-2
7- Tricarico	15- IS-CE-3	23- Quercia Appignano
8- Pascià	16- Vittoria	24- Palazzo San Gervasio

Figure 3.14 - PCA and loading plot for the dimensional traits of chickpea seeds of spring season for horizontal (**a**) and lateral side (**b**). PCA and loading plot were performed considering Desi and Kabuli types together. Red, Desi type, blue Kabuli type.

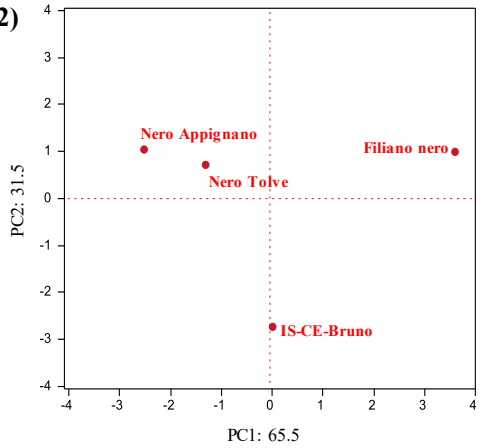
A PCA analysis was also carried out based on the reflectance values obtained by the multispectral camera (**Figure 3.15**). These data can help in discriminating genotypes on the basis of their seed color. The PCA was performed considering data recorded for the horizontal side for all the genotypes (**Figure 3.15a1** and **b1**) and separately for Desi and Kabuli types (**Figure 3.15a2** and **b2**). The PC1 and PC2 explain 97.8 % and 1.4 % of the total variance, respectively, for the autumn sowing season experiment, and 97.3% and 2%, respectively, for the spring sowing season experiment. The PC1 is positively correlated to all the reflectance values in both the sowing seasons. The PC2 is positively correlated to avgR 667 nm and avgR 715 nm (**Figure 3.15a1** and **b1**). The Desi types were clearly separated from all the other genotypes, being characterized by higher reflectance values (**Figure 3.15a1** and **b1**). By performing the PCA separately for Desi and Kabuli accessions it was possible to highlight the relationships among genotypes belonging to these two types on the basis of their color. In particular, it was possible to separate all the four Desi types, with IS-CE-Bruno genotype showing the highest reflectance value for avgR 667 nm and avgR 715 nm and the Nero Appignano genotype having the lowest reflectance values for almost all the different wavelengths and being the darkest chickpea seeds analysed (**Figure 3.15a2** and **b2**) in both autumn and spring sowing field trials. Filiano nero and Nero Tolve behaved a little bit differently in autumn and spring sowing trials (**Figure 3.15a2** and **b2**). Among Kabuli types, genotypes resulted dispersed and mainly separated on the basis of PC1. Some genotypes behaved similarly in both autumn and spring sowing season trials, with Ituchi, Bianco scalo, IS-CE-5 and Principe genotypes showing the highest reflectance values for most of the different wavelengths (white color of seeds), while the opposite was for Ares, IS-CE-2, Filiano bianco, and Palazzo San Gervasio.

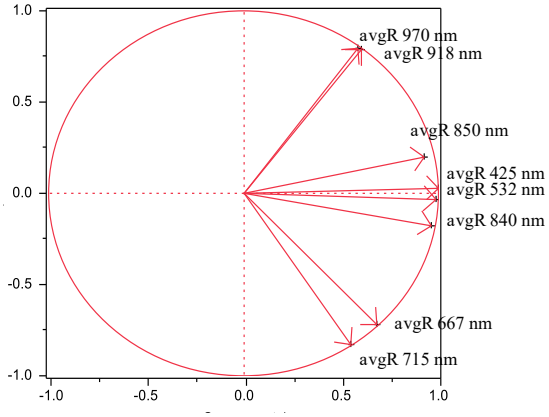
(a1)

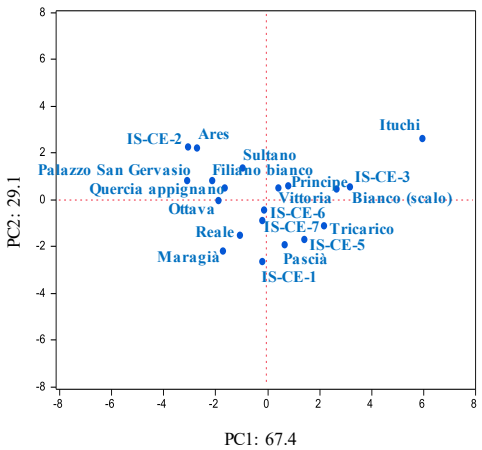

PC2: 1.4
PC1: 97.8

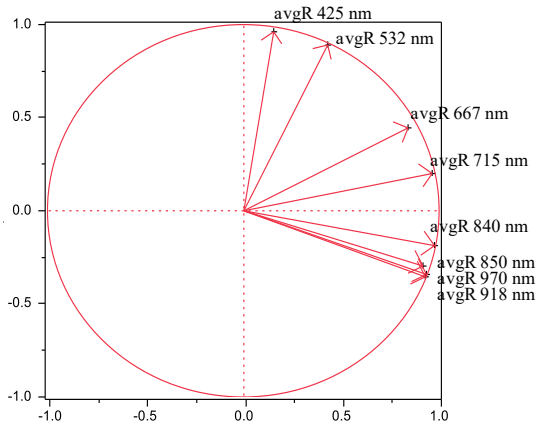

avgR 667 nm
avgR 715 nm
avgR 532 nm
avgR 425 nm
avgR 840 nm
avgR 850 nm
avgR 918 nm
avgR 970 nm

(a2)


Nero Appignano
Filiano nero
Nero Tolve
IS-CE-Bruno
PC2: 31.5
PC1: 65.5


avgR 970 nm
avgR 918 nm
avgR 850 nm
avgR 425 nm
avgR 532 nm
avgR 840 nm
avgR 667 nm
avgR 715 nm


Ituchi
IS-CE-2
Ares
Sultano
Palazzo San Gervasio
Filiano bianco
IS-CE-3
Principe
Quercia appignano
Vittoria
Bianco (scalo)
Ottava
IS-CE-6
IS-CE-7
Tricarico
Reale
IS-CE-5
Maragià
Pascià
IS-CE-1
PC2: 29.1
PC1: 67.4


avgR 425 nm
avgR 532 nm
avgR 667 nm
avgR 715 nm
avgR 840 nm
avgR 850 nm
avgR 970 nm
avgR 918 nm

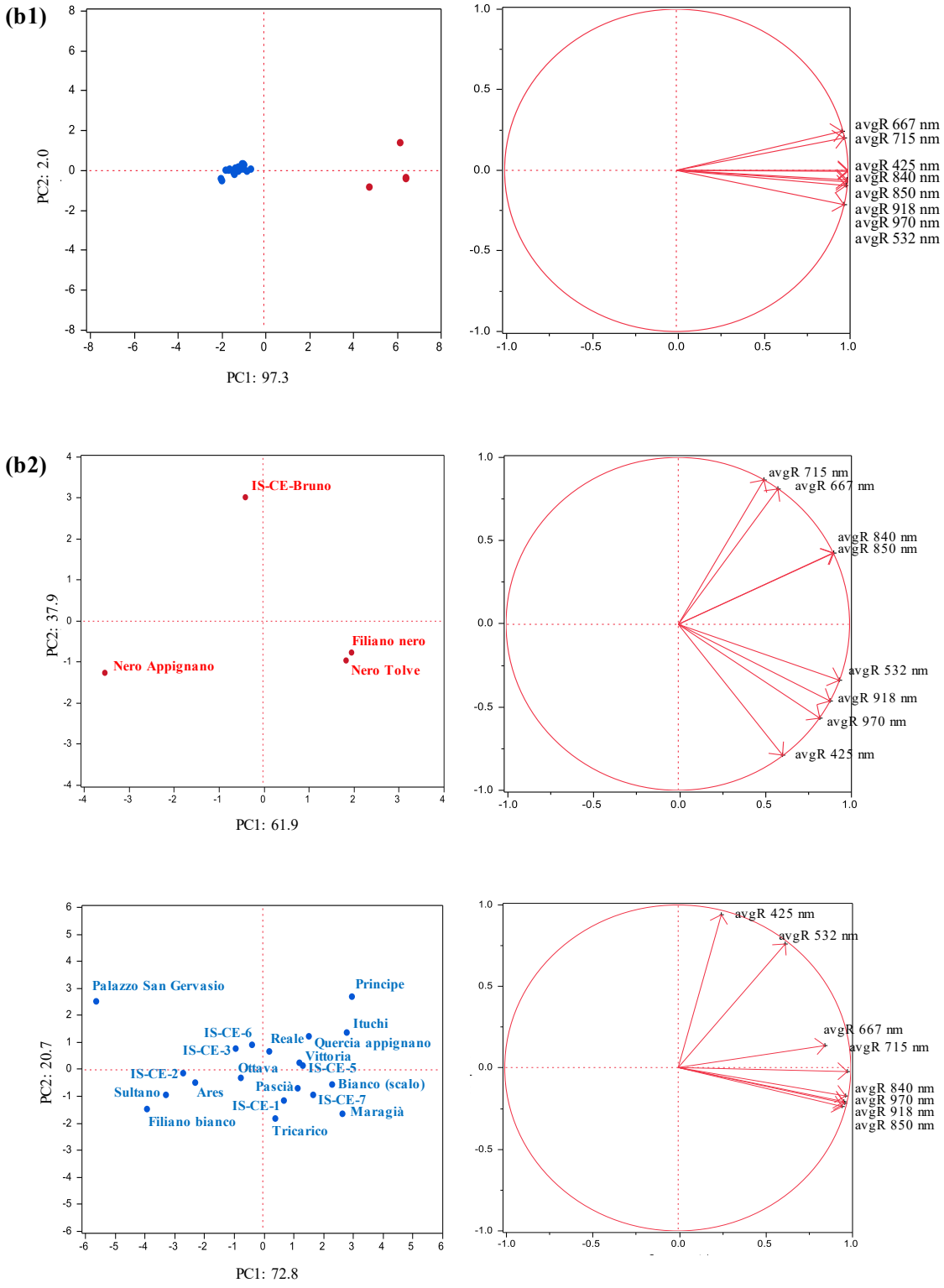


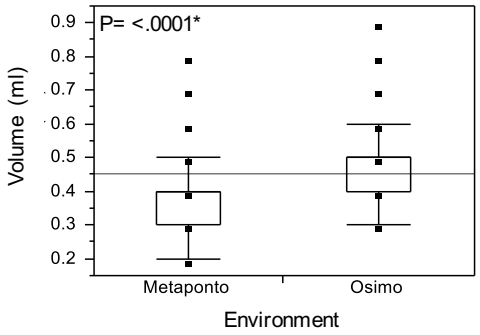
Figure 3.15 - PCA and loading plot for the color trait of chickpea seeds of autumn (**a**) and spring season (**b**). PCA and loading plot were performed considering Desi and Kabuli types together (**a1**, **b1**) and Desi and Kabuli types separated (**a2**, **b2**). Red, Desi type, blue Kabuli type.

LENTIL

Seeds of all the lentil accessions (for each of the three replicates), grown in the two localities (Osimo and Metaponto) in 2019, were characterized for average 1000 seed weight (g), size (SSI), volume (ml), hydration (%), shape (mm) and color (nm).

Analysis of variance components was carried out for 1000 seed weight; a significant higher 1000 seed weight was found for seeds obtained in Metaponto compared to Osimo locality (P=0.017) (**Figure 3.16**). No significant cross interactions were detected as showed by the interaction plot (**Figure 3.16**). Heritability was not so high, with 53.5% of total variance explained by the genotype. The model inferred 24.5% of the total variance to GEI and 7.1% to effects of environmental factors.

Component	Percent of Total Variance
Genotype	58.7
Environment	17.7
Genotype*environment	9.8
Residual	13.8
Total	100.0



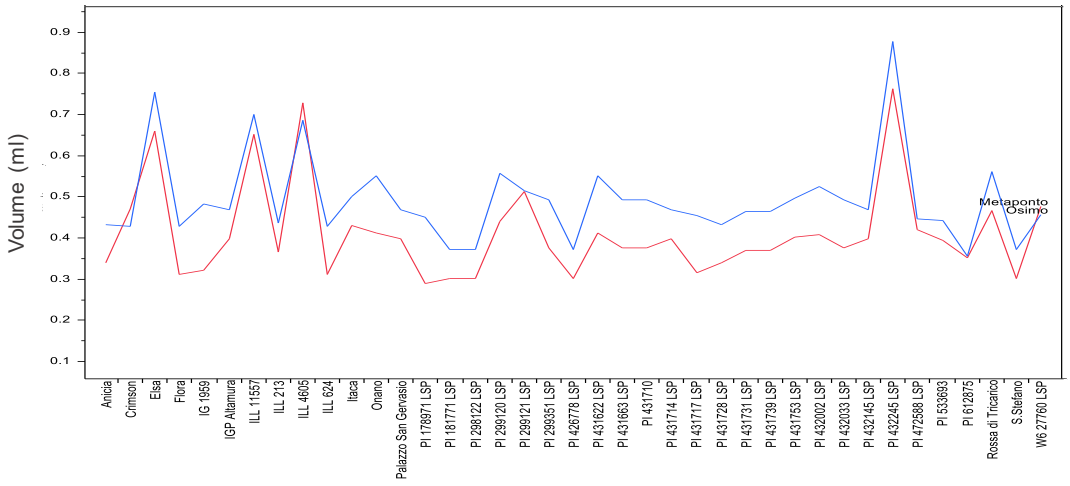


Figure 3.16 - Interaction plot GEI analysis and variance components for 1000 seed weight.

Distribution and broad sense heritability (h^2) were calculated for SSI and volume of seeds from Osimo and Metaponto trials (**Figure 3.17**). High estimates were obtained for SSI (h^2 = 93.0% and 96.8% for Osimo and Metaponto trials, respectively) and volume (h^2 = 76.5% and 87.8% for Osimo and Metaponto trials, respectively).

