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Plant Biology

How almonds became sweet

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ABSTRACT

How many times have you enjoyed sweet, healthy almonds and, suddenly, all this sweetness became erased by a taste of a single bitter one? Almond genome has enlightened how a single point mutation turned the previously bitter almonds sweet.

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Salads, vegan milk, yogurt, marzipan - all these products contain healthy sweet almonds. The almond ancestor, which still grows in the wild, carries bitter almonds. Consumption of its bitter kernels can be lethal to us and to wild herbivores. The bitterness comes from the presence of amygdalin, a compound that can release toxic cyanide and that almonds accumulate as a defence against herbivores and pests. The key event enabling us to grow almonds as a crop was the selection of a tree that cannot produce amygdalin and therefore has sweet kernels. Agriculture maintained the *sweet kernel* (*Sk*) trait for several thousand years, enabling us to enjoy their delicious taste and generating a business of 7.5 billion US dollars per year.

For years, breeders have analysed numerous crosses between sweet and bitter varieties to understand how almonds inherit their taste. They discovered that the *Sk* trait is controlled by a change in a single gene, however when and where the it arose remained unknown. Each individual almond tree carries either sweet or bitter almonds, never a mix of the two. Knowing the gene that gives almonds their sweet taste, we could develop an easy genetic test to select for the sweet kernel taste already at the seedling stage and accelerate the breeding process. We therefore set out to identify the mysterious *Sk* gene.

In our previous research, we discovered the entire recipe the almond uses to make amygdalin. It consists of four steps: the first two steps take place in the seed coat (the thin brown layer that covers the kernel) and the last two in the kernel itself. Specific enzymes are necessary for completing the recipe:

their mission is to transform one compound into another. Genes (DNA) encode information about how to make the enzymes (proteins), but before a gene becomes an enzyme it has to temporarily become a messenger (RNA). When we compared the levels of the four messengers in the sweet and bitter varieties, we discovered that no messengers for the two first enzymes were present in the sweet seed coats. The levels of messengers are controlled by another gene encoding formation of a transcription factor. **Clue number 1: we were looking for an altered transcription factor.**

In this study, the sequence of the almond genome - the complete set of all its genes was determined, composed of almost 28 thousand genes, distributed on eight chromosomes. Comparing the sweet and bitter varieties, we narrowed down the position of the *Sk* gene to an eleven-gene region on chromosome five. Similar to digging in an archaeological cave, we found that five of these genes encoded transcription factors called bHLH 1 through 5. **Clue number 2: in the sweet varieties, one of the five bHLH factors carried a unique mutation.**

We then analyzed the amount of messenger corresponding to each of the five bHLH transcription factors and showed that only those of bHLH1, 2 and 4 were present in the seed coat. To identify which of these were involved in production of amygdalin, we replicated the transition from DNA to protein in a test tube instead of in the intact almond. Only the bHLH2 encoding sequence from the bitter variety was able to produce the messengers for the amygdalin-producing genes. **Our main suspect was the transcription factor bHLH2.**

When we compared the gene sequences encoding the bHLH2 transcription factor in sweet and bitter almond, we discovered that they differed by a single letter. We exchanged that specific letter between the two varieties and saw that it enabled the sweet almond to make amygdalin, and prevented the previously bitter version from doing so. **Eureka! We confirmed that the *Sweet kernel* gene encodes the bHLH2.**

Thousands of years ago, the mutation in bHLH2 made it possible to introduce almonds into our diet without any toxicity risks. Having identified the gene responsible for sweetness has rendered it possible to set-up an easy DNA test that already, at the almond seedling state, identifies the seedlings, which will develop into almond trees carrying sweet kernels. In the past, removal of almond trees carrying bitter almonds would only be possible after three to four years, when the tree flowered and carried the first set of almonds. And more importantly, with the complete sequence of the almond genome, we now have tools to find other genes responsible for important agronomic traits like flowering time, pest resistance and drought tolerance. This could help to introduce new almond varieties, for example, ones that adapt better to the effects of climate change.