FROM SEED TO PASTA IV

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DIPARTIMENTO DI SCIENZE AGRARIE E FORESTALI

CONSORZIO DI RICERCA GIAN PIETRO BALLATORE

Evaluation of genetic diversity of Sicilian autochthonous tetraploid wheat varieties by gluten protein analysis

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Introduction

In the latter years, there has been a renewed interest in "ancient grains" because they are perceived by consumers as more natural and healthier than modern ones (Di Francesco *et al*., 2020). The term "ancient grains" indicates the old relatives, the landraces and the cultivar of the genus *Triticum* cultivated before the Green Revolution when they have been replaced by modern varieties (De Vita *et al*., 2007). Since 2009, in Italy, landraces and historical varieties which are "naturally adapted to the local and regional conditions and threatened by genetic erosion" can be registered in the National Catalogue of agricultural plant species as "Conservation varieties" (Legislative Decree No. 149 of 2009, now repealed and replaced by Legislative Decree No. 20 of 2021). Sicily is a territory especially rich in durum wheat landraces and they are very important for the local economy because their products have a high-value market (Varia *et al.*, 2021). Moreover, they are appreciated by farmers because they can be grown in marginal areas and low-input farming systems and they have a higher tolerance to biotic and abiotic stresses (Fiore *et al.*, 2019). Currently, 22 out of 27 of the durum wheat conservation varieties registered in the National Catalogue originate from Sicily. Storage protein composition is a key trait for wheat as it determines its technological quality; in addition, it can be used to study genetic variability and for traceability purposes (Visioli *et al.*, 2021). This study aimed to analyse the storage protein composition of the 22 Sicilian durum wheat conservation varieties registered in the National Catalogue to detect genetic variability and to figure out if it is possible to use such data to discriminate among them.

Materials and methods

We collected 55 accessions out of the 22 Sicilian durum wheat conservation varieties from several custodian farmers, together with one historical variety, Cappelli, and 3 modern varieties, Core, Iride and Saragolla. They were grown in 2019 in Palermo (Italy) and in 2020 in Vicari (Italy). 10 individual spikes and 1 bulk of every accession were analysed for electrophoretic analyses. A-PAGE and SDS-PAGE were conducted as described in Pfluger *et al.* (2001) in order to study the electrophoretic patterns of gliadin and glutenin. The percentage of Unextractable Polymeric Protein (%UPP) was calculated on a subset of eight of the most cultivated conservation varieties and we compared them with one modern variety, Saragolla, and one historical variety, Cappelli. This value was obtained using size-exclusion chromatography (SE-HPLC) as reported by Gagliardi *et al.* (2020) (Fig.1). For SE-HPLC all samples were analysed at least in duplicate, all statistic analyses were conducted using R (R Core Team, 2022).

