ECONOMIC EXPLOITATION OF GENOMICS: GENETIC AND PHYSIOLOGICAL REGULATION OF SKIN RED COLOUR DEVELOPMENT ON APPLES UNDER HIGH TEMPERATURE ENVIRONMENTS

PIRSES-GA-2008: 230857

Acronym: REDHOTGEN

Project Coordinator: Dr. Ignasi Iglesias

Contact person: ignasi.iglesias@irta.cat website: www.irta.cat

Project partners: FEM-IASMA (Italy): www.iasma.net
Plant & Food Research (New Zealand): www.plantandfood.co.nz

SUMMARY

Warm summer temperature can cause a lack of colouration of apple skin

Major apple producing regions are located in warm areas of South European countries, such as the Ebro Valley (Northern Spain), Emilia Romagna (Italy), Midi Pyrénées and Languedoc-Roussillon (Southern France). Spain is the country where almost all the apple production is located in warm regions and this negatively affects the fruit quality obtained from economically significant cultivars such as ‘Golden Delicious’ (lack of firmness) and bicoloured cultivars such as ‘Gala’ and ‘Fuji’ (lack of skin colouration). The lack of apple’s adaptation to warm temperature has caused a dramatic decrease of production in Spain from 920k ton to 540k ton in the 1985-2010 period. Poor coloured fruit is perceived by the consumer as a lack of quality. Consequently, the economic return to the fruit growers can be reduced by as much as 60% compared to the premier class apples.

Apple skin colouration occurs during the last stages of fruit development and for early to mid-season cultivars this is during the late summer months in temperate regions. These months are particularly warm in some apple producing regions such as Northern Spain and these high temperatures cause a lack of red skin colouration. It is likely that this issue will increase due to global warming, i.e. be found in other apple producing regions in the world. As there is no sustainable horticultural solution to overcome this problem, one strategy is to develop new varieties that still develop red colour well under warm summer temperatures. To achieve this, a more in-depth physiological knowledge and genomics tools were required for faster and more efficient breeding of new apple varieties with good red skin colour. The scientific objective of the programme was therefore to establish the genetic and physiological bases of the regulation of skin red colour development on apples under high temperature

environments as a model case of economic exploitation of genomics knowledge. Having cultivars that can express high colour and quality even under the climatic conditions of warm environments is the most important and sustainable option for high quality apple production in these countries, thus increasing both the benefit to growers and apple consumption. On a more practical aspect, this project was a first example of the use of genomics technologies to understand a complex character and develop tools applicable for more efficient and faster breeding of new cultivars.

Physiological control of skin colouration

Our results have enabled us to develop a better understanding of the response of apple skin to warm temperatures. We studied the physiological control of skin coloration by comparing skin colour of the same cultivars grown in different environments: New Zealand (cool summer) and Spain (warm summer). Apple skin red colouration is due to anthocyanin pigments. We demonstrated that anthocyanin concentration in the apple fruit skin is affected by warm summer temperature. This was confirmed by warming fruit *in vivo* in New Zealand. We made a complete inventory of the genes coding for the enzymes involved in the anthocyanin synthesis pathway, as well as the genes regulating these enzymes using the apple genome sequence. We paid special attention to regulatory genes as they are likely to respond to environmental conditions and then up- or down-regulate the enzymes that synthesize pigments. We studied the gene expression of such synthetic enzymes and regulatory genes in a range of cultivars with contrasting red skin colour patterns, as well as cultivars grown under warming (in NZ) and cooling (in Spain) conditions (Figure 1). Our results demonstrated the down regulation of the anthocyanin biosynthetic and regulatory genes by warm temperature.

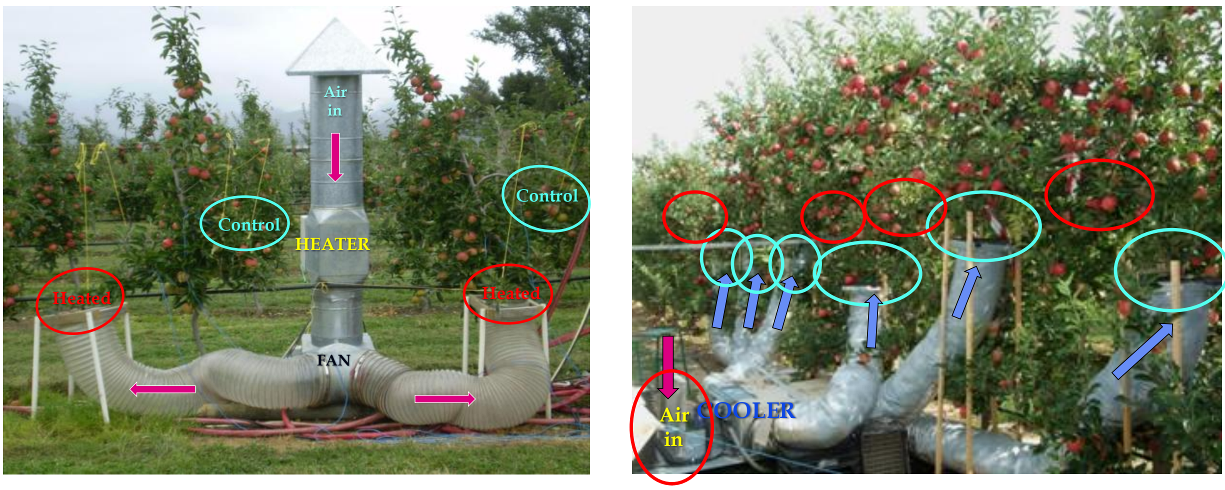

Figure 1: Cooling 'Gala' apples in Nelson (New Zealand) to simulate the temperatures of warm climates as Spain (left); and cooling 'Gala' apples in Lleida (Spain) to simulate the temperatures of mild climates as New Zealand (right). The gene expression of synthetic enzymes and regulatory genes was studied in this modified conditions compared to the control without modification.