For the same traits, one-way ANOVA highlighted significant differences among the different lentil accessions both for Osimo (**Figure 3.18**) and Metaponto (**Figure 3.19**) trials. High variability for SSI and Volume was detected among accessions for both the localities. SSI of lentils from Osimo ranged from 3.56 (Santo Stefano) to 5.20 (PI 432245 LSP), while it ranged from 3.58 (PI 426778 LSP) to 5.31 (ILL 4605) within lentils from Metaponto. Volume ranged from 0.33 ml (PI 612875 LSP) to 0.9 ml (PI 432245 LSP) ml for lentils from Osimo and from 0.27 ml (PI 178971 LSP) to 0.77 ml (ILL 4605) ml for those from Metaponto trial.

Analysis of the variance components for the two localities was also performed as showed in **Figure 3.20**. SSI did not show significant difference between the two localities. The relative interaction plot shows no cross interaction, the only exception being ILL 4605 genotype, for which SSI is higher in Metaponto compared to Osimo locality. Variance components show high heritability for SSI, with 82.7% of the total variance explained by the genotype. The Volume resulted significant higher in Osimo compared to Metaponto (P = <0.0001*). The interaction plot shows not cross interactions. Variance components show 58.7% of the total variance explained by the genotype, 17.7% to locality, 9.8% to GEI and 13.8% to environmental factors.

The parameters of Hydration max (Hmax) and Hydration rate (Hrate) obtained from the model are not replicated. **Figure 3.21** and **Figure 3.22** show the parameters for all the genotypes analyzed in both the localities. PI 432245 LSP and Elsa genotypes from Osimo trial showed the highest values of Hydration max; PI 432245 LSP showed the highest Hydration max also in Metaponto trial, followed by ILL 4605 and Elsa genotypes. Santo Stefano and PI 612875 from Osimo and Santo Stefano and PI 426778 LSP from Metaponto resulted the genotypes for which the seeds showed the lowest Hydration max **(Figure 3.21**). PI 431622 LSP, PI 431731 LSP, PI 431714 LSP, PI 431717 LSP (Osimo) and ILL 213, IG 1959, PI 431622 LSP, and PI 431731 LSP (Metaponto) showed the highest values for Hydration rate, while W6 27760 LSP, PI 299121 LSP (Osimo) and Rossa Tricarico and PI 426778 LSP (Metaponto) the lowest values for such trait. The correlated response between Osimo and Metaponto was significant (P < 0.0001) with high coefficients (0.82 and 0.76) for the hydration max and hydration rate, respectively.

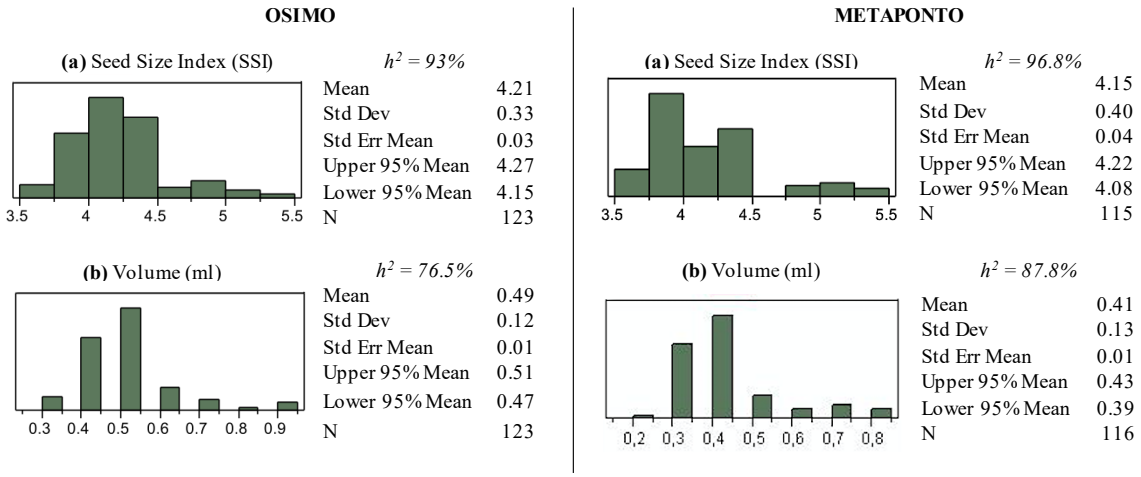


Figure 3.17 - SSI (**a**) and volume (**b**) distribution and heritability estimation of Osimo and Metaponto sowing.

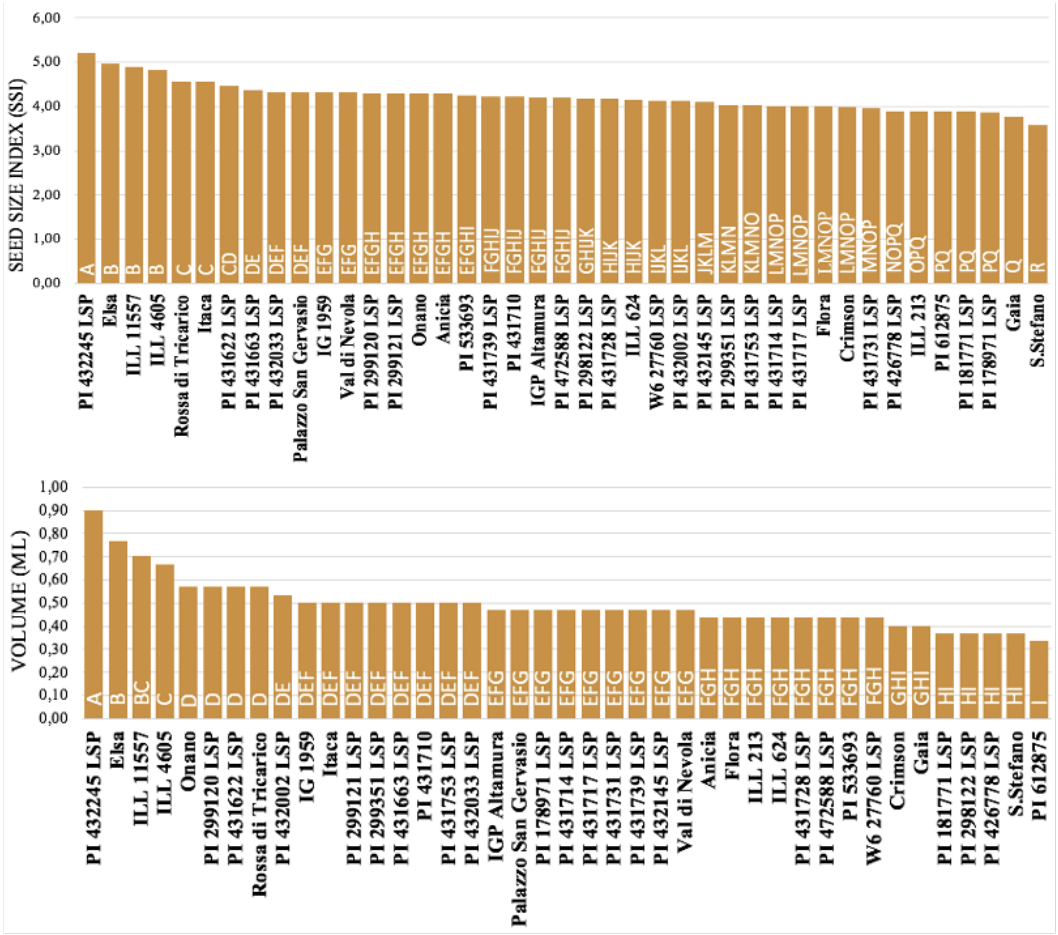


Figure 3.18 - Average SSI (**a**) and Volume (**b**) for Osimo trial; levels not connected by same letter are significantly different ($P < 0.05$; T student test).

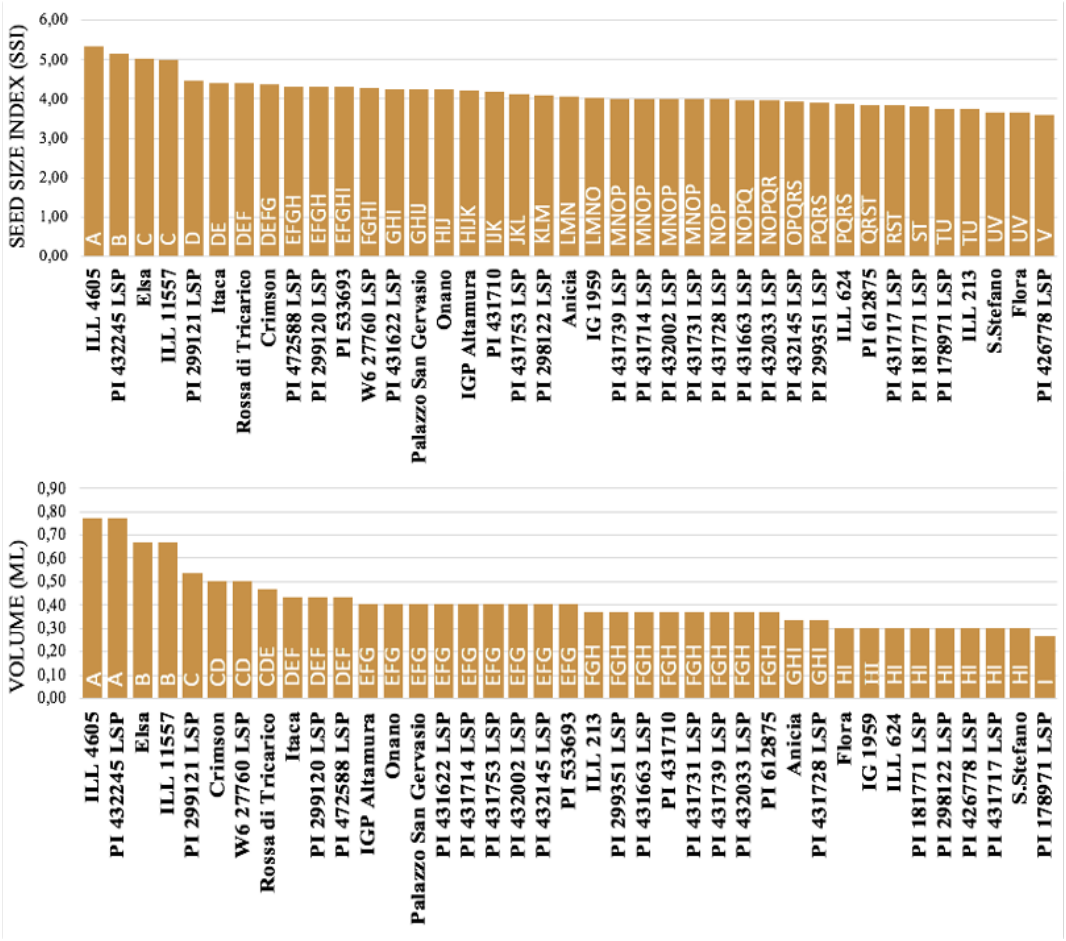
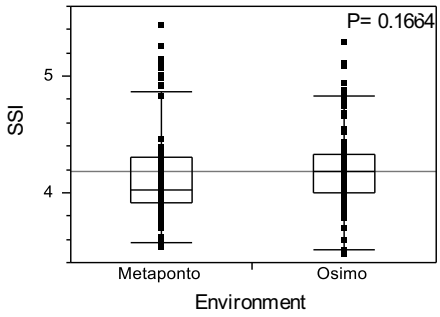
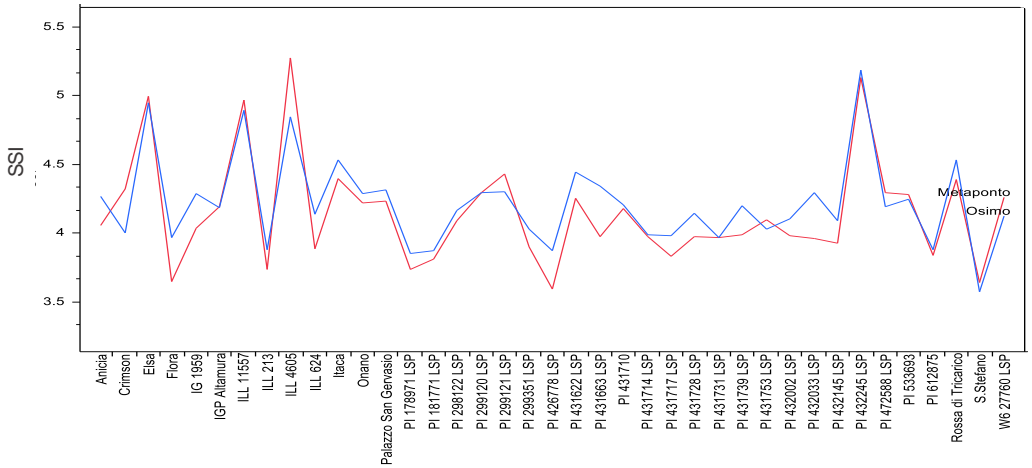


Figure 3.19 - Average SSI (**a**) and Volume (**b**) for Metaponto trial; levels not connected by same letter are significantly different ($P < 0.05$; T student test).

