DEVELOPMENT OF MRM METHODS TO QUANTIFY PROTEIN ISOFORMS OF POLYPHENOL OXIDASE

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Protein quantification using multiple-reaction monitoring (MRM) is an emerging technology with promising application in high throughput protein biomarker assay in complex biological samples. Other assays such as enzymatic activity and western blotting endow shortfalls that introduce uncertainty in the assay results. Enzyme assays in crude extracts are contributed by the activity of the different isoforms and such contribution cannot be resolved straightforward. Western blotting techniques would resolve the isoform heterogeneity in complex samples provided that highly isoform specific antibodies are developed, which would demand lots of resources and time. MRM-based assays could overcome these shortfalls by directly detecting and quantifying proteolytic peptides unique to particular isoforms, i.e. proteotypic peptides at isoform level, with high specificity, sensitivity, accuracyand assay speed. We have focused on Polyphenol Oxidase (PPO), a plant conspicuous enzyme encoded by multigene families as demonstrated in several species, including tree plants of the Rosaceae family such as loquat (*Eriobotrya japonica* Lindl.). PPO is responsible for the enzymatic browning of fruits and vegetables, making them more attractive to seed dispersal agents but also being a major cause of important economical losses in agriculture and food industry. PPO also protects plants from biotic stress, thus having a benefit on agriculture. An adequate management of PPO at plant breeding level would maximize the benefits and minimize the disadvantages of this enzyme, but it would require a precise knowledge of the biological role played by each isoform in the plant. Thus, for the functional study of the PPOs we have cloned, characterized and overexpressed fragments of three PPO isoforms from loquat to develop MRM-based methods for quantification of each isoform. In this study we present the results of the development of an MRM method to characterize and quantify different PPO isoforms in loquat and other species sharing the same proteotypic peptides.