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# Wheat landraces identification through glumes image analysis

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ABSTRACT

A new practical method able to identify wheat local landraces was implemented. It is based on computerized image analysis techniques and statistical identification, for the first time on the basis of glumes size, shape, colour and texture.

Ears of 52 different Sicilian wheat landraces were reaped for three consecutive years. Digital images of the glumes were acquired, processed and analysed, measuring 138 quantitative morpho-colorimetic variables. The data were statistically analysed applying a Linear Discriminant Analysis. All the statistical comparisons, distinguished for systematic rank, given perfect identification performances; while an overall percentage of correct identification of 89.7% was reached when all the landraces were compared all together.

Finally, the identification system was tested with an unknown glume sample, later entirely identified as Vallelunga, one of the Sicilian landraces.

This work represents the first attempt of wheat landraces identification based on glume phenotypic characters, applying image analysis techniques. Considering the growing interest in local old wheat landraces, strongly linked to the renewed appreciation in traditional and typical local products, the obtained results support the application of the image analysis system not only for grading purposes, but also to define the product traceability, in order to get a "market card" for wheat landraces.



## 1. Introduction

Wheat (*Triticum* subsp.) is one of the main food sources in the world. Its world production for 2016/17 is approximately expected in 740 million tons, exceeding the 2015/16 record by 1.2%, and covering about 15% of the world's arable surface (FAO, 2017). Durum wheat production reaches around 30 million tons in about 16 million hectares, accounting approximately 5–6% of the total world wheat production (Cebola Lidon et al., 2014). It is commonly grown in most of the countries around the world, although the Mediterranean region produces about 60% of world durum wheat production (Morancho, 2000), being the EU (Italy, Spain, France and Greece) the leading global producer (Cebola Lidon et al., 2014). On this scenario, south Italy is one of the regions historically most voted to the cereal crops, where the durum wheat varietal biodiversity is particularly high.

Sicily, with an area of 25,711 km$^2$, is the largest island in the Mediterranean sea and due to its geographical position and extremely diversified ecological condition, always hosted an ideal environment for the cultivation of cereals and in particular durum wheat. This is due to the extreme variability of altitude and pedo-climatic conditions, characterized by clayish to sandy fields, by variable orography, distance from sea and wind regime (Lombardo, 2004). Some socio-cultural aspects had also contributed enriching the varietal heritage, such as the great amount of invasions that, during the centuries, conquered wide Sicilian areas, favoured by the strategic geographical position of the island. All these conditions, together with the mass selection historically conducted and the more recent genetic improvement programs based on artificial crosses, had contributed to build the extremely wide varietal panorama currently existing. On the other hand, Sicily is known as "Republic granary" since III-II century b.C., as reported by Caton the censor (234–149 b.C.).

In Sicily are currently cropped a few of tens of old and new durum wheat varieties officially recorded and regulated with national and communitarian protocols, but also many ancient landraces or populations characterized by specific bio-morphological traits and qualitative features (Spina et al., 2008; Sciacca et al., 2014). A cropped variety or cultivar, is an intra-specific taxonomic entity characterized by high level of homozygosis, specially for the genes that control the selected traits, consequently, the individuals belonging to the same variety show homogeneous morphological and/or productive traits. Nevertheless, some differences in

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genetically controlled biochemical traits may exist within a same variety (e.g. protein components) (Peruffo et al., 1985). These variations were defined "biotypes". Differently from varieties, landraces are natural populations put in cultivation and as such, they are characterize by wide adaptability to various environments including irrigated and dry land conditions (Jones et al., 2008; Camacho et al., 2005). Considering all the abiotic factors, the high probability of inter-population crosses and their heterozygosis condition, from the genetic point of view, these populations result to be more than a mixture of different pure lines (Zeven, 1998; Landjeva et al., 2015).

Up to 100–150 years ago, the landraces were the only one kind of wheat cultivar available for the farmers; afterward, knowledge and new technologies launched the genetic improvement as understood today. It reflected on a marked genetic and phenotypic homogeneity, useful for the mechanization of many agronomic practices; but it also reflected on a greater phenological synchrony, helpful for the application of herbicides, pesticides and fertilizers. Homogeneity also positively affects on the value of the productions addressed to industry, and from the legal point of view, facilitates the unequivocal varietal identification.

The extreme homogeneity also implicates the negative aspects related to the biotic and abiotic stresses. Wild plants are indeed unlikely subjected to epidemics and pathogenic attacks. Moreover, changing old varieties genetically heterogeneous with new ones certainly more homogeneous, damaging local populations, activates genetic erosion phenomena (Guarda et al., 2004; Newton et al., 2010).

Broadly speaking, genetic improvement has always existed, but what has changed in the last century is not only the nature of the selection, but the nature and range of genetic variability (Frankel, 1970).

