Polyphenols Communications 2016

XXVIIIth
International Conference
on Polyphenols

XXVIIIth
International Conference
on Polyphenols

2₀16

VIENNA
July 11-15
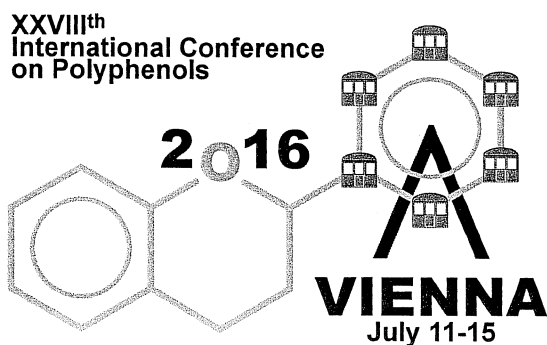
July 11th – 15th, 2016

Institute of Chemical Engineering
TU Wien

Austria

groupe polyphénols

http://www.icp2016vienna.org/

GENETIC AND GENOMIC INVESTIGATION ON PHENOLIC ACIDS IN DURUM WHEAT

Barbara Laddomada [a,*], Domenica Nigro [b], Antonella Pasqualone [c],
Laura Nunzia Delvecchio [c], Rosanna Simeone [b], Agata Gadaleta [d], Miriana Durante [a],
Leone D'Amico [a], Giovanni Mita [a], and Antonio Blanco [b]

[a] Institute of Sciences of Food Production, ISPA, CNR, Italy
[b] Department of Soil, Plant & Food Sciences, Plant Breeding Section, University of Bari, Italy
[c] Department of Soil, Plant & Food Sciences, Food Science Section, University of Bari, Italy
[d] Department of Agricultural & Environmental Science, University of Bari, Italy

MAIN CONCLUSION

We carried out the first comprehensive study of the genetic variability for phenolic acids in a collection of tetraploid wheat genotypes. We also identified genomic regions attributable to individual phenolic acids and total soluble phenolics by means of a genome wide association study (GWAS).

INTRODUCTION

Wheat species contain a variety of polyphenols including phenolic acids, flavonoids, proanthocyanidins, condensed tannins, catechins and lignans. Phenolic acids are derivatives of hydroxycinnamic or hydroxybenzoic acid (Figure 1) and represent the most common phenolic compounds found in whole wheat grains that can be found as:

Figure 1 General formulas and names of the main wheat phenolic acids identified and quantified in this study

i) soluble free; ii) soluble conjugates that are esterified to saccharides and other low molecular mass components (e.g. organic acids); and iii) insoluble bound forms that are linked to polymers of the plant cell wall. Despite their high value for human health, only few studies have been carried out on the genetics and genomics of these bioactive compounds in durum wheat.

MATERIALS AND METHODS

The collection analyzed in this study was composed of a large set of tetraploid wheat genotypes (111 accessions) including durum wheat cultivars, landraces and wild accessions of different *Triticum turgidum* L. subspecies [1].
The plant material was grown under conventional farming in the experimental field of the University of Bari, at Valenzano (Bari, Italy) in the 2011-12 and 2012-13 growing seasons using a randomized complete block design. Phenolic acids were extracted from 250 mg of whole-meal flour. After delipidation, 10 μl of internal standard (3,5-dichloro-4-hydroxybenzoic acid) were added to the residue prior to NaOH hydrolysis. After centrifugation, the supernatant was acidified to pH 2 with 12 M HCl and phenolic acids were extracted into ethyl acetate. After ethyl acetate evaporation, phenolic acids were dissolved in
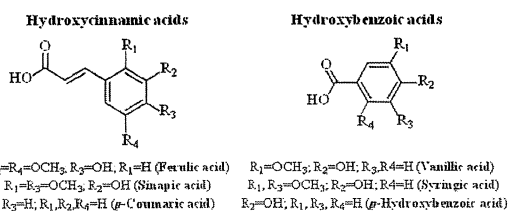
* barbara.laddomada@ispa.cnr.it

80 % methanol and analyzed by using an Agilent 1100 HPLC equipped with a photodiode array detector [2]. The total soluble phenolic compounds were extracted by adding 1 mL of methanol to 0.1 g whole meal flour, then purging with the stream of nitrogen, keeping on orbital shaker at 200 rev min^{-1}, for 2 h, in the dark, and centrifuging at 7,000 x g for 5 min. The recovered supernatants was subjected to Folin-Ciocalteu reaction [3].

The GWAS was based on the detection of correlations between genotype and phenotype in the durum germplasm collection by using a high-density wheat SNP iSelect array including gene associated SNPs.

RESULTS AND DISCUSSION

A total of six major phenolic acids were quantified across the durum wheat genotypes, namely: ferulic, sinapic, p-coumaric, vanillic, syringic and p-hydroxybenzoic acids. The concentration of total individual phenolic acids ranged from 341 to 1700 µg g^{-1} d.m., with a mean value of 800 µg g^{-1}.

Source of variation	d.f.	p-Hydroxy benzoic acid	Vanillic acid	Syringic acid	p-Coumaric acid	Ferulic acid	Sinapic acid	Total
Year (Y)	1	3.702***	19.445***	1.098***	401.927***	476395.935***	3391.107***	604268.532***
Genotype (G)	110	6.541***	50.426***	7.382***	391.356***	71766.494***	5112.566***	94561.467***
Y x G	110	1.776***	11.102***	3.964***	133.104***	15333.427***	2513.087***	27884.704***
Error	222	0.016	0.133	0.039	1.234	155.780	13.374	235.223
h²$_B$		0.65	0.69	0.48	0.60	0.70	0.50	0.63

Table 1 Combined analysis of variance and heritability (h²$_B$) of phenolic acids.
*** Significant differences at 0.001 p value. d.f: degree of freedom.

The soluble free fraction ranged from 1280 to 3150 µg g^{-1} as ferulic acid equivalents. The analysis of variance (ANOVA) revealed significant effects of genotype, year and year x genotype. The ratio of genotypic variance to total variance was moderately high suggesting that phenolic acids concentration can be further improved by breeding approaches in durum wheat (Table 1).

Results of the genome wide association analysis showed several significant marker-trait associations (MTA), identifying several quantitative trait loci (QTL) associated with phenolic acids concentration. Conservation of syntheny between SNPs and the annotated genes and proteins in *Brachypodium distachyon, Oryza sativa* and *Sorghum bicolor* can allow the identification of candidate genes coincident with QTLs for phenolic acid concentration.

REFERENCES

[1] Giovanni Laidò et al. PLoS One 8: e67280 PMID: 23826256, **2013**.

[2] Barbara Laddomada et al, *Gen Res Crop Evol*, **2016**, *in press*, doi 10.1007/s10722-016-0386-z..

[3] Antonella Pasqualone et al, *Agric Food Sci* , **2014**, *23*, 307–316.

ACKNOWLEDGMENT
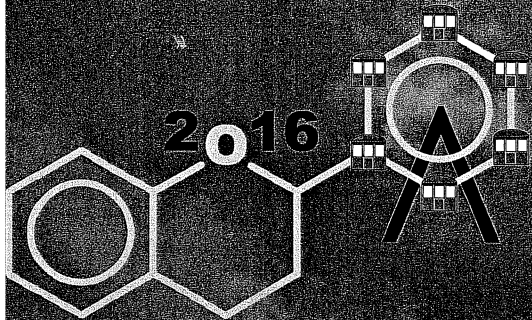
The XXVIIIth International Conference on Polyphenols
July 11-15, 2016
Vienna / Austria

2o16

groupe.polyphenols