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BTL-LIKE PROTEINS AS MOLECULAR MARKERS TO EVALUATE POLYPHENOL ACCUMULATION POTENTIAL AND MATURATION RATE IN GRAPE

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Abstract — Flavonoid accumulation is a phenomenon associated to grape maturation and could be ascribed to several active and passive mechanisms. A more detailed knowledge of these processes could provide outputs about to improve the nutritional values of grape and its derivatives. This goal will be obtained assessing an immunochemical detection device able to point out the expression level of membrane transporters in grape skin and, thus, to estimate the polyphenol accumulation potential. In addition, this innovative test could allow to determine the best time for grape harvest, following in the field the dynamics of flavonoid accumulation systems. This parameter will implement the usual parameters applied by agronomical techniques, which are based on sugar content and acidity measurement. The evaluation of the phenolic maturity represents an efficient tool for the assessment of grape quality, since it takes into account the concentration of secondary metabolites as fundamental antioxidant molecules.

Index Terms — TRANS2CARE, grape, flavonoid accumulation, membrane transporters, bilitranslocase-like proteins, maturation marker

1 BACKGROUND

In agronomic practices polyphenol content is one of the most critical characters to assess grape quality. The accumulation of these secondary metabolites (flavonoids) has a significant impact on fruit nutritional value, as a source of antioxidants for the diet and on the subsequent stages of wine processing. Polyphenol synthesis and accumulation in grape cells could positively affect their bio-availability in mammals, where the absorption into cells is ensured, among others, by bilitranslocase (BTL), localized at the intestinal and stomach level, in liver and kidney epithelia as well as in vascular endothelium.

2 OBJECTIVES

The aim of the present project was to study and understand which are the mechanisms underlying the accumulation of secondary metabolites in plant cells, such as polyphenols, particularly flavonoids. It has widely accepted that there are several mechanisms and transporters responsible for this process, either active or passive. The characterization of the transporters involved is essential to develop cultivation techniques, useful to maximize the content of flavonoids in grapes in the field. In addition, it will allow to choose the best time to harvest, when flavonoid accumulation is high and, simultaneously, sugar content has not reached its maximum level yet. This target is in agreement with the new market guidelines aiming at obtaining wine products with high nutritional quality, lowering sugar content and, consequently, alcohol production during winemaking.

3 APPROACH & METHODS

General approach

The application of a molecular approach is proposed, using antibodies produced against mammalian BTL to identify similar transporters of flavonoids in grape berry. The antibody was built against the transporter specific sequence involved in recognition and binding with the polyphenolic substrates.

Methods

ELISA test and immunochemical methods will be applied. A diagnostic kit will be developed to easily detect and possibly to quantify the presence of grape transporters in berry skin. The device will be developed in a stick format for ease-of-use.

4 RESULTS

The results obtained so far show that in grape berry skin there are at least five proteins able to cross-react with an Ab raised against a specific sequence (234-245) of mammalian BTL (Figure 1). All these proteins are potential candidates in interaction with flavonoids, since the BTL sequence is known to be involved in flavonoid binding and transport. In addition, a transport activity, measured with spectrophotometric assay, is detectable in grape and this process is inhibited by the addition of the anti-BTL Ab (Figure 2). Moreover, on the basis of ELISA assay, it is shown that the expression level of BTL-like proteins increases during berry maturation, exhibiting a pattern similar to that detectable for flavonoid accumulation (Figure 3).

5 POTENTIAL NEW PRODUCTS & SERVICES

The results will allow the realization of an immunochemical kit able to determine the presence of the carrier of flavonoids with a simple colorimetric assay performed on skin extracts. If the sequence recognized by the Ab would appear to be conserved in the plant kingdom, it could provide cross-reaction with other enzymes involved in active transport of polyphenols. Therefore, the method will

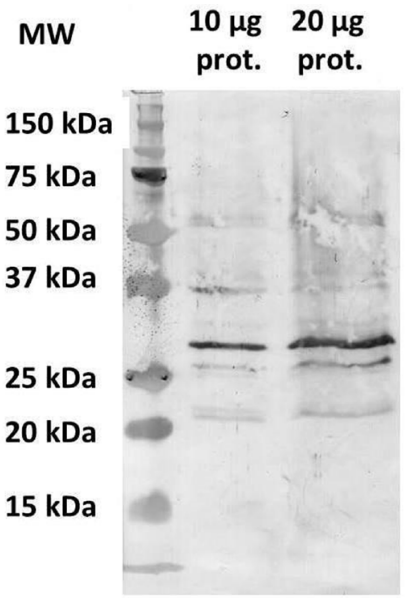

Figure 1: Immuno-chemical analysis of BTL-like proteins in microsomes from berry skins.