(a)

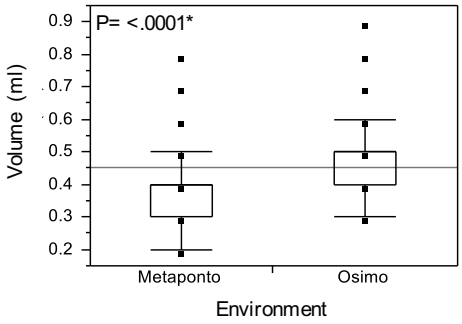
Component	Percent of Total Variance
Genotype	82.7
Environment	1.5
Genotype*environment	11.2
Residual	4.6
Total	100.0





(b)

Component	Percent of Total Variance
Genotype	58.7
Environment	17.7
Genotype*environment	9.8
Residual	13.8
Total	100.0



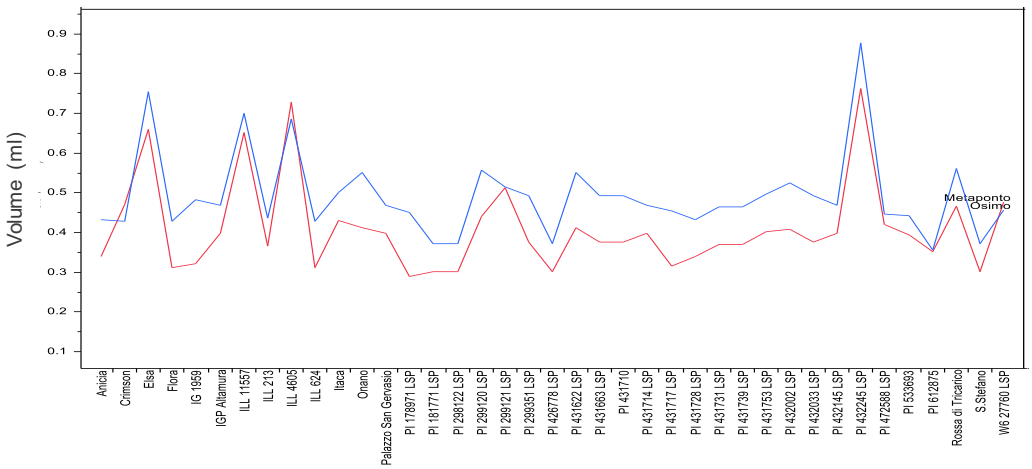
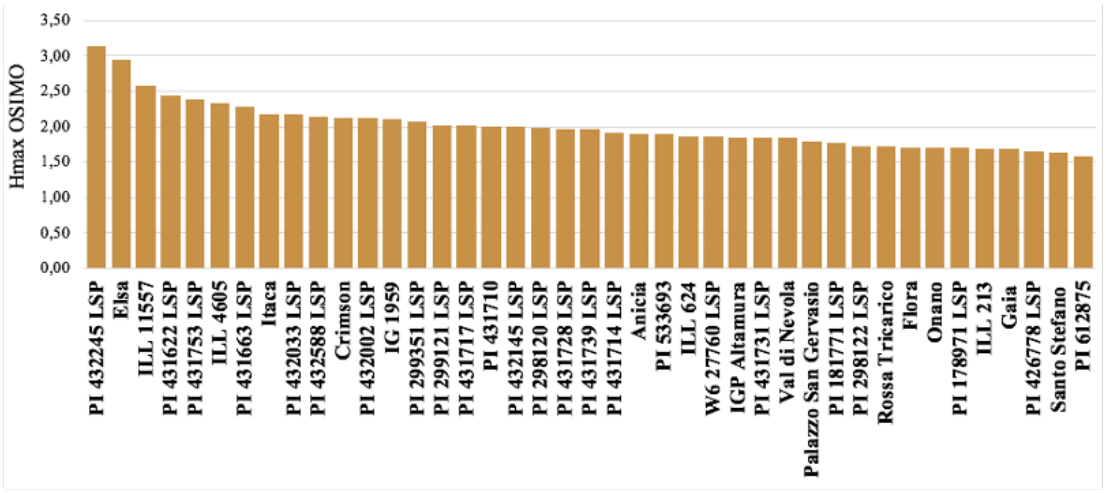
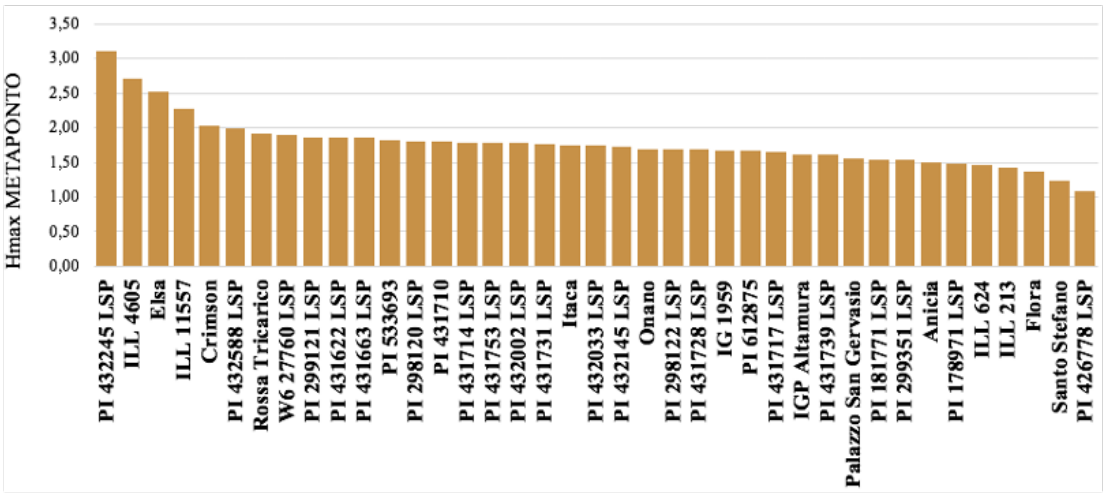


Figure 3.20 - Interaction plot GEI analysis and variance components for SSI (**a**) and Volume (**b**).





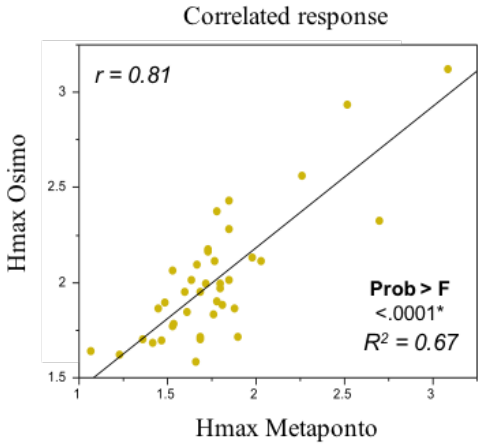
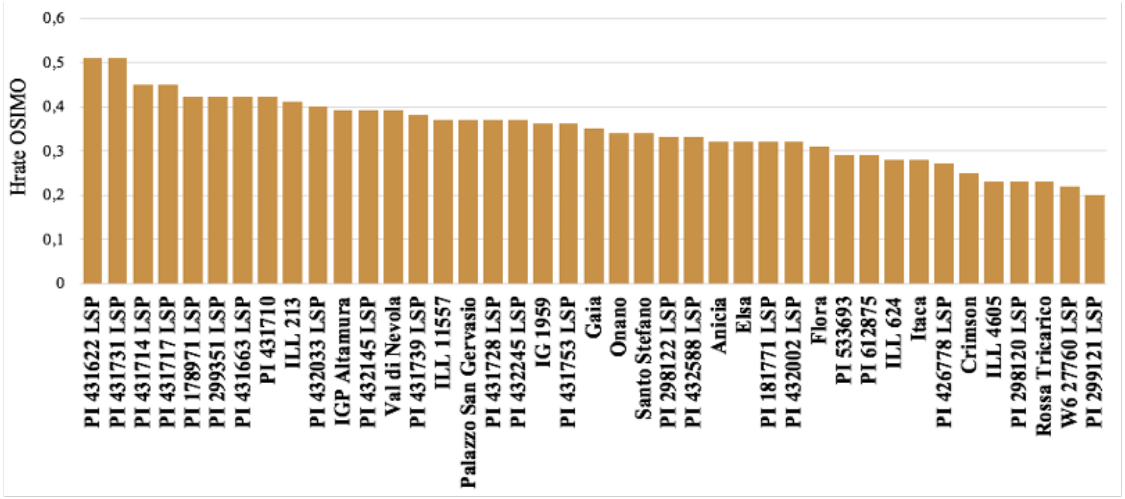
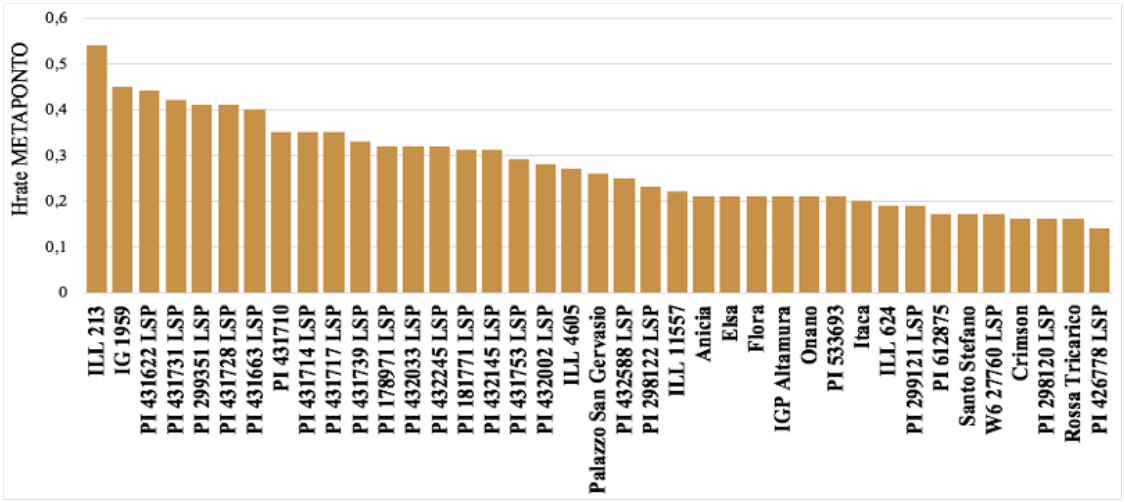


Figure 3.21 - Hydration max for all genotypes of Osimo and Metaponto.





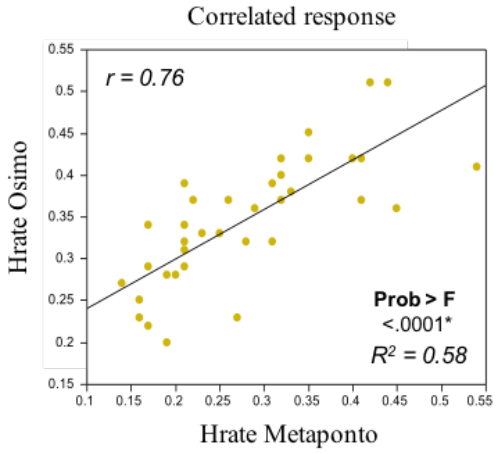
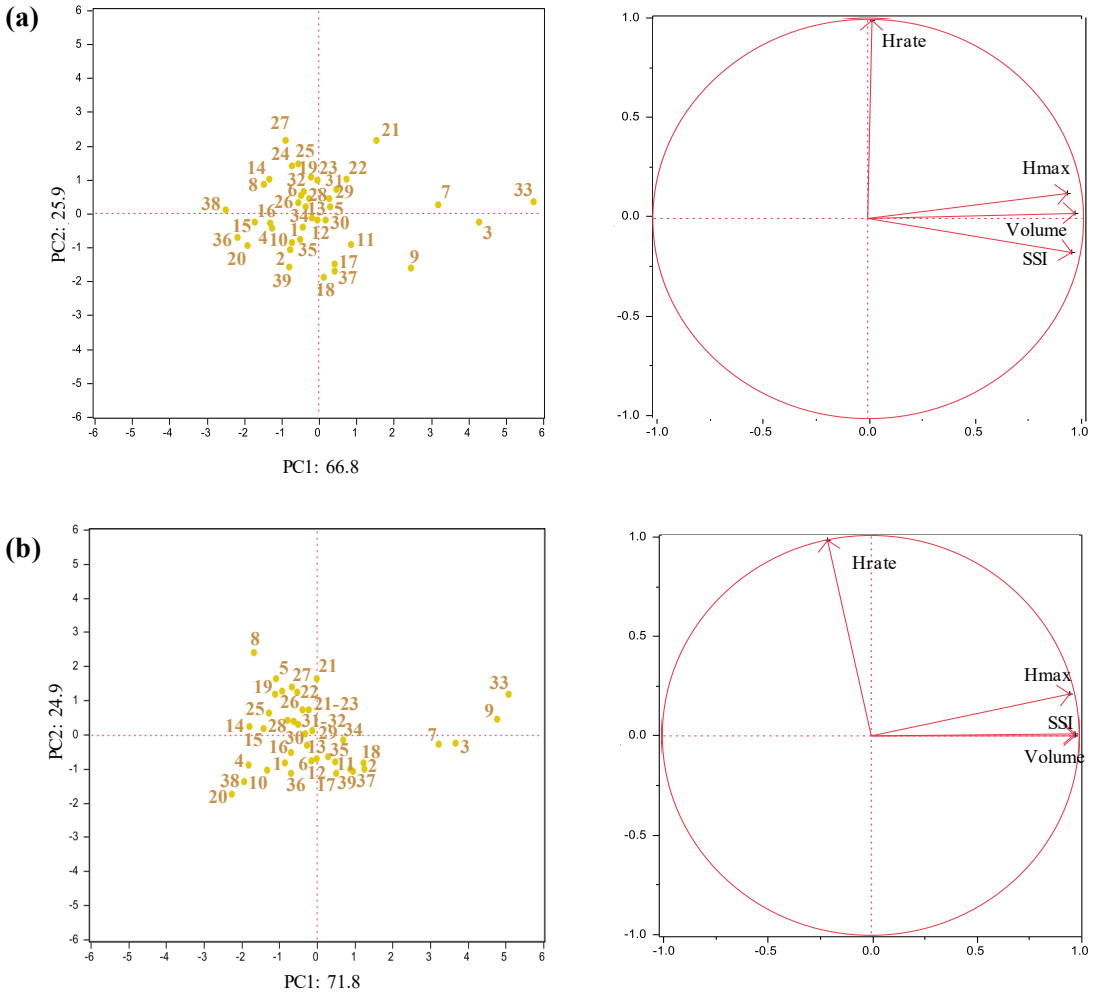


Figure 3.22 - Hydration rate for all genotypes of Osimo and Metaponto.

