

METABOLITE CHARACTERIZATION OF DURUM WHEAT GRAIN FOR ASSOCIATION STUDIES: EXPLORING THE NATURAL VARIATION OF FREE ASPARAGINE CONTENT

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Acrylamide, a neurotoxic molecule that is also suspected to have a carcinogenic activity, has been detected in many foods after high temperature processing. In wheat derivatives, free asparagine content has been identified as a key factor in acrylamide formation. Therefore, the control of free asparagine levels in wheat seeds is of considerable interest in contemporary crop and food sciences. Free asparagine concentration in the grain is affected by several factors, including environmental cues, such as abiotic and biotic stresses, agronomic practices, and genetic factors. Currently, few information is available about the genetic control of this trait. Our study aims to explore the natural variation of free asparagine levels in durum wheat (*Triticum turgidum* L. ssp. *durum* (Desf.)) grains to identify candidate genes

controlling this trait. To this purpose, 201 durum wheat genotypes, including 193 landraces, were selected from the Global Durum Panel (GDP), an international genotyped collection. Population structure analysis indicated that the selected panel maintained a high level of genetic variability, comparable to that observed for the GDP. Clustering analysis identified four groups, matching the geographic region of origin. The 201 genotypes were sown in an experimental field located in Fiorenzuola d'Arda (Italy) for three years. Wholemeal flour was obtained from harvested grains and free asparagine content was measured using an enzymatic method. The analysis highlighted a good variability of free asparagine levels. Both single-locus and multi-locus genome-wide association studies were performed to identify genomic regions associated with free asparagine content. Six QTNs (Quantitative Trait Nucleotides) on chromosomes 6A, 7A, 2B, 4B, 7B associated with grain free asparagine content were identified. A total of 396 genes were found in LD blocks and are under analysis, since some of them could be related to the metabolic pathways involved in free asparagine accumulation in durum wheat seeds. To deeply characterize metabolite variations associated to asparagine content, the whole grain metabolome of the 201 durum wheat genotypes was investigated by Ultrahigh Pressure Liquid Chromatography coupled to High Resolution Mass Spectrometry (UPLC-HRMS). Moreover, 100 selected samples were analysed through High Resolution Proton Nuclear Magnetic Resonance ($^1\text{H-NMR}$) spectroscopy. Both spectroscopic determinations showed a good correlation with the enzymatic method for free asparagine content quantification, confirming the good variability observed in our experimental set. These analyses will allow to identify metabolic pathways associated to free asparagine accumulation and to characterize the metabolite composition of the whole grain of the 201 durum wheat genotypes.