

INSIGHTS INTO MOLECULAR ROUTES LEADING TO GABA ACCUMULATION IN PACKAGED BROCCOLI-RAAB/“CIME DI RAPA”

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Broccoli-raab, known as “cime di rapa”/“friarielli”, belong to *Brassica rapa* susp. *sylvestris* (L.) Jansch; they are shoots made of leaves, stem and florets, eaten as cooked vegs, and have traditional and economic relevance in south-central Italy. Seed multiplication is still based on open-pollinated landraces and the packaged product has expanded in large scale retailing. Florets are most appreciated if closed (stay-green), with pungent taste, and healthy for nutritional composition. In a context of valorisation of organic local product and shelf-life performance (supported by the Project TOC – “Gruppi di Ricerca” by Regione Lazio and Enza Zaden), metabolic and transcriptomic variations were addressed in two genotypes (unregistered hybrid and a market spread “novantino”) by comparing field-harvested (FH, control) against stored fresh (SF) and stored packaged florets (SP). Overall, 28 hydrosoluble compounds were quantified by untargeted NMR approach (amino acids, carbohydrates, organic acids, vitamins, quercetin, others) and principal component analysis neatly pictured the variables that distinguished FH, SF and SP florets, while it was less effective in genotype separation. Interestingly, gamma aminobutyric acid (GABA), a non-protein amino acid of health importance, was over 30-fold accumulated in bagged florets compared to the field-harvested ones. Parallel RNA-seq analyses showed that both genotypes had over 4000 differentially expressed genes in SF vs FH that raised up to over 8000 in SP vs FH. Focusing on SP vs FH florets, weighted gene expression correlation analysis allowed the selection of modules significantly linked to GABA variations and pointed at candidate genes underpinning GABA

regulation. After constructing the GABA pathways (synthesis, catabolism and transport), its accumulation in packaged florets of both genotypes could be significantly correlated to the reduced expression of *GABA-transaminase*, which is responsible for GABA degradation. A modest repression/unvaried transcription of *glutamate decarboxylase (GAD)* - encoding the enzyme of GABA direct synthesis from glutamate - was also observed, suggesting its minor contribution to GABA accumulation in the four-day SP florets. This indicates that *GAD* up-regulation could have occurred at earlier stages (e.g. during washing, drying, and bagging).