Figure 3.23 shows the PCA carried out combining SSI, volume, hydration max and hydration rate traits of Osimo (**Figure 3.23a**) and Metaponto (**Figure 3.23b**) sowing. The Principal Component 1 (PC1) and Principal Component 2 (PC2) obtained from Osimo sowing explain 66.8% and 25.9% of the total variance. For Metaponto sowing, PC1 and PC2 explain 71.8% and 24.9% of the total variance. Independently by the locality, the PC1 is positively correlated to SSI, volume and hydration max, while it showed slightly negative correlation with th hydration rate in Metaponto sowing. The PC2 showed positive correlation with the hydration traits, independently by the locality. Slightly negative correlation was found between PC2 and SSI of Osimo sowing. Elsa, ILL 11557, ILL 4605 and PI 472588 LSP resulted separated from the other genotypes analyzed showing the highest values for SSI, volume and hydration max, independently by the locality.

Correlations between SSI, volume, hydration max and hydration rate of the two localities were also investigated (**Figure 3.24**). Significant positive correlation was found between SSI, volume and hydration traits, of the two localities, as well as between SSI and volume ($r = 0.94$ and $r = 0.88$ for Osimo and Metaponto sowing respectively), SSI and hydration max ($r = 0.91$ and $r = 0.79$ for Osimo and Metaponto sowing respectively), volume and hydration max ($r = 0.84$ and $r = 0.91$ for Osimo and Metaponto sowing respectively) ($P < 0.05$). No significant negative correlation was found between analyzed traits.



1- Anicia	9- ILL 4605	17- PI 299120 LSP	25- PI 431717 LSP	33- PI 472588 LSP
2- Crimson	10- ILL 624	18- PI 299121 LSP	26- PI 431728 LSP	34- PI 533693
3- Elsa	11- Itaca	19- PI 299351 LSP	27- PI 431731 LSP	35- PI 612875
4- Flora	12- Onano	20- PI 426778 LSP	28- PI 431739 LSP	36- Rossa Tricarico
5- IG 1959	13- Palazzo S. Gervasio	21- PI 431622 LSP	29- PI 431753 LSP	37- S. Stefano
6- IGP Altamura	14- PI 178971 LSP	22- PI 431663 LSP	30- PI 432002 LSP	38- PI W6 27760 LSP
7- ILL 11557	15- PI 181771 LSP	23- PI 431710	31- PI 432145 LSP	39- Gaia
8- ILL 213	16- PI 298122 LSP	24- PI 431714 LSP	32- PI 432245 LSP	40- Val di Nevola

Figure 3.23 - PCA and loading plot for SSI, volume, hydration max and hydration rate traits of lentil seeds of Osimo (**a**) and Metaponto sowing(**b**).

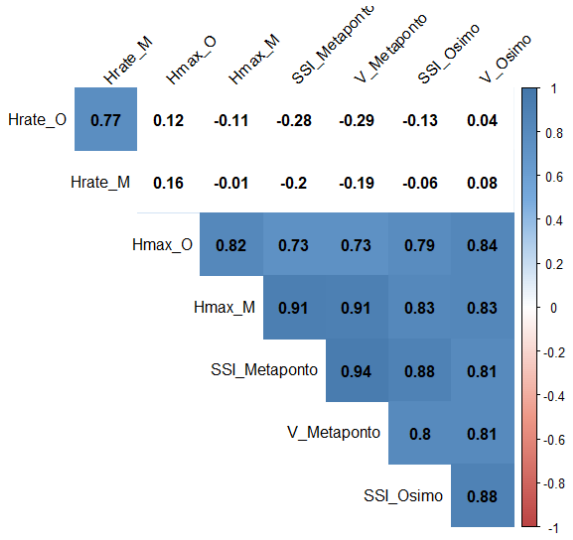


Figure 3.24 – Correlation (Pearson's coefficient, r) between SSI, Volume, hydration max and hydration rate of Osimo and Metaponto sowing.

Distribution and broad sense heritability (h^2) were also investigated for seeds from both the localities for the following shape traits: area (mm^2), major axis (mm), minor axis (mm), r0 (mm), r1 (mm) and r2 (mm) (**Figure 3.25**). Differently from chickpea seeds, these traits were recorded only for the horizontal side of the seeds. Considering the Osimo trial, the highest heritability estimates were obtained for minor axis (h^2 = 75.2%), area (h^2 = 74.9%), major axis (h^2 = 72.2%) and r0 (h^2 =70.1%) (**Figure 3.25**). Intermediate heritabilities were found for the r1 (h^2 = 49.7%) and r2 (h^2 = 53.1%). Seeds from Metaponto trial showed higher heritability values compared to Osimo trial for all the shape parameters, except for r2 (h^2 = 45.9% for Metaponto) (**Figure 3.25**).

For the same traits, one-way ANOVA (**Figure 3.26** and **Figure 3.27**) and PCA (**Figure 3.29**) were performed. One-way ANOVA highlighted significant differences among the different accessions for both Osimo and Metaponto trials for all the dimensional traits analyzed (P = <0.0001*). For Osimo trial, area showed a range of variability from 14.73 mm^2 (PI 426778 LSP) to 23.79 mm^2 (ILL 11557), while for Metaponto, such trait varied from 14.91 mm^2 (PI 432145 LSP) to 24.46 mm^2 (PI 432245 LSP) (**Figure 3.26** and **Figure 3.27**). In Osimo, the highest values for major and minor axis were 5.68 mm and 5.33 mm, respectively for ILL 11557 genotype, while the lowest values were recorded for PI 426778 LSP genotypes (4.51 mm and 4.16 mm for major and minor axis, respectively) (**Figure 3.26**); in Metaponto, the highest values for major and minor axis, 5.67 mm and 5.37 mm, respectively, were detected for PI 432245 LSP genotype and (**Figure 3.27**). r0, r1 and r2 showed a range of variability from 0.8 mm to 0.9 mm, 1.0 mm to 1.02 mm, 1.0 mm to 1.02 mm respectively, independently by the locality.

The PCA was performed highlighting the groups obtained with the clustering analysis (**Figure 3.28**). In Osimo locality (**Figure 3.29a**), the PCA divided accessions with higher SSI, volume and hydration max from those characterized by higher hydration rate. In particular, ILL 4605 showed different characteristics from all the groups. The PC1 and PC2 explain 50.1% and 17.0% of the total variance, respectively. The PC1 is positively correlated to SSI, volume, hydration max, area, r1, r2 and major and minor axis. The PC2 is positively correlated to hydration rate, r2, r1 and major axis and negatively correlated to r0, minor axis and SSI. In Metaponto locality (**Figure 3.29b**), the PCA divided the group showing the highest SSI, volume and hydration max from the other with opposite characteristics. The PC1 and PC2 explain 57.0% and 26.3% of the total variance, respectively. The PC1 is positively correlated to hydration max, major and minor axis, volume and area. The PC2 is positively correlated to r2, r1 and hydration rate and negatively correlated to r0.

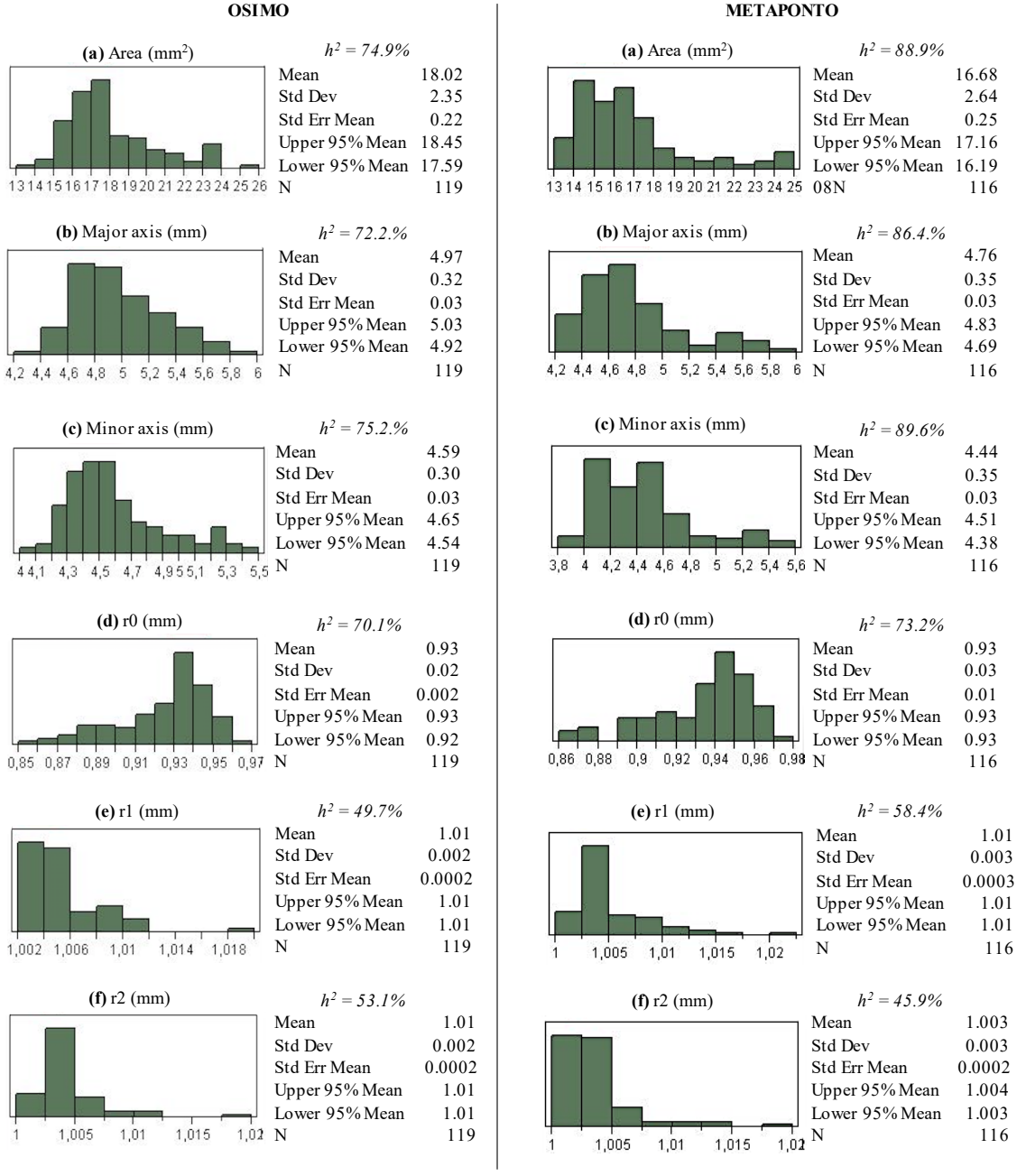
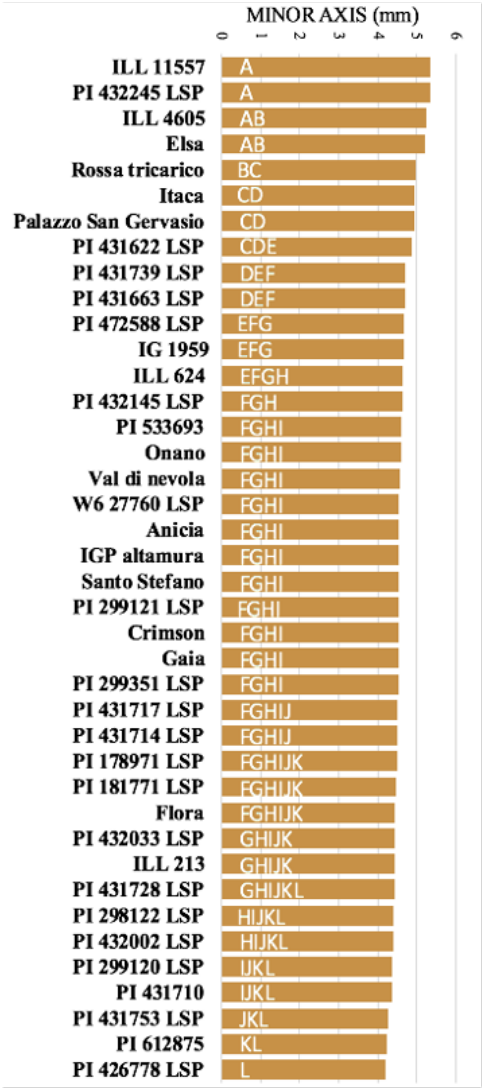
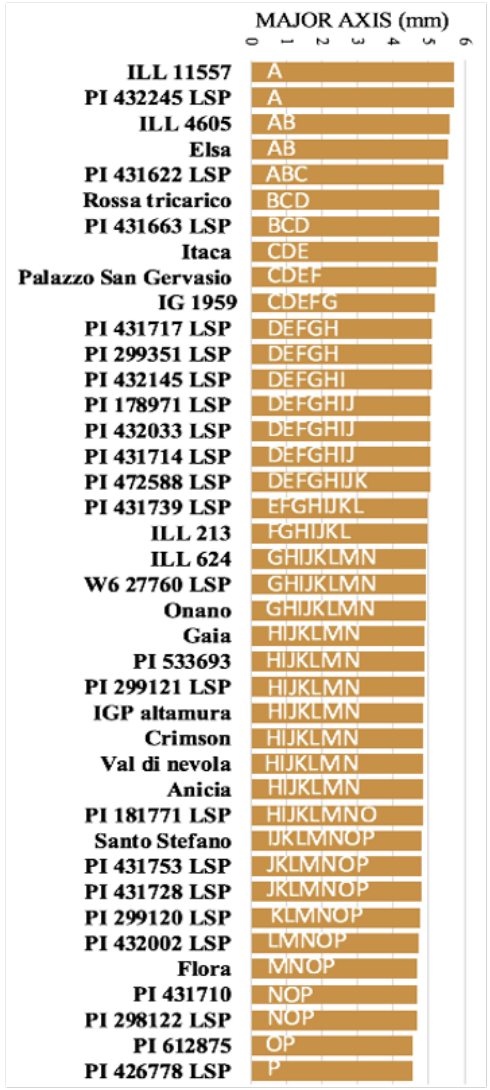
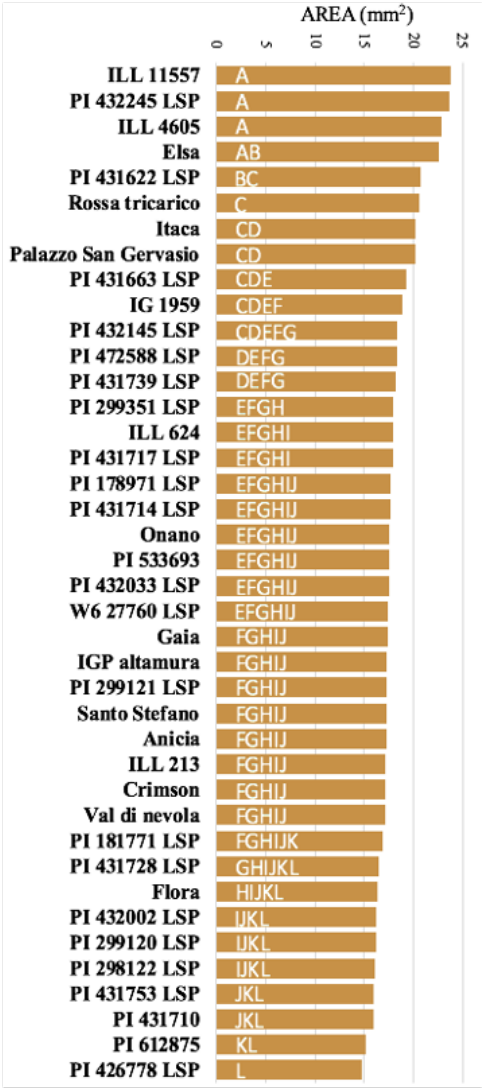


Figure 3.25 - Area, major axis, minor axis, r0, r1 and r2 distribution and heritability estimation of Osimo (left) and Metaponto locality (right). Seed shape parameters were measured for horizontal side.