At the beginning of the XX century, in Sicily as well as in the rest of the world, the strong interest in biodiversity conservation powered up the accurate search of cropped species germplasm. In Europe, many researchers started to collect ex-situ seed materials in germplasm banks. Vavilov (1957), at time one of the most operative investigators, found many seed material belonging to cultivated plant species, establishing the origin and speciation centres of a great part of the currently cropped species. Thanks to the work of these scientists, a lot of endangered local varieties were saved and currently made available for breeding programs and typical products making.

In recent years, in Sicily as well as in the rest of Europe, the attention paid to local and traditional productions and is growing, especially in the agro-food sector. For economic, social and nutritional reasons, this trend has led to the rediscovery and reuse of landraces both of wheat and other crops, responding to requests for more and more demanding market. The rising price of these local productions and the consequent increased satisfaction of farmers, is proving to be an interesting professional opportunities also for young workers. Moreover, many recent studies testify the high healthy and nutraceutical value of old landraces, both for high amount of antioxidant compounds and for their natural aptitude to organic production (Gallo et al., 2004; Pasqualone et al., 2014; Migliorini et al., 2016; Lo Bianco et al., submitted for publication).

This growing interest in local old landraces has inspired to find effective and objective identification methods, able to distinguish old landraces (Grillo et al., 2016).

In the recent past, many DNA-based methods have been set up, for wheat-derived products, to trace cultivars in starting seed stocks, semolina, bread and pasta (Pasqualone et al., 1999, 2000; Fujita et al., 2009). Giancaspro et al. (2016) described the denaturing high performance liquid chromatography technique for setting up a single nucleotide polymorphism based method to achieve the varietal traceability of the durum wheat cultivar "Timilia", reaching no very high but promising percentage of detection.

Anyway genetic approach is not the only one. Substantial work dealing with the use of different morphological (size and shape) features for classification of wheat grains and varieties has been reported in the literature (Keefe and Draper, 1986; Zayas et al., 1989; Barker et al., 1992; Arefi et al., 2011; Zapotoczny, 2011). Modern phenotyping methods proved to be a helpful tool both in plant identification and classification and in quality assessment (Venora et al. 2009; Guevara-Hernandez and Gomez-Gil, 2011; Smykalova et al., 2011, 2013). Pourreza et al. (2011) applied machine vision techniques to classify nine common wheat varieties based on seeds; while recently, Szczypiński et al. (2015) implemented an identification system to discriminate among 11 barley varieties based on image-derived shape, colour and texture attributes of individual kernels, reaching an accuracy included between 67% and 86%. Many other researches, based on image analysis technology, were recently conducted in order to distinguish wheat and other cereal varieties (Szczypiński and Zapotoczny, 2012; Mebatsion et al., 2013; Chaugule and Mali, 2016). Although seeds and kernels proved to be the right matrix to study in order to discriminate among varieties, problems arise increasing the varietal sample amount and above all when no genetically defined samples, such as populations or landraces, have to be identified.

The aim of this paper is to establish a practical method based on computerized image analysis techniques and statistical identification capable to identify wheat local landraces, for the first time on the basis of glumes size, shape, colour and texture.

## 2. Material & methods

### 2.1. Samples details

Ears of 52 different wheat local varieties or landraces were reaped, at the time of maximum ripening, from the fields of the Stazione Sperimentale di Granicoltura per la Sicilia, sited in Santo Pietro – Caltagirone [37 °07′12″N; 14 °31′17″E; 313 m a.s.l.] (CT, Sicily, Italy) (Table 1; Fig. 1). In order to include a widest morphological and environmental variability, the wheat ears were collected during three consecutive years (2012, 2013, 2014).

From three to six ears were sampled and from two to four glumes were removed from the spikelets of the ear middle section and from the both sides of each ear. The glumes were stored at room temperature under controlled conditions (20 °C and 50% RH).

Applying the same sampling approach, one more unknown landrace, collected in 2015 from Gangi (PA, Sicily, Italy) in the Madonie mountains (C-N Sicily), locally named "Nivuru", was used to test and validate the identification system.

### 2.2. Glume image analysis

Digital images of glumes samples were acquired using a flatbed scanner (ScanMaker 9800 XL, Microtek Denver, CO) with a digital resolution of 400 dpi and a scanning area not exceeding 1024 × 1024 pixel. Before image acquisition, the scanner was calibrated for colour matching following the protocol of Shahin and Symons (2003) as suggested by Venora et al. (2009). Images consisting of few wheat glumes were captured, disposing them on the flatbed tray, distinguishing in right and left side of the ear and used for the digital image analysis. Morpho-colorimetric features were only measured for sound intact glumes, rejecting that ones with broken beak or shoulder. A total of 4253 wheat glumes were analysed.