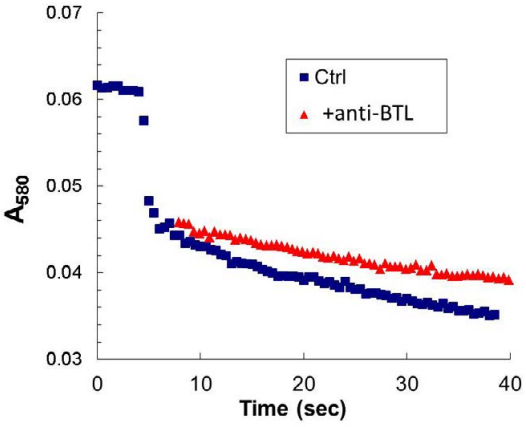

Figure 2: Spectrophotometric transport assay in microsomes from grape berry pulp. Inhibitory effect due to anti-BTL Ab addition.

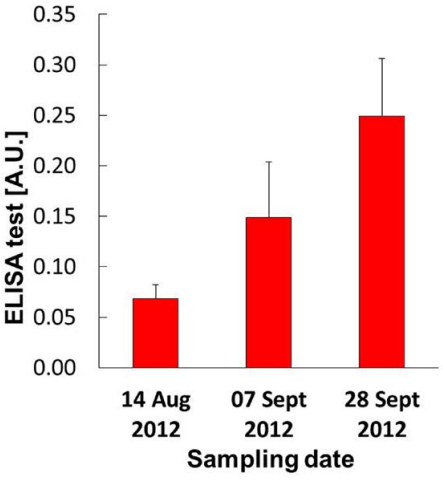

Figure 3: ELISA test performed on berry skin extracts obtained from berries at different developmental stages.

also provide an effective mean to assess the potential of phenolic metabolite production in grape cultivar. Such a determination would represent a useful application in genetic selection and breeding of new accessions, to identify the most promising clones to be propagated.
The main stakeholders of the project will be farmers and breeders, with whom the setup and development of the diagnostic kit would be necessarily performed.

6 CURRENT COLLABORATIONS

With other researchers: during these first steps the involvement of researchers from University of Trieste (Dept. Life Sciences - LP, Trans2Care) Nova Gorica University (Slovenia - PP3, Trans2Care), Agricultural Institute of Slovenia (Ljubljana, Slovenia), Dept. of Plant Physiology (SAS, Bratislava, Slovakia) and University of Hohenheim (Faculty of Natural Science, Germany) has been established.
With SMEs: it would be crucial the involvement of local farms to test the feasibility and operation of the project.
With hospitals: blood Transfusion Centre of Slovenia (Ljubljana, Slovenia - PP10, Trans2Care).
With associations: A close collaboration with professional associations (both agricultural and enological operators) is required to assess the prototype and develop field test. These contacts are partially already available ("Consorzio Tutela Vini Collio e Carso" e "ZDRUŽENJE KONZORCIJ KRAŠKIH PRIDELOVALCEV TERANA").

7 CONTACT OR COLLABORATIONS NEEDED

A collaboration is needed with biotechnology laboratories to implement and develop the diagnostic kit.

8 COMMUNICATION TOOLS

Websites: Trans2care, ResearchGate, LinkedIn

9 FUNDS NEEDED

9.1 For basic research (investigation of biological mechanisms): 20.000 €

9.2 For applied research (solutions for real-world problems): 100.000 €

9.2 For pilot & demonstrator activities (to develop a prototype): 150.000 €

10 CONCLUSION

The development of large scale production of the innovation will require subsequent steps, since the current technology readiness corresponds to a laboratory level. The next implementation of the project will need the involvement of an enterprise with biotechnological competence, with the aim of producing a diagnostic kit developed in a stick format for ease-of-use.
This device could provide to winegrowers useful directions to plan the harvest time and to select accessions characterized by high polyphenol content.

ACKNOWLEDGEMENT

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