MINOR AXIS (mm)
0 1 2 3 4 5 6
ILL 11557 A
PI 432245 LSP A
ILL 4605 AB
Elsa AB
Rossa tricarico BC
Itaca CD
Palazzo San Gervasio CD
PI 431622 LSP CDE
PI 431739 LSP DEF
PI 431663 LSP DEF
PI 472588 LSP EFG
IG 1959 EFG
ILL 624 EFGH
PI 432145 LSP FGH
PI 533693 FGHI
Onano FGHI
Val di nevola FGHI
W6 27760 LSP FGHI
Anicia FGHI
IGP altamura FGHI
Santo Stefano FGHI
PI 299121 LSP FGHI
Crimson FGHI
Gaia FGHI
PI 299351 LSP FGHI
PI 431717 LSP FGHIJ
PI 431714 LSP FGHIJ
PI 178971 LSP FGHIJK
PI 181771 LSP FGHIJK
Flora FGHIJK
PI 432033 LSP GHIJK
ILL 213 GHIJK
PI 431728 LSP GHIJKL
PI 298122 LSP HIJKL
PI 432002 LSP HIJKL
PI 299120 LSP IJKL
PI 431710 IJKL
PI 431753 LSP JKL
PI 612875 KL
PI 426778 LSP L


MAJOR AXIS (mm)
0 1 2 3 4 5 6
ILL 11557 A
PI 432245 LSP A
ILL 4605 AB
Elsa AB
PI 431622 LSP ABC
Rossa tricarico BCD
PI 431663 LSP BCD
Itaca CDE
Palazzo San Gervasio CDEF
IG 1959 CDEFG
PI 431717 LSP DEFGH
PI 299351 LSP DEFGH
PI 432145 LSP DEFGHI
PI 178971 LSP DEFGHIJ
PI 432033 LSP DEFGHIJ
PI 431714 LSP DEFGHIJ
PI 472588 LSP DEFGHIJK
PI 431739 LSP EFGHIJKL
ILL 213 FGHIJKL
ILL 624 GHIJKLMN
W6 27760 LSP GHIJKLMN
Onano GHIJKLMN
Gaia HIJKLMN
PI 533693 HIJKLMN
PI 299121 LSP HIJKLMN
IGP altamura HIJKLMN
Crimson HIJKLMN
Val di nevola HIJKLMN
Anicia HIJKLMN
PI 181771 LSP HIJKLMNO
Santo Stefano IJKLMNOP
PI 431753 LSP JKLMNOP
PI 431728 LSP JKLMNOP
PI 299120 LSP KLMNOP
PI 432002 LSP LMNOP
Flora MNOP
PI 431710 NOP
PI 298122 LSP NOP
PI 612875 OP
PI 426778 LSP P


AREA (mm²)
0 5 10 15 20 25
ILL 11557 A
PI 432245 LSP A
ILL 4605 A
Elsa AB
PI 431622 LSP BC
Rossa tricarico C
Itaca CD
Palazzo San Gervasio CD
PI 431663 LSP CDE
IG 1959 CDEF
PI 432145 LSP CDEFG
PI 472588 LSP DEFG
PI 431739 LSP DEFG
PI 299351 LSP EFGH
ILL 624 EFGHI
PI 431717 LSP EFGHI
PI 178971 LSP EFGHIJ
PI 431714 LSP EFGHIJ
Onano EFGHIJ
PI 533693 EFGHIJ
PI 432033 LSP EFGHIJ
W6 27760 LSP EFGHIJ
Gaia FGHIJ
IGP altamura FGHIJ
PI 299121 LSP FGHIJ
Santo Stefano FGHIJ
Anicia FGHIJ
ILL 213 FGHIJ
Crimson FGHIJ
Val di nevola FGHIJ
PI 181771 LSP FGHIJK
PI 431728 LSP GHIJKL
Flora HIJKL
PI 432002 LSP IJKL
PI 299120 LSP IJKL
PI 298122 LSP IJKL
PI 431753 LSP JKL
PI 431710 JKL
PI 612875 KL
PI 426778 LSP L

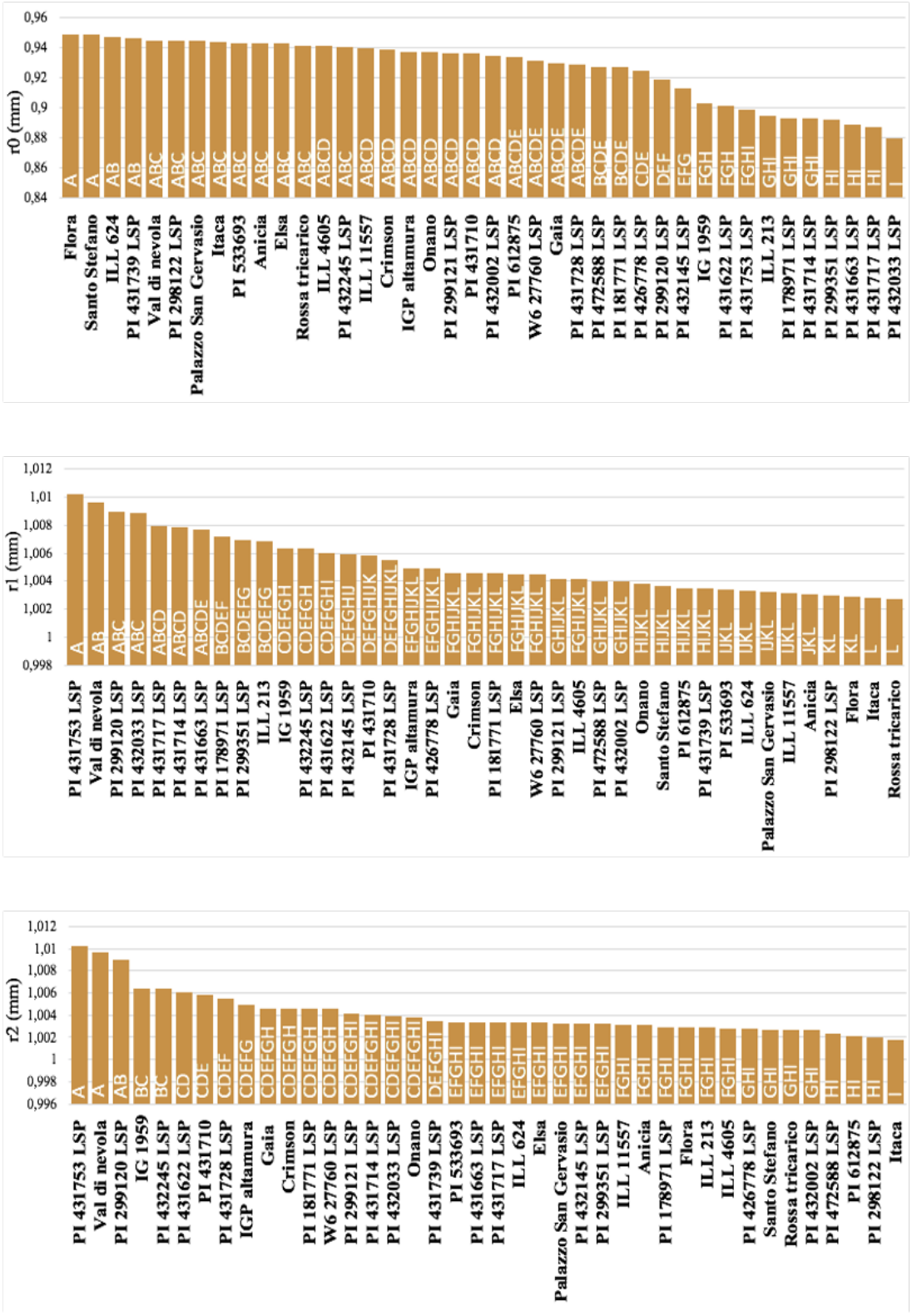
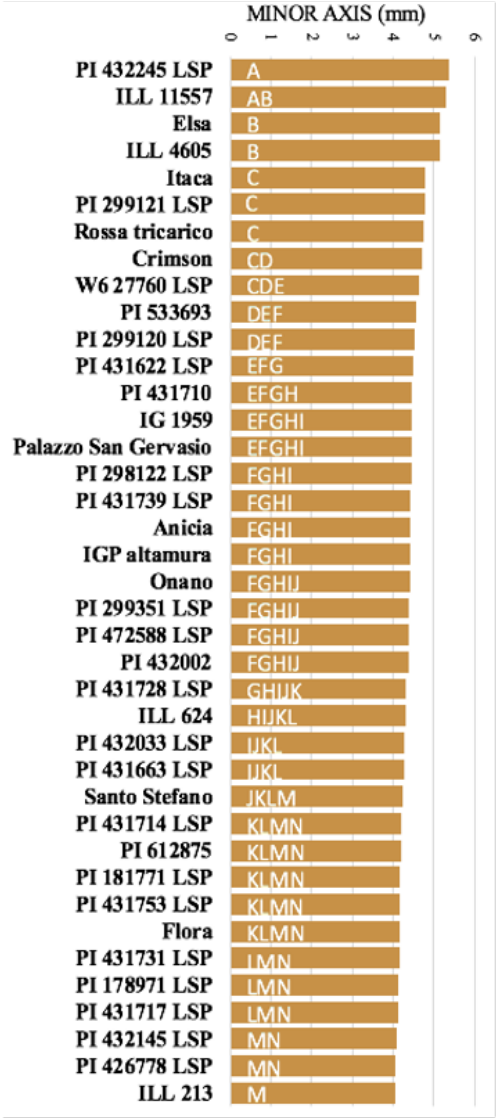


Figure 3.26 - One-way Anova compare different mean for area, major axis, minor axis, r0, r1 and r2 of Osimo sowing for horizontal side; levels not connected by same letter are significantly different ($P < 0.05$; T student test).


MINOR AXIS (mm)
0 1 2 3 4 5 6
PI 432245 LSP A
ILL 11557 AB
Elsa B
ILL 4605 B
Itaca C
PI 299121 LSP C
Rossa tricarico C
Crimson CD
W6 27760 LSP CDE
PI 533693 DEF
PI 299120 LSP DEF
PI 431622 LSP EFG
PI 431710 EFGH
IG 1959 EFGHI
Palazzo San Gervasio EFGHI
PI 298122 LSP FGHI
PI 431739 LSP FGHI
Anicia FGHI
IGP altamura FGHI
Onano FGHIJ
PI 299351 LSP FGHIJ
PI 472588 LSP FGHIJ
PI 432002 FGHIJ
PI 431728 LSP GHIJK
ILL 624 HIJKL
PI 432033 LSP IJKL
PI 431663 LSP IJKL
Santo Stefano JKLM
PI 431714 LSP KLMN
PI 612875 KLMN
PI 181771 LSP KLMN
PI 431753 LSP KLMN
Flora KLMN
PI 431731 LSP LMN
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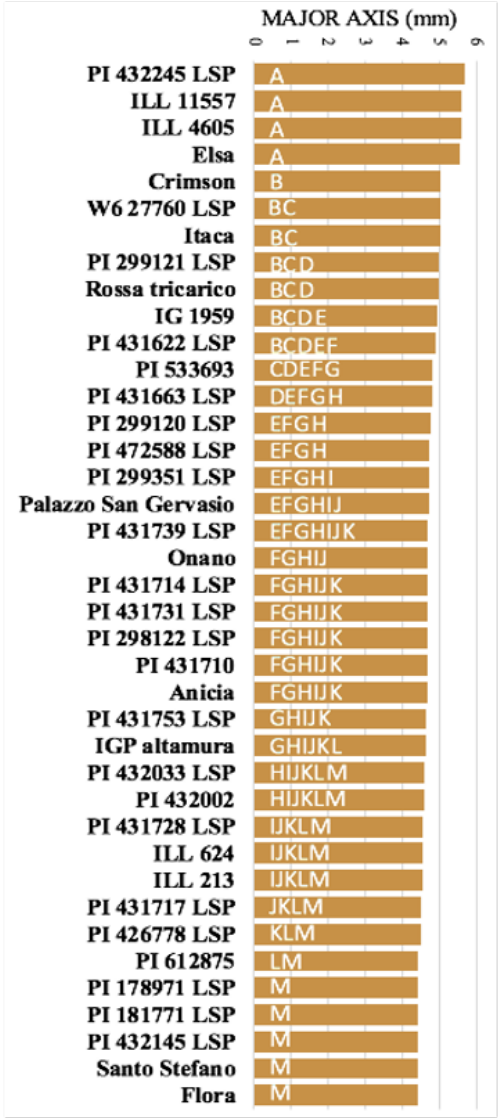
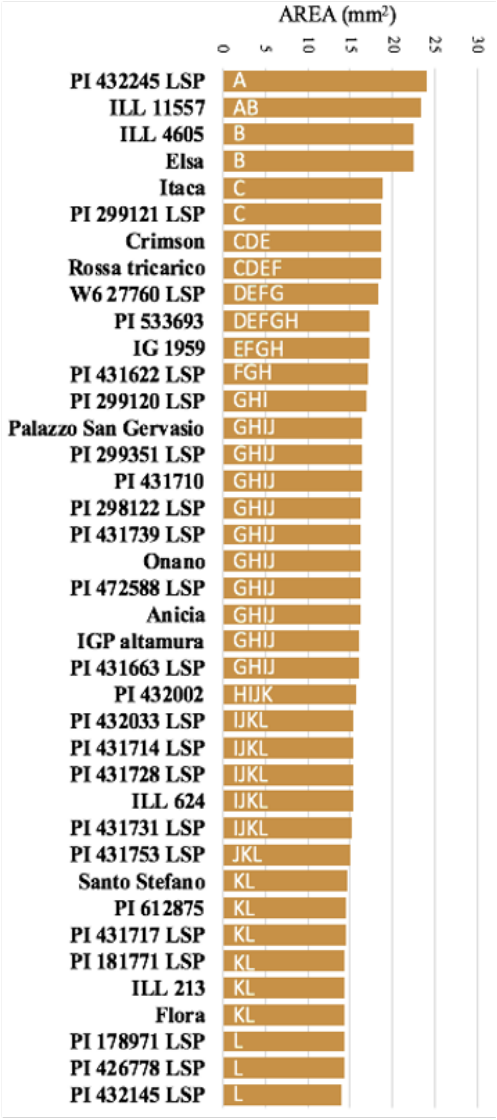
PI 431717 LSP LMN
PI 432145 LSP MN
PI 426778 LSP MN
ILL 213 M