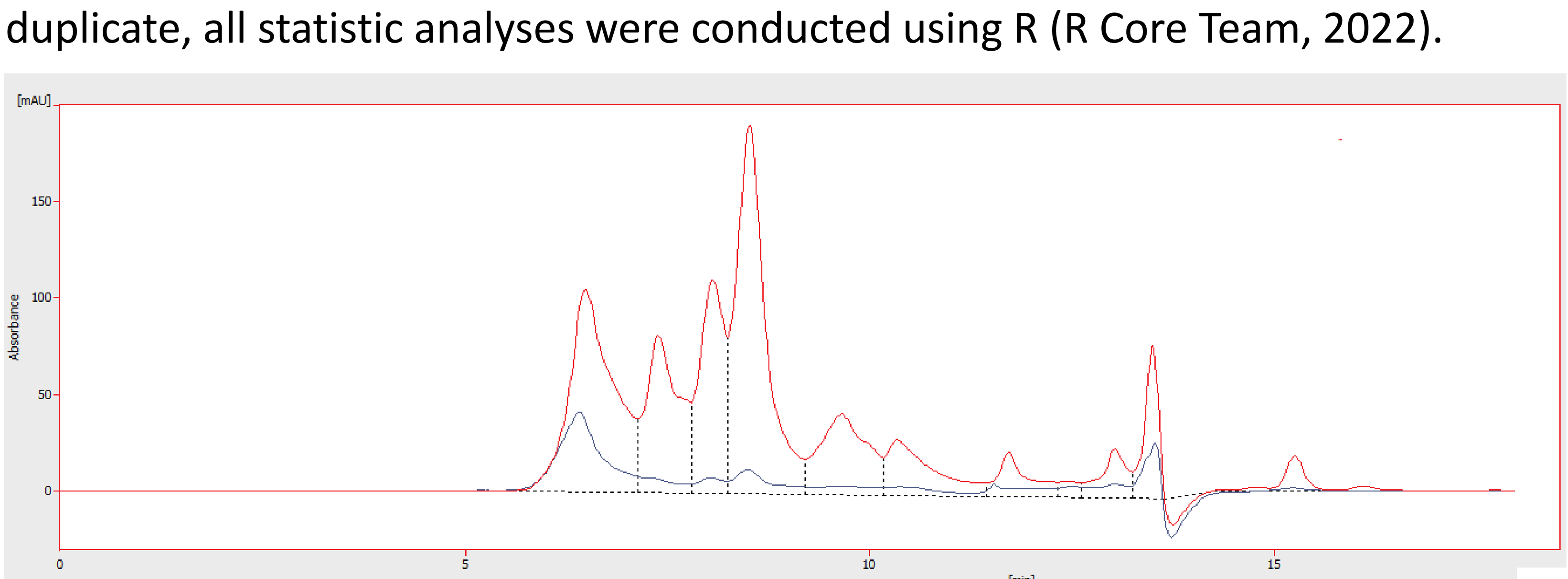

Fig. 1 – Example of a chromatogram obtained with SE-HPLC: the red line represents SDS extractable protein fraction and the blue line represents the SDS unextractable protein fraction (absorbance measured at 214 nm). The %UPP is determined as the ratio between the area of the first peak of the unextractable fraction and the sum of the area of the first peaks of both fractions.

Acknowledgments

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References

- De Vita, P. et al. Breeding progress in morpho-physiological, agronomical and qualitative traits of durum wheat cultivars released in Italy during the 20th century. European Journal of Agronomy 26, 39–53 (2007).
- Di Francesco, A. et al. Qualitative proteomic comparison of metabolic and CM-like protein fractions in old and modern wheat Italian genotypes by a shotgun approach. Journal of Proteomics 211, 103530 (2020).
- Fiore et al. High-Throughput Genotype, Morphology, and Quality Traits Evaluation for the Assessment of Genetic Diversity of Wheat Landraces from Sicily. Plants 8, 116 (2019).
- Gagliardi, A. et al. Effects of genotype, growing season and nitrogen level on gluten protein assembly of durum wheat grown under Mediterranean conditions. Agronomy 10, (2020).
- Legislative Decree No. 149, of the 29/10/2009 GU n. 254. 31 October 2009. https://www.fao.org/faolex/results/details/en/c/LEX-FAOC090693
- Legislative Decree No. 20, of the 02/02/2021 GU n. 49. 27 February 2021. https://www.gazzettaufficiale.it/eli/id/2021/02/27/21G00022/sg
- Pflüger, L. A. et al. Characterisation of high- and low-molecular weight glutenin subunits associated to the D genome of Aegilops tauschii in a collection of synthetic hexaploid wheats. Theoretical and Applied Genetics 103, 1293–1301 (2001).
- R Core Team. R: A Language and Environment for Statistical Computing. (R Foundation for Statistical Computing, 2022).
- Varia, F., Macaluso, D., Vaccaro, A., Caruso, P. & Guccione, G. D. The Adoption of Landraces of Durum Wheat in Sicilian Organic Cereal Farming Analysed Using a System Dynamics Approach. Agronomy 11, 319 (2021).
- Visioli, G., Giannelli, G., Agrimonti, C., Spina, A. & Pasini, G. Traceability of sicilian durum wheat landraces and historical varieties by high molecular weight glutenins footprint. Agronomy 11, (2021).