Using apple genomics to solve a complex physiological issue and address climatic change in apple growing regions.

Apple genomics can help discover the genetic variants causing trait variation, such as reddening *v.* non-reddening. Our study on the response of skin colouration to warm temperature is a first example of how the apple genome can help understand a trait with significant economic issue. A draft genome sequence was developed and published by IASMA-FEM during the project. However, the genome sequence data was available to the project researchers prior to its publication.

The apple genome sequence enabled the development of large sets of genetic markers to be tested on a genetic apple seedling population with contrasting phenotype for red skin coloration under warm summer temperature. These genetic markers were then screened in an apple progeny segregating for skin colouration and then correlated with the phenotypic variation.

Genetic control of skin colouration under warm temperature

There is a variable response to warm temperature in different apple cultivars. Mutants of 'Gala' have contrasting skin colour phenotypes. For example, 'Royal Gala' and Galaxy™ apples are less coloured than 'Brookfield™ Gala' under warm Spanish conditions, while all colour adequately in New Zealand. Indeed 'Brookfield™ Gala' can be excessively coloured in the cooler growing regions of New Zealand.

We used genetic populations obtained from controlled crosses using red-skinned cultivars as parents. A segregation of skin colouration was observed in the progeny, with some seedlings showing an absence of skin coloration and other seedlings showing strong red colouration under warm temperature. The experiment was repeated over two years to verify our results. Skin colour was measured by visual assessment, colorimetry and anthocyanin pigment concentration across the population and this data was compared with a set of 300 genetic markers evenly distributed through the genome. This enabled us to construct a genetic map and identify genomic regions that are linked to the red colouration of the skin (Figure 2). The genomic region explaining most of the variability in skin colouration is at the base of chromosome 9, where a gene coding for a regulator of the anthocyanin biosynthesis pathway enzymes is located (*MYB10*). The association between a marker located close to *MYB10* and skin colouration was confirmed in a wider set of apple germplasm.

In addition to being correlated with skin colour in a segregating apple progeny and in a set of apple breeding material, *MYB10* expression is also affected by heating fruit in NZ and under control hot conditions in Spain, while it is more highly expressed concomitant with the appearance of pigments under cooled conditions and unheated conditions in NZ. This strongly suggests a major involvement of *MYB10* in the determination of apple skin colouration.

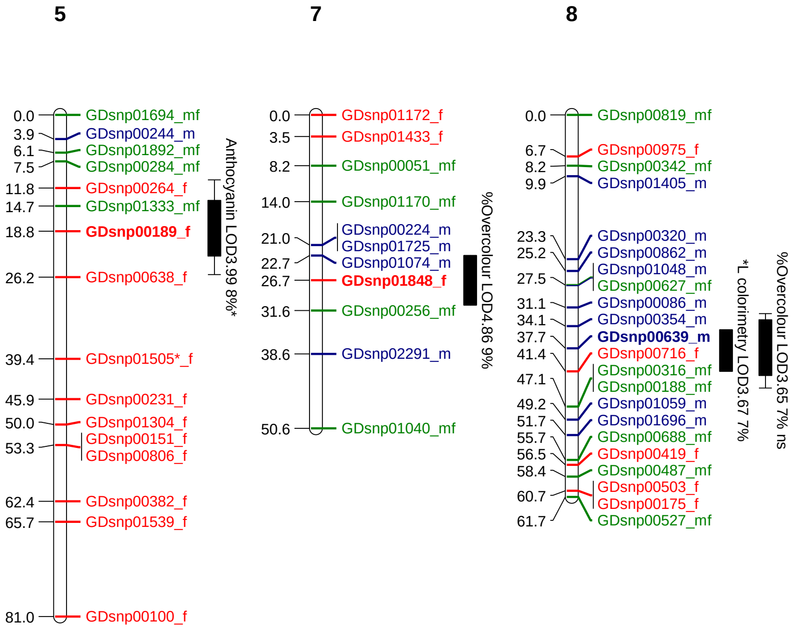

Figure 2: QTL detection in LG 5, 7, 8, for red skin colouration in the 'A121R18T089' x 'Gala' ('Galaxy' + 'Brookfield') population.

Impact of our research

Genetic markers linked to red skin colouration in apple have been identified, including markers located close to *MYB10*, a gene controlling the anthocyanin synthesis pathway and responding to warm temperature. It is likely that allelic variants of *MYB10* are less sensitive to warm temperature. Such alleles can be selected for in breeding populations to enable the selection of apple seedlings that are resistant to warm summer temperature producing red colour on the skin. A genetic marker derived from *MYB10* must be developed and tested on breeding populations to validate its efficiency as a tool for faster breeding using marker-assisted selection.

The information obtained could be after further development as a useful tool for breeders to improve the efficiency of breeding programs focusing on the development of new apple cultivars with high potential of fruit colour development even in warm and hot climates typical of Mediterranean areas.