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Assuring the integrity of the food chain: Turning science into solutions

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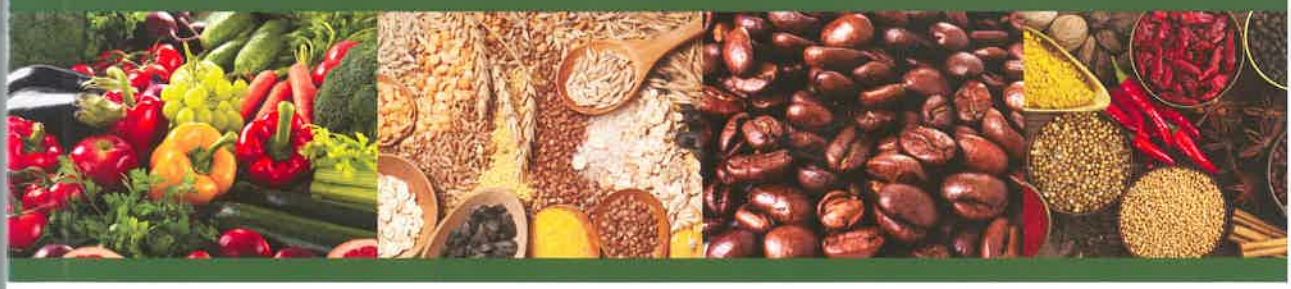

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EXPLOITING POLYMORPHISMS IN THE WAXY AND ALK GENES OF ITALIAN RICE VARIETIES TO IDENTIFY DNA MARKERS FOR CARNAROLI AUTHENTICATION

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Rice (Oryza sativa L.) is one of the most important cereals in the world, being Italy its main producer in Europe with nearly 200 different varieties present in the germplasm [1]. Italian rice varieties have different characteristics, from which the starch composition is a highly relevant parameter. Starch is composed of two polysaccharides, amylose and amylopectin, whose ratio is determinant for the rice cooking properties. After cooking, high amylose content varieties have dry, firm and separate grains, while low amylose ones usually have tender, cohesive and glossy texture [2]. Gelatinisation temperature and gel consistency are also important properties that are related to the amylopectin content [3]. Amylose synthesis is catalysed by the granule bound starch synthase (GBSS) that is encoded by the Waxy gene (Wx) [2], while the amylopectin synthesis is driven by the starch synthase IIa (SSIIa) encoded by the ALK gene [3], both located on the chromosome 6. Various nucleotide polymorphisms have been associated with the Wx [2] and ALK [3] genes, namely (CT)n repeats and several single nucleotide polymorphisms (SNP). This work intends to exploit nucleotide polymorphisms in both genes aiming at identifying molecular markers of authenticity of Italian varieties, focusing on Carnaroli rice. The Carnaroli rice is a high quality and priced variety belonging to the group of Japonica, produced mainly in Piedmont. It is considered one of the finest Italian rice varieties due to its excellent cooking resistance, given by a low tendency to lose starch and a good ability to absorb liquid while creaming, being, therefore, ideal for the preparation of traditional risotto.
In the present work, the Italian rice varieties of Carnaroli, Sant'Andrea, Carnise, Karnak, Gladio, Volano, Barone, Ronaldo, Gloria and Sole CI where obtained from producers. DNA from rice grains was extracted with NucleoSpin food kit. In silico analysis was performed in the Wx gene to design primers targeting the (CT)n microsatellite, the G/T first intron and the A/C SNP in exon 6. In the ALK gene, primers targeting SNP in exon 8 (A/G at 4041 bp and GC/TT at 4172 bp) were used [4]. In the Wx gene, the preliminary sequencing results suggest that Gladio has a different number of CT repeats

from all the other varieties; Carnaroli, Carnise, Karnak and Gladio show a G in the first intron SNP, while other varieties have T; Carnaroli, Carnise and Karnak present a C in the exon 6 and the others A. In the ALK gene, Carnise and Sole CL display an A in the exon 8 SNP, while the others show G; Carnise, Gladio, Ronaldo and Sole CL have a GC polymorphism in the exon 8, while the others present TT. These findings are being exploited for the development of a method based on high resolution melting (HRM) analysis as a promising and high-throughput tool to differentiate closely related species or even varieties.

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References

1. Cai, X., Fan, J., Jiang, Z., Basso, B., Sala, F., Spada, A., Grassi, F., & Lu, B.-R. (2013). The puzzle of Italian rice origin and evolution: determining genetic divergence and affinity of rice germplasm from Italy and Asia. PLoS ONE 8 e80351.
2. Biselli, C., Cavalluzzo, D., Perrini, R., Gianinetti, A., Bagnaresi, P., Urso, S., Orasen, G., Desiderio, F., Lupotto, E., Cattivelli, L., & Valè, G. (2014). Improvement of marker-based predictability of Apparent Amylose Content in japonica rice through GBSSI allele mining. Rice, 7(1), 1.
3. Zhou, Y., Zheng, H., Wei, G., Zhou, H., Han, Y., Bai, X., Xing, Y., & Han, Y. (2016). Nucleotide Diversity and Molecular Evolution of the ALK Gene in Cultivated Rice and its Wild Relatives. Plant Molecular Biology Reporter, 34(5), 923-930
4. Kadaru, S. B., Yadav, A. S., Fjellstrom, R. G., & Oard, J. H. (2006). Alternative ecotilling protocol for rapid, cost-effective single-nucleotide polymorphism discovery and genotyping in rice (Oryza sativa L.). Plant Molecular Biology Reporter, 24(1), 3-22.