Results and discussions

In general, we found that A-PAGE of gliadins is a more effective technique than SDS-PAGE of glutenins to identify polymorphism. This was expected because it is well known that gliadins are highly variable. All the gliadin electrophoretic patterns of landraces showed intra-accession polymorphism except for Bivona and Ciciredda, but in these latter cases we analysed only one accession (Fig. 2). On the other hand, historical varieties that are registered as "Conservation varieties", exhibited uniformity, and this was expected because they have undergone a selection process (Fig. 2).

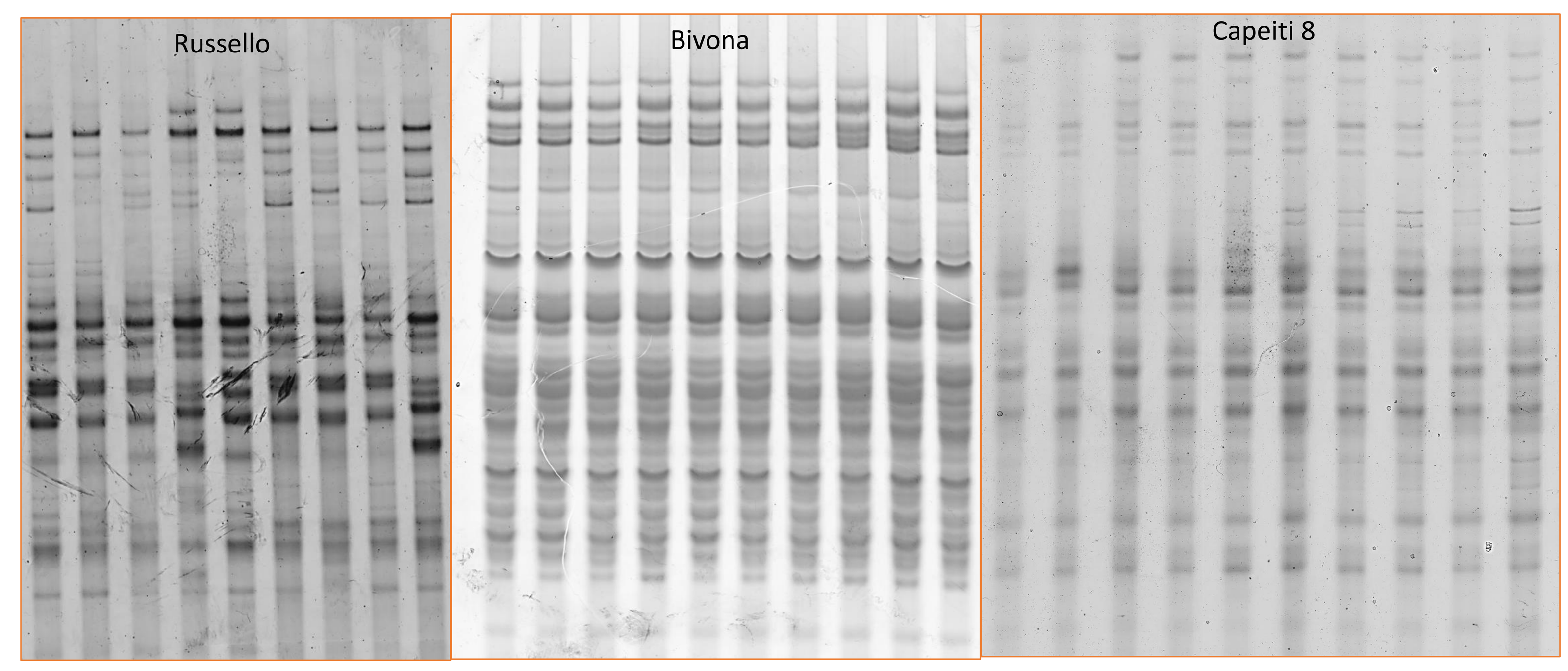

Fig. 2 – Three examples of gliadin electrophoretic patterns of ten individual spikes of three conservation varieties: Russello is a landrace and presents intra-accession eterogeneity; Bivona is a landrace but presents intra-accession homogeneity; Capeiti 8 is a historical variety and presents intra-accession homogeneity.