MAJOR AXIS (mm)
0 1 2 3 4 5 6
PI 432245 LSP A
ILL 11557 A
ILL 4605 A
Elsa A
Crimson B
W6 27760 LSP BC
Itaca BC
PI 299121 LSP BCD
Rossa tricarico BCD
IG 1959 BCDE
PI 431622 LSP BCDEF
PI 533693 CDEFG
PI 431663 LSP DEFGH
PI 299120 LSP EFGH
PI 472588 LSP EFGH
PI 299351 LSP EFGHI
Palazzo San Gervasio EFGHIJ
PI 431739 LSP EFGHIJK
Onano FGHIJ
PI 431714 LSP FGHIJK
PI 431731 LSP FGHIJK
PI 298122 LSP FGHIJK
PI 431710 FGHIJK
Anicia FGHIJK
PI 431753 LSP GHIJK
IGP altamura GHIJKL
PI 432033 LSP HIJKLM
PI 432002 HIJKLM
PI 431728 LSP IJKLM
ILL 624 IJKLM
ILL 213 IJKLM
PI 431717 LSP JKLM
PI 426778 LSP KLM
PI 612875 LM
PI 178971 LSP M
PI 181771 LSP M
PI 432145 LSP M
Santo Stefano M
Flora M


AREA (mm²)
0 5 10 15 20 25 30
PI 432245 LSP A
ILL 11557 AB
ILL 4605 B
Elsa B
Itaca C
PI 299121 LSP C
Crimson CDE
Rossa tricarico CDEF
W6 27760 LSP DEFG
PI 533693 DEFGH
IG 1959 EFGH
PI 431622 LSP FGH
PI 299120 LSP GHI
Palazzo San Gervasio GHIJ
PI 299351 LSP GHIJ
PI 431710 GHIJ
PI 298122 LSP GHIJ
PI 431739 LSP GHIJ
Onano GHIJ
PI 472588 LSP GHIJ
Anicia GHIJ
IGP altamura GHIJ
PI 431663 LSP GHIJ
PI 432002 HIJK
PI 432033 LSP IJKL
PI 431714 LSP IJKL
PI 431728 LSP IJKL
ILL 624 IJKL
PI 431731 LSP IJKL
PI 431753 LSP JKL
Santo Stefano KL
PI 612875 KL
PI 431717 LSP KL
PI 181771 LSP KL
ILL 213 KL
Flora KL
PI 178971 LSP L
PI 426778 LSP L
PI 432145 LSP L

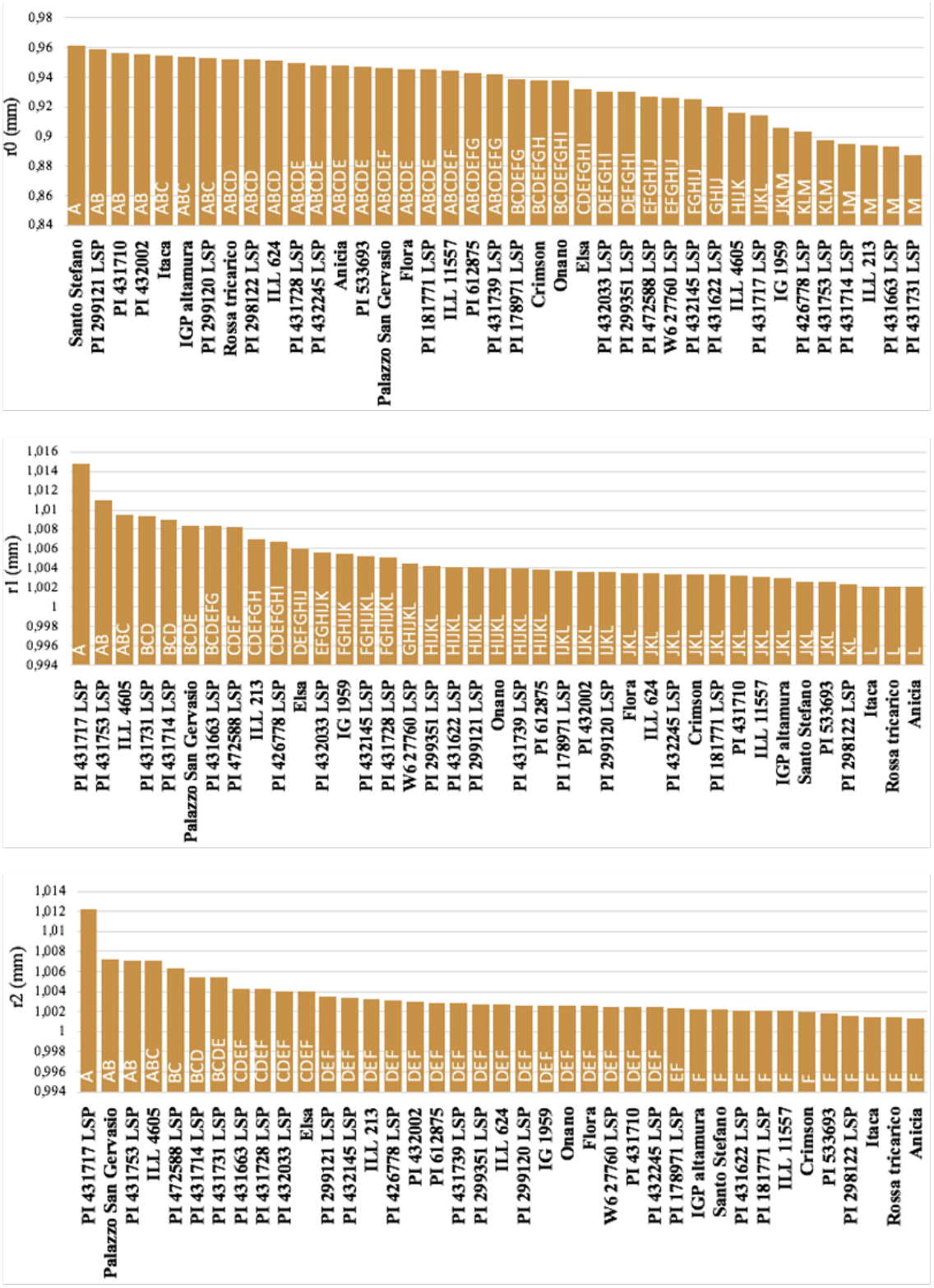
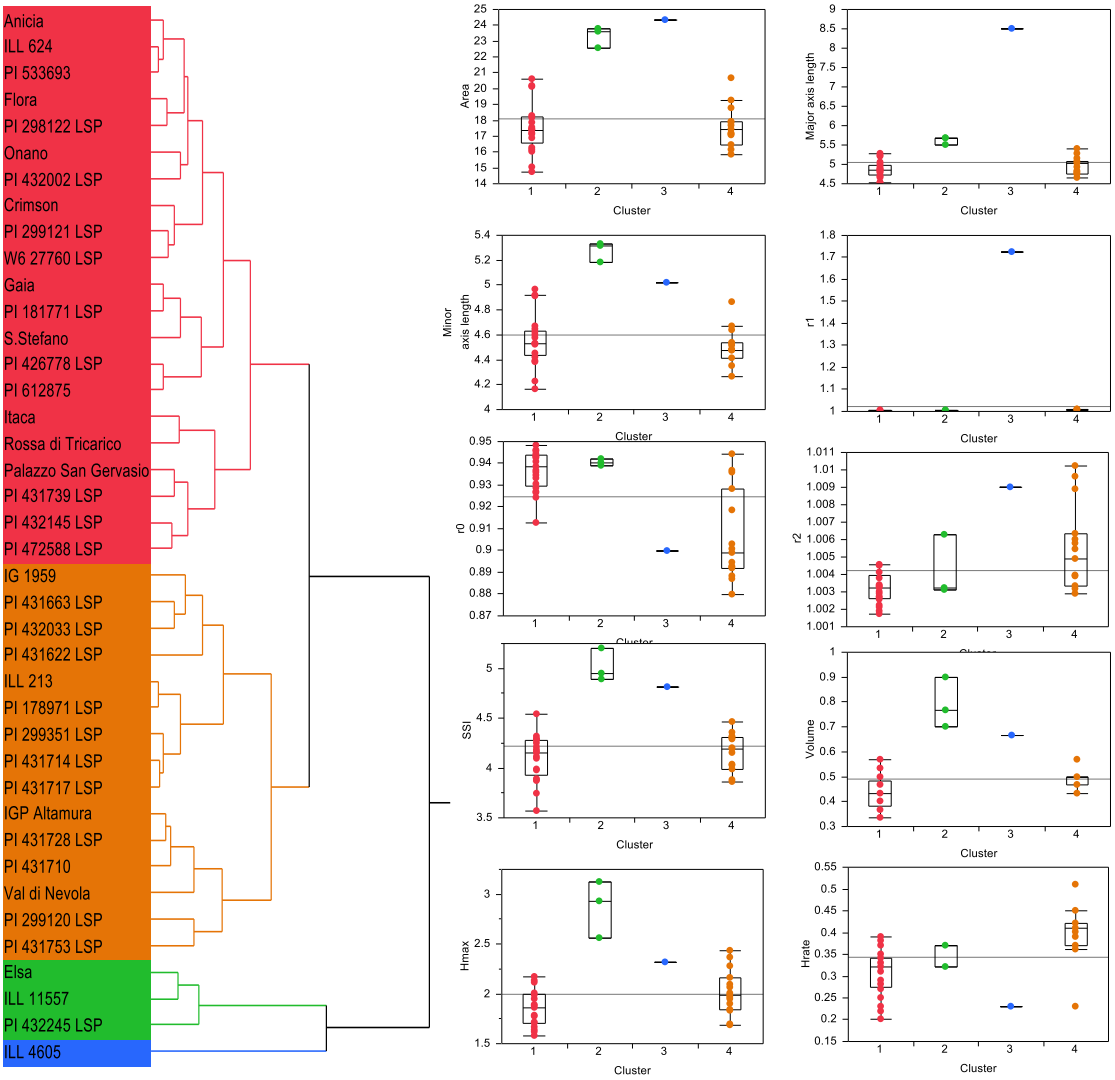


Figure 3.27 - One-way Anova compare different mean for area, major axis, minor axis, r0, r1 and r2 of Metaponto sowing for horizontal side; levels not connected by same letter are significantly different ($P < 0.05$; T student test).

