

## METHYLATION PATTERNS IN *BRASSICA OLERACEA* L.: CHANGES DURING TIME AND BETWEEN DIFFERENT AGROCLIMATIC CONDITIONS

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Among the *Strategies for Organic and Low-input Integrated Breeding and Management* (SOLIBAM) project aims, there is the identification of epigenetic modifications caused by farming under different agronomic and/or pedoclimatic conditions. SOLIBAM project is funded by the European Commission under the Seventh Framework Programme (GA 245058, coord. Véronique Chable, INRA, scientific responsible Valeria Negri, UNIPG).

Plants of two hybrid varieties of broccoli (*Brassica oleracea* L. var. *italica* Plenck), Iron Man (IM) and Santee (SN) were grown in three experimental fields under different climatic and management conditions: low input (LI) and organic agriculture (OA). Young and healthy leaves were collected from both varieties and from each experimental field at three different dates during winter time. Differences in methylation state were identified applying the *Methylation-Sensitive Amplified Polymorphism* technique (M-SAP) using 5 different primer combinations on genomic DNA extracted from 36 samples (2 plants for each of the 2 hybrids, grown in 3 different experimental fields and collected at 3 different times).

From a total of 210 produced amplicons (with an average of 42 for each primer combinations) 25 were specific of IM, 13 of SN and 172 were found in both hybrids. Seventy two amplicons in Iron Man (36,5%) and 70 amplicons in Santee (37,8%) were from non-methylated regions. Among the amplicons from methylated regions, 41 (21,4%) in IM and 51 in SN (27,6%) showed the same profile at the three growing stages. Presence *vs* absence of 83 amplicons in IM and 64 in SN underlined changes in methylation patterns in relationship to different sampling dates, while 17 in Iron Man and 19 in Santee are putatively related to the different agro climatic growing conditions. Intriguing enough, 9 of these amplicons are common between the two analyzed hybrids. Changes in methylation state have generally occurred from the non-methylate or the hypermethylated pattern to the intermediate states or *vice versa*. Direct shifts between hyper-methylated to non-methylated state were rarely recorded.

Considered the large number of polymorphic amplicons obtained, the M-SAP technique appears to be an appropriate method to identify the methylation state modification. The technique will be used in further studies aimed at assessing broccoli epigenetic modifications due to different climatic and management conditions.