We analysed the electrophoretic pattern of bulk flours of every accession and bulk flours of every variety as well. We found that all the accessions of a variety have a nearly identical gliadins electrophoretic pattern but, most importantly, that the patterns are different between varieties and thus they are discriminant (Fig. 3).

We obtained the same results for bulk flours of varieties except for Gioia and Castiglione Glabro, that are not distinguishable. In fact, the electrophoretic pattern of one accession of Gioia is the same as those of Castiglione Glabro and they are also morphologically very similar. This is confirmed by molecular markers (SSR) as well (Delogu et al, in prep).

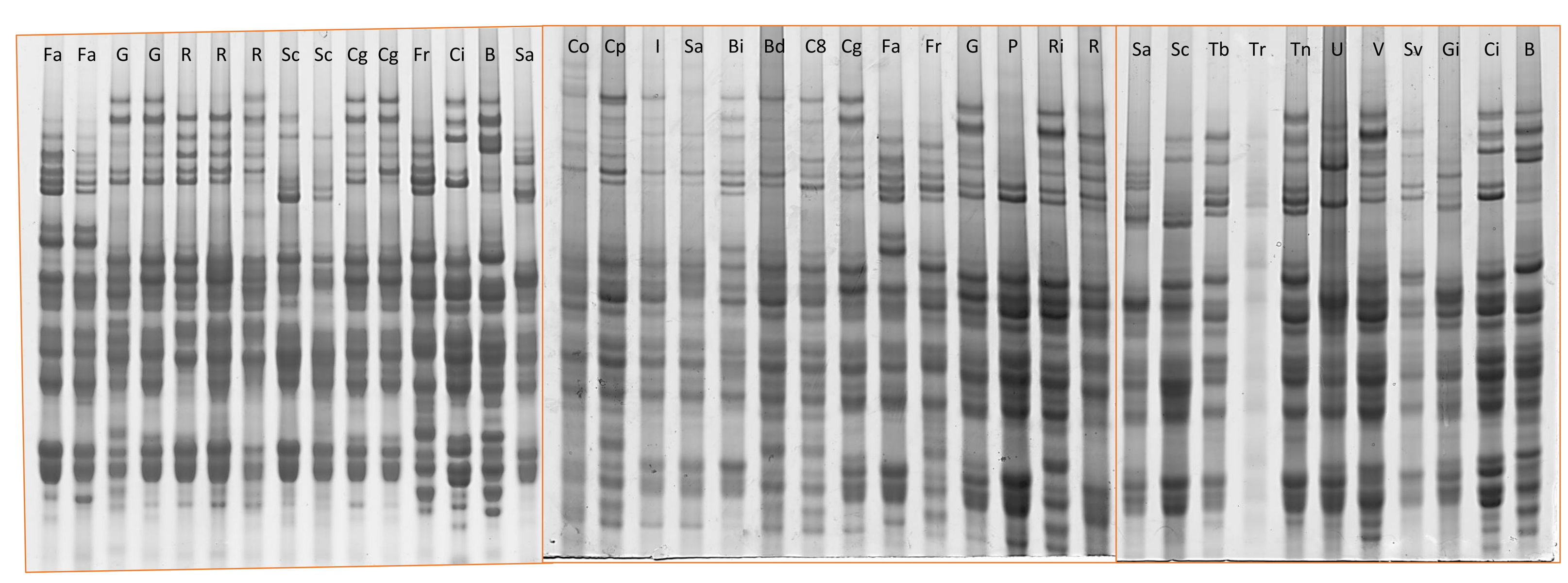

Fig. 3 – On the left one example of gliadin electrophoretic patterns of different accessions of several varieties. On the middle and on the right the gliadin electrophoretic patterns of 21 conservation varieties and the 4 varieties used as a control (Fa = Faricello, G = Gioia, R = Russello, Sc = Scorsonera, Cg = Castiglione Glabro, Fr = Francesa, Ci = Ciciredda, B = Bivona, Sa = Sammartinara, Co = Core, Cp = Cappelli, I = Iride, Sa = Saragolla, Bi = Biancuccia, Bd = Bidì, C8 = Capeiti 8, P = Perciasacchi, Ri = Russello Ibleo, Tb = Timilia Reste Bianche, Tr = Tripolino, Tn = Timilia Reste Nere, U = Urria, V = Vallelunga, Sv = Scavavuzza, Gi = Giustalisa).