(a)


Anicia
ILL 624
PI 533693
Flora
PI 298122 LSP
Onano
PI 432002 LSP
Crimson
PI 299121 LSP
W6 27760 LSP
Gaia
PI 181771 LSP
S.Stefano
PI 426778 LSP
PI 612875
Itaca
Rossa di Tricarico
Palazzo San Gervasio
PI 431739 LSP
PI 432145 LSP
PI 472588 LSP
IG 1959
PI 431663 LSP
PI 432033 LSP
PI 431622 LSP
ILL 213
PI 178971 LSP
PI 299351 LSP
PI 431714 LSP
PI 431717 LSP
IGP Altamura
PI 431728 LSP
PI 431710
Val di Nevola
PI 299120 LSP
PI 431753 LSP
Elsa
ILL 11557
PI 432245 LSP
ILL 4605
Area
Major axis length
Minor axis length
r1
r0
r2
SSI
Volume
Hmax
Hrate
Cluster

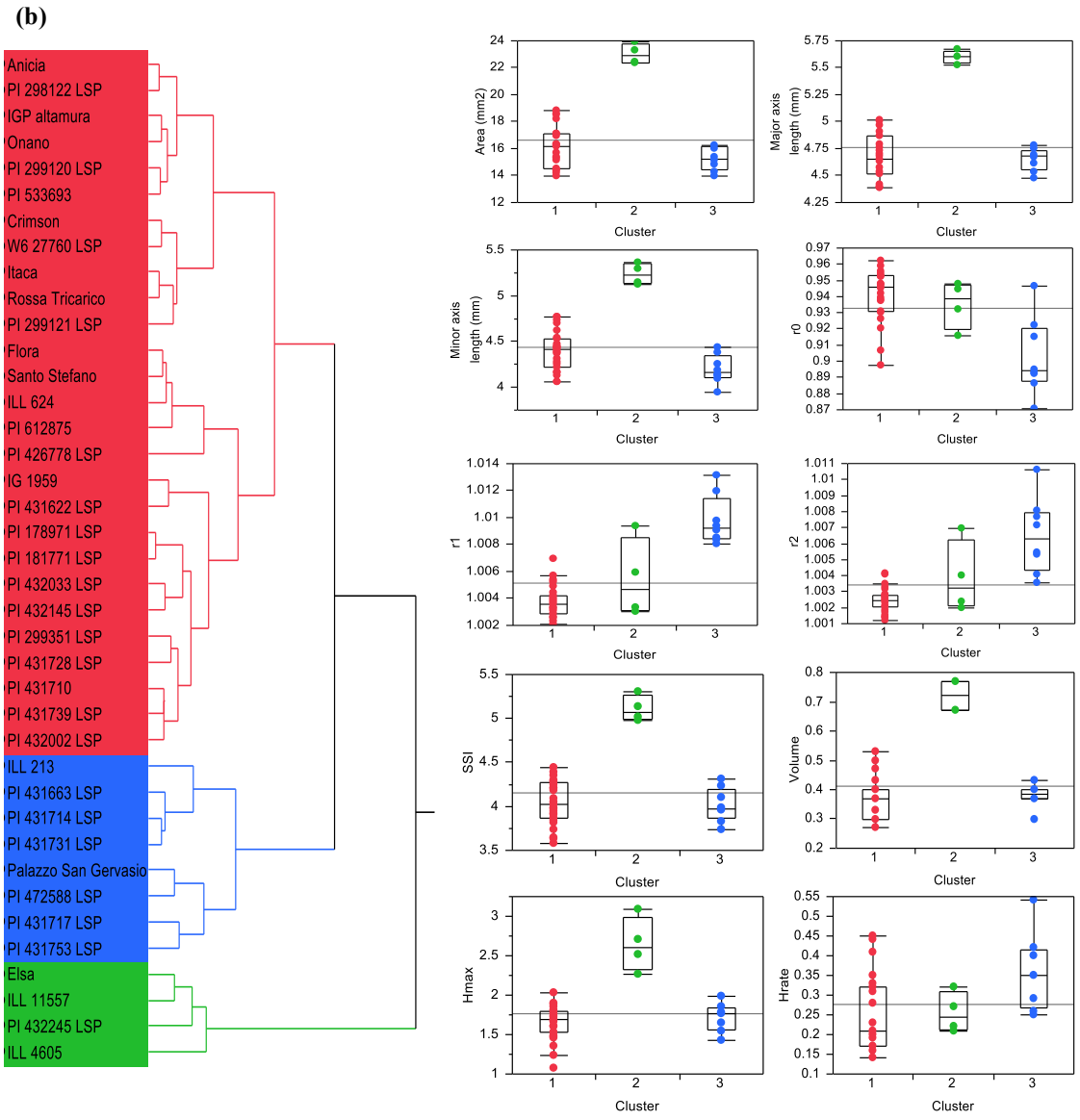


Figure 3.28 – Cluster analysis for quality traits for Osimo (**a**) and Metaponto (**b**) sowing.

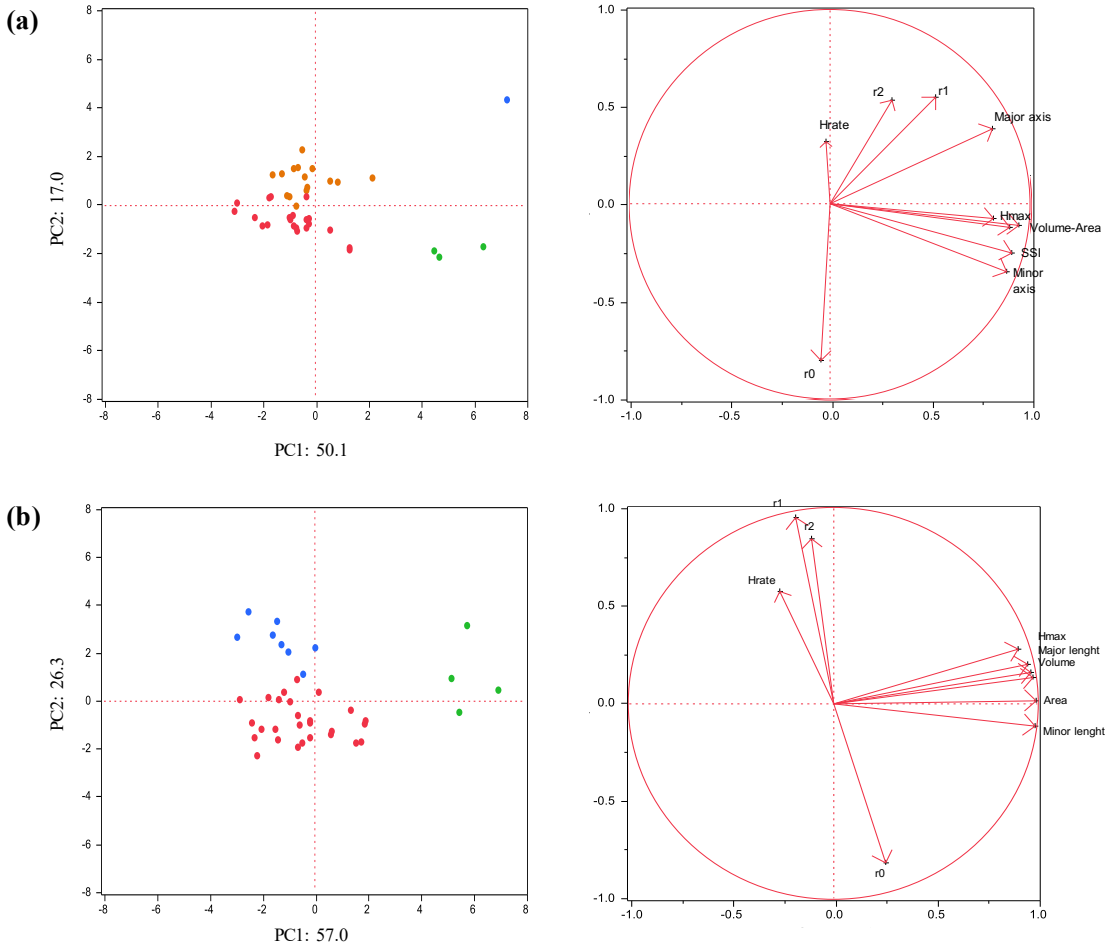


Figure 3.29 - PCA and loading plot for the dimensional traits of lentil seeds of Osimo (**a**) and Metaponto (**b**) sowing.

A PCA analysis was also carried out based on the reflectance values (**Figure 3.30**). The PC1 and PC2 explain 61.5% and 20.4% of the total variance, respectively, in Osimo (**Figure 3.30a**). The PC1 and PC2 explain 78.7% and 20.1% in Metaponto (**Figure 3.30b**). The PC1 is positively correlated to all the reflectance values for both the locality. The PC2 is positively correlated to avgR 715 nm, avgR 840 nm, avgR 850 nm and avgR 918 nm in Osimo (**Figure 3.30a**) and to avgR 840 nm and avgR 850 nm in Metaponto (**Figure 3.30b**). For the PCA analyses, it was possible to identify a differentiation among groups of accessions based on the color. In particular, accessions PI 431710, PI 432245 LSP, Val di nevola, PI 431753 LSP, PI 299120 LSP, PI 431728 LSP, PI 299121 LSP, IGP Altamura, Onano and Elsa, the ones with the lowest reflectance values, were separated from the other genotypes for PC1 in Osimo trial (**Figure 3.30a**). The same can be observed for the same last seven genotypes plus further nine genotypes, Flora, PI 426778 LSP, PI 432145 LSP, PI 431714 LSP, PI 431717 LSP, PI 472588 LSP, PI 431731 LSP, Palazzo San

Gervasio and ILL 4605, in Metaponto trial (**Figure 3.30b**). Especially in PCA based on Osimo data, a further separated group of accessions (PI 432002 LSP, PI 432145 LSP and PI 431714 LSP) showed higher values for avgR 840 nm, avgR 850 nm wavelengths. The same results are highlighted considering the cluster analysis (**Figure 3.28**) as showed in **Figure 3.31.** The results confirmed the variability present among the analyzed accession (**Figure 3.32**).

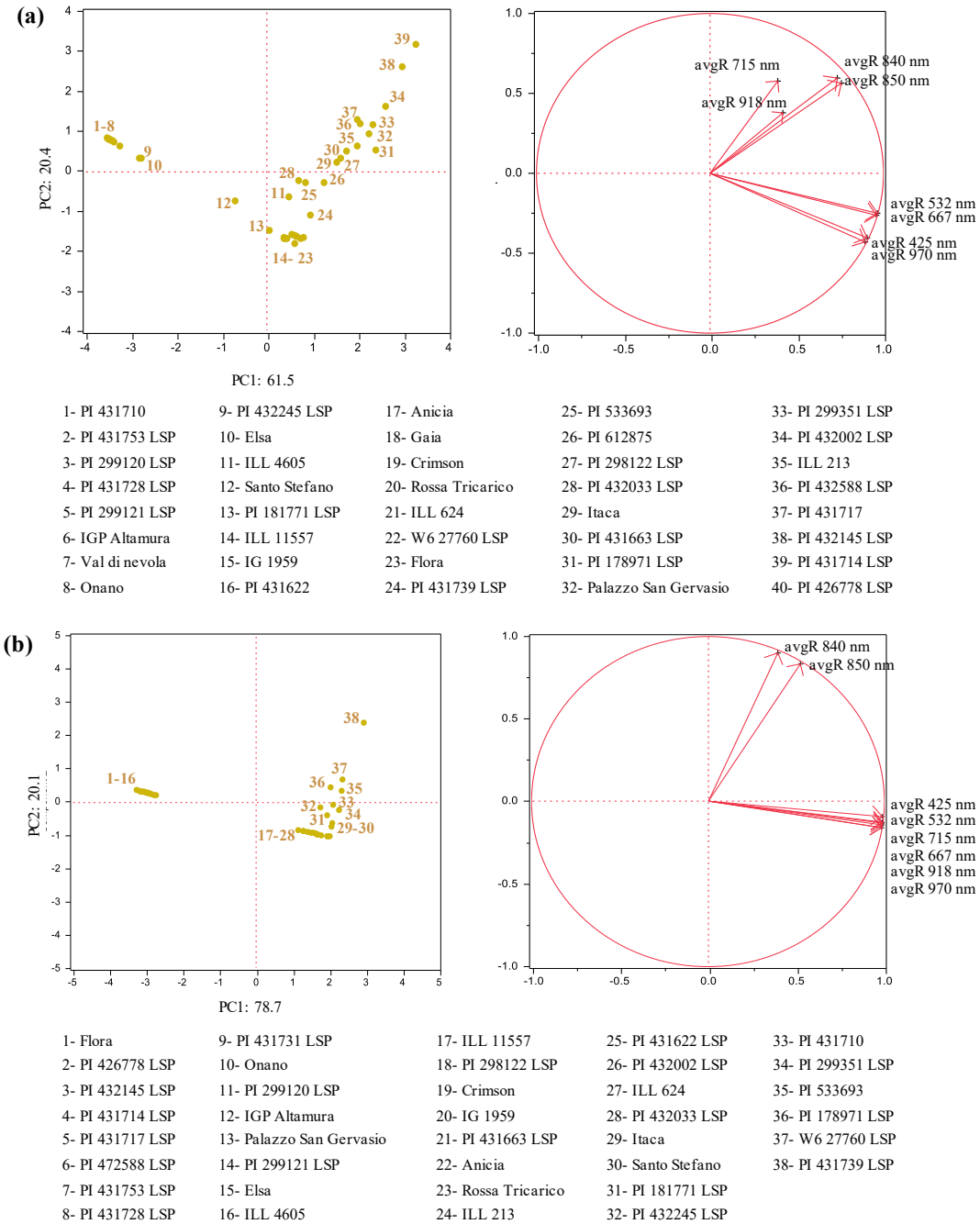


Figure 3.30 - PCA and loading plot for the color trait of lentil seeds of Osimo (**a**) and Metaponto locality (**b**).

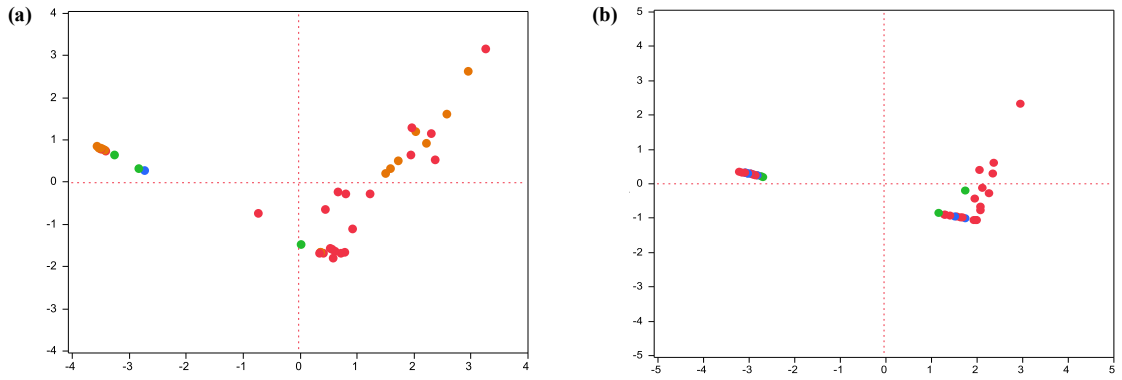


Figure 3.31 - PCA for the color trait of lentil seeds of Osimo (**a**) and Metaponto locality (**b**) considering the cluster analysis.