Regarding the chromatographic analyses we found that in 2019, conservation varieties showed a %UPP comparable to Saragolla, but in 2020, Saragolla had an %UPP significantly higher, as expected (Fig. 4). In general, in 2020, the %UPP was higher than 2019, so a possible explanation is that in less-than-ideal conditions ancient grains perform as usual whereas modern varieties performs worst, thus technological qualities of modern and ancient varieties are comparable in adverse conditions. Focusing on individual varieties, the most interesting data is that Russello in both years had a %UPP comparable to Saragolla (Fig. 4).

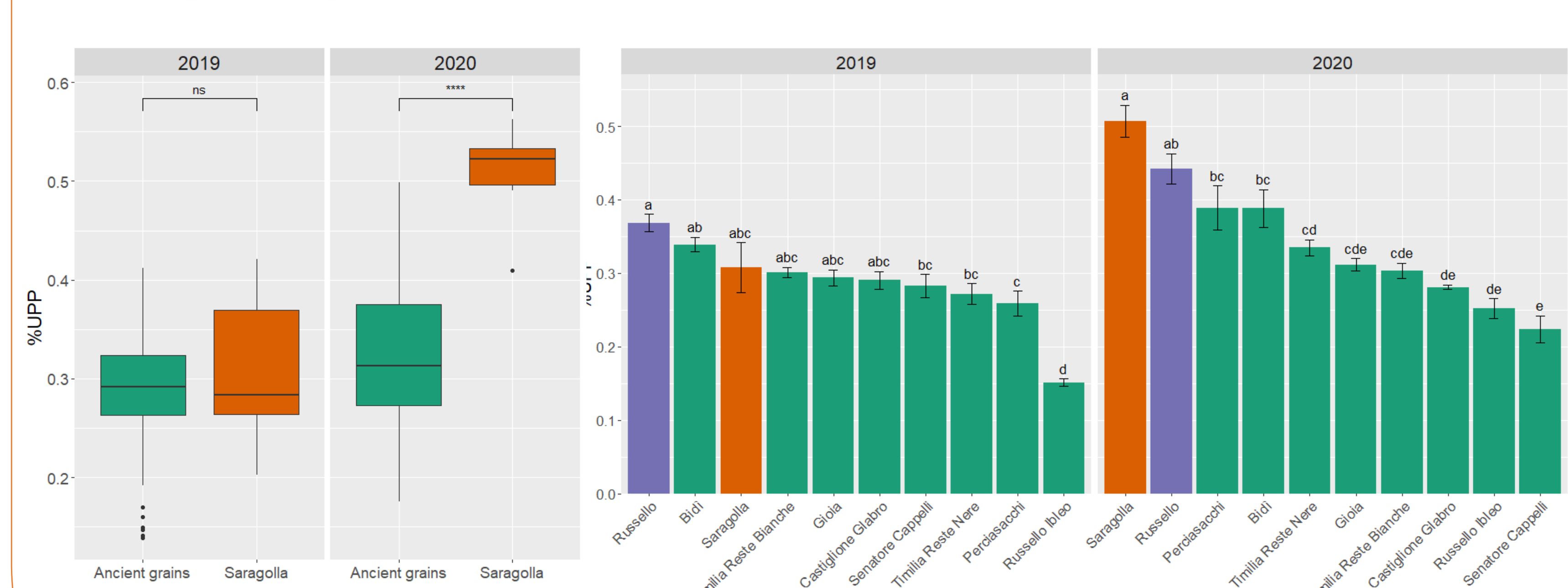

Fig. 4 – On the left, boxplot of %UPP of ancient grains and Saragolla in both years (Student t-test, α=0.05; ns = p-value > 0.05, **** = p-value < 0.001). On the right, bar chart of %UPP of individual varieties in both years; the error bars indicate two times the standard error, means not sharing any letter are significantly different (ANOVA and HSD test, α=0.05).