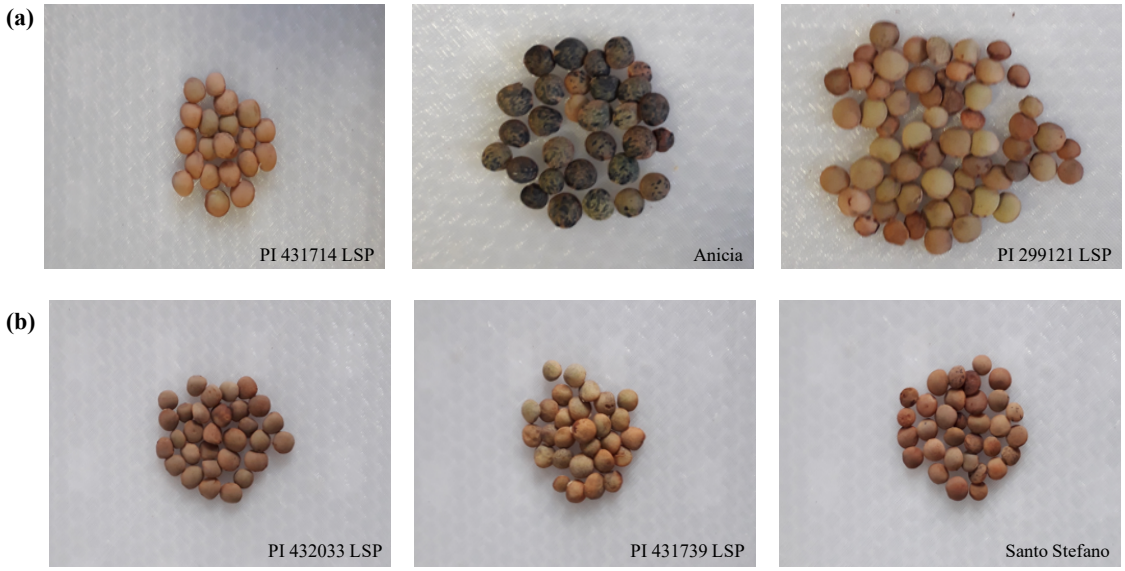


Figure 3.32 – Lentil seeds sample analyzed for Osimo (a) and Metaponto (b) sowing.

DISCUSSION

Plant genetic resources of a crop include all the genetic variability present in landraces, modern cultivars, wild relatives, breeding lines and genetic stocks, which are available for humans to be used. They represent the most important components of agro-biodiversity which, providing basic material for selection and improvement through breeding, has to be preserved in order to ensure food security and to develop novel varieties able to overcome agriculture's challenges such as climate changes (Upadhyaya et al. 2008). Phenotypic characterization of plant genetic resources, including agronomic, morphological and quality traits, is essential to provide information on available genetic diversity and to allow the use and exploitation of such germplasm by breeders to develop improved varieties, indeed recording of data on important characteristics which distinguish accessions within a species, enables an easy and quick discrimination among phenotypes and allows identification of gaps and retrieval of valuable germplasm for breeding programs, resulting in better insight about the collection genetic diversity.

Food security and quality represent the main tasks for agriculture. The challenge is to guarantee them in the context of climate change and of sustainable farming system. Only recently, humans have started to realize that a significant change of direction in the way they do agriculture is strongly needed, indeed the pressure on earth system is not more sustainable. In this regard, Rockström et al. (2009) introduced the concept of 'planetary boundaries', which are defined by critical values for one or more control variables representing key planet's biophysical subsystems or processes. To not exceed such thresholds, allows to remain in a "safe operating space" for humanity with respect to the Earth system. Nowadays agriculture is putting majors constrains on four planet boundaries: biosphere integrity, land system change, freshwater use and nitrogen flow as reported by Gerten et al. (2020); in their work they indicate that if these boundaries are strictly respected, which means maintaining the current agricultural system, it is possible to feed only 3.4 billion of people, while promoting a transition towards more sustainable production and consumption could support the growing population that will reach level over 9 billion, while respecting the four planetary boundaries. Among the key actions to do it, they indicated a spatially redistributed cropland, an improved water–nutrient management, the food waste reduction, and dietary changes.

According to Poore et al. (2018) dietary change of consumers can have environmental benefits on a scale not achievable by producers; this change is achievable only moving towards vegetable-based diet and/or drastically reducing animal products. The 2019 report of the International Panel for Climate Change (IPCC) indicated that the plant-based diet is an important opportunity for mitigating climate change, while generating significant co-benefits in terms of human health.

In this complex scenario, food legume crops represent valuable resources for diet change and reduction of environmental impact of protein production (Stagnari et al., 2017). Since animal protein supply has been ever-decreasing and its cost has been ever-increasing, today vegetable proteins have had further importance (Sozen et al. 2017). Edible legumes are the main vegetable-protein sources and as the world population increases, the importance of increasing the food demand, and so their production, is becoming increasingly necessary.

This study aimed to characterize seeds of a set of 24 and 41 different genotypes of chickpea and lentil, respectively, for morphological and physical quality traits. A strength of this study is that the seeds were obtained in designed experimental field trials, with replicates, carried out in different environmental conditions (autumn and spring season for chickpea in Osimo locality; Osimo and Metaponto localities for lentil). This allowed *i)* to apply a formal procedure of statistical inference able to properly compare different accessions and to highlight significant differences among them, *ii)* to compute heritability of quality traits, which is the percentage of phenotypic variance that is attributed to genetic variance. High heritability indicates that the environmental influence is minimal on characters and the higher the heritability of a trait the more progress could be made in selection. Heritability is crucial for plant breeders because traits with higher heritability can be improved more rapidly using fewer resources than traits with lower heritability (Smalley et al. 2004); and, finally, *iii)* to test the potential genotype-by-environment interaction (GEI). Both genetic and environmental factors affect qualitative and quantitative characters of legumes. They can interact to each other increasing or decreasing the impact of the latter. The genotype-by-environment interaction analysis is an important pre-requisite for recommendation of selection for large-scale production (Horn et al. 2018).

The main outcome of the present study is the variability that we observed for almost all the traits among the different genotypes evaluated in the field experiments. This aspect is crucial because gives the possibility to identify interesting genotypes with specific characteristics and adaptation traits for cultivation in different environments as well as suitable to meet the different needs of different stakeholders such as quality pasta producers or canning industry.

Independently by the environmental conditions, high heritability was found for seed size traits (SSI and Volume), seed coat weight, and seed coat content for chickpea seeds (h^2 >77%) and for seed size traits for lentil seeds (h^2 >76%). Such high heritability values indicate that the influence of environment on these traits is low and that breeding can be faster. Kabuli chickpea genotypes are characterized by larger seed size than desi types; in particular, Ituchi variety has the largest seed compared to all the other analyzed genotypes, independently by the season. Desi varieties are mainly traded locally because international markets favor larger-seeded kabuli varieties (Shiferaw et al. 2007). Indeed, the consumers' preferences of larger seed size have provided an excellent opportunity for a premium price and higher profitability (Yadav et al. 2007). However, according to Gaur et al. (2016), the seed size is negatively correlated to protein content. Generally, a more uniform seed size is desired within a pulse sample not only for appearance (which affects marketing) and consumer acceptance, but also for optimal processing efficiency, particularity for that species that undergo milling or canning. It is important also to achieve even levels of cooking and softness for every seed within each can and for the processor who wants easy preparation and greater yields. Seed size affects cooking time because both moisture and heat will take longer to penetrate and thermally change a larger mass. Many authors have noted that larger seeds generally take longer to cook; this seems similar across most, if not all, pulse species (Stavreva 1987; Bressani et al. 1988; Nielsen et al. 1993), as long as seeds do not possess un-hydratable or hard-to-cook defects that can change this relationship.

On seed coat, our analyses confirmed the contrasting characteristics found in the literature (Wood et al. 2011) between desi and kabuli types. Indeed, it results much thicker in desi types with Filiano nero genotypes showing the highest seed coat content (%) followed by Nero Tolve, IS-CE-Bruno and Nero Appignano genotypes. Moreover, the desi chickpea seeds varied considerably in their ease of decortication, the reason why they can be consumed whole, but the majority are generally decorticated and used for making splits (dhal) and flour, contrary to kabuli types. The lighter coating that characterize kabuli types is explained by the presence of fewer tissue layers and a weak cell structure compared to the stronger, thickened cell structure of desi seed coat (Wood et al. 2011). The seed coat characteristics are important parameters for breeders since they affect cooking time. The time required to cook these products is an important quality attribute for food processors and consumers because longer cooking time are inconvenient requiring more electricity or fuel and therefore, are more costly to the industry, and being more time consuming for consumers. There are specific breeding programs interested in breeding for quicker-cooking pulse varieties achieving premiums in the marketplace because of higher demand (Wood 2016). The seed coat represents a physical barrier to the initial water absorption, as we

can see from our results higher values of seed coat has been associated with higher hydration rate. Removal of seed coat can lead higher water access to cotyledons, speeding up the cooking process and avoiding presoaking before cooking and increasing the taste and smoothness of the texture of seeds. In addition, the generated waste, given the high levels of dietary fiber, mineral and potential health-promoting phytochemicals present in the seed coat, could be used as a novel natural functional food ingredient (Zhong et al., 2018).

GEI analysis highlighted a higher performance of almost all the considered chickpea genotypes for autumn sowing compared to the spring sowing for 1000 seed weight, with Ituchi genotypes showing the highest values. The same analysis was performed for lentil seeds showing significant higher 1000 seed weight in Metaponto compared to Osimo sowing. Test weight is a very important indicator of the general seed quality which is effective on sprouting potential, seeding growth and plant performance. This quality is dependent on the size of embryo and reserved nutrients quantity used for sprouting and growth (Deivasigamani et al. 2018). Moreover, 1000 seed weight is useful for producers when calculating seeding rates and harvest losses. The analysis was also performed for SSI, volume, seed coat weight, seed coat content for chickpea seeds, and for SSI and volume for lentil seeds, showing low GEI that combined with high heritability suggest the environmental stability of the seed traits considered.

Correlations among autumn and spring sowing for chickpea, Osimo and Metaponto for lentil were found for the hydration parameters analyzed. This allowed to understand the relationship existing between the different environmental conditions. To quantify the magnitude of this relationship we measured the correlation coefficient which resulted significant for hydration max and hydration rate of chickpea and lentil seeds. A positive correlation suggests that the considered variables, independently by the environmental conditions, are incline in increasing in a parallel way. Hydration parameters are important quality parameters that would be useful for many industrial applications, especially canning, where seeds that swell to larger volumes have economic benefits. At this regards, kabuli types showed the highest estimates of hydration max as well as PI 432245 LSP genotype for lentil, independently by the environmental conditions.

Correlation between traits were also investigated. As expected, in chickpea seeds the seed size index showed significant positive correlation with volume, hydration rate and hydration max, independently by the season. Hydration traits showed significant negative correlation with seed coat content in chickpea. In lentil seeds, a significantly positive correlation was found between SSI, volume and hydration parameters and no negative correlations were found between traits and localities.

A multivariate analysis confirmed the contrasting characteristics of desi and kabuli types as regard color already visible to naked eyes. Desi types showed darker colors (mostly black/brown) compared to kabuli types (white or cream colored). Polyphenolic compounds such as anthocyanins, proanthocyanidins, and certain flavonol glycosides and isoflanonoids are known to contribute to the color of legume seed coats. Desi chickpea seeds have been shown to contain polyphenolic compounds (Singh et al. 1984). Results from lentil seeds showed high variability between the analyzed genotypes, with some genotypes clearly separated from the others. The seed coat color has overriding importance in determining market quality and acceptance and hence breeders need access to diverse genetic resources (DVSSR Sastry et al. 2013). The multivariate analysis was also performed to obtain information about the seed shape. In chickpea, shape is an important qualitative character that determine the seed appearance and uniformity and is often used as quality indicator for consumers and importers. From our results, we can suggest desi types Nero Tolve and Filiano nero for producers that want more flattened and less rounded seeds, and kabuli types for smaller-rounded seeds. The recognition of different seed shapes can offer the prospect of significant genetic gain and improved selection efficiency. For example, Shia and Slinkard (1977) associated the rounded shape in pea with increased yield and seed size.

Color and shape data were obtained exploiting a multispectral imaging system which allowed to greatly improve the classical phenotyping based on human eye (Elmasry et al. 2019). This system allowed to deeply analyze the phenotypic variation for seed morphology of the different accessions analyzed and, represent a very useful tool to establish protocols for the identification of different varieties to be used as a traceability system and online classification protocols, as showed by Elmasry et al. (2019), which demonstrated the potential of the multispectral imaging system in the ultraviolet, visible and shortwave near infrared range to provide the required information necessary for the discrimination of individual cowpea seeds of different classes.

This project was commissioned by Barilla. The Company was interested in evaluating different genotypes of chickpea and lentil from an agronomic and quality of seeds point of view. In particular, the main aim of the study is to understand which traits can affect the quality of pasta produced with 100% legume flour. They are characterizing the same seeds for additional quality traits such as protein, fiber, and ash content, humidity, dehulling efficiency, texture analysis (cooking quality). As well as they are using flours obtained by the different genotypes to produce pasta and look at differences. Nutritionally improved pasta has been started to be prepared by replacing wheat, in whole or in part, with other materials such as legumes. Kaur and Singh (2006) observed that physicochemical properties of chickpea seeds were significantly correlated to functional, gelatinization and pasting properties of their flours and physicochemical properties of

their starches. In this regard, it is interesting that a positive correlation of water solubility index (WSI) of chickpea flours with seed mass, volume and hydration capacity of seeds was observed.

CONCLUSION

The physical properties of seeds are important for processing and storage germplasm samples as well as assessing seed quality. Some traits are fundamental attributed defining the consumers demands and market premiums. Consumption demand of legumes is segmented depending on their end use and our analyses permitted to measure specific seed traits in order to discriminates different genotypes in order to meet specific market demands. We performed analyses on different chickpea and lentil genotypes, however the methods used in this study can also be used on larger populations of different legumes and can be extended to several aspects. Most of the investigated traits showed high heritability estimates which indicates that the breeders' action could give an important genetic improvement. Further, a significant high variability was found among the different accessions for all the analyzed trait indicating the possibility to use the most interesting genotypes for specific industry purposes. Desi and kabuli chickpea showed a clear distinction for important traits such as seed coat, color and hydration. The multispectral imaging technique have proven to be useful in providing important information about seed quality traits and phenotyping parameters in the study of various morphological traits of different common varietal seeds. The identification and characterization of seed types which is traditionally done by eye, can be done more efficiently using the multispectral image analysis.

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