Tomato **diversified** by modern plant breeding

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The diversity among glasshouse tomatoes in NW Europe was low in the 1950s, and even lower in the 1960s, due to inbreeding and selection. But from the 1970s onwards the diversity increased considerably because of introgressions of disease resistances from wild donors, and introgressions for fruit quality since the 1990s in response to the 'Wasserbombe' crisis.

A paper in the Nature Biotechnology journal on domestication of tomato voiced the general belief that "breeding of crops over millennia for yield and productivity has led to reduced genetic diversity. As a result, beneficial traits of wild species, such as disease resistance and stress tolerance, have been lost (...). Despite the increases in yield conferred by domestication, the breeding focus on yield has been accompanied by a loss of genetic diversity and reduced nutritional value and taste" (Nat. Biotech. 36, 1211-1216, 2018).

The loss of genetic variation in crops has been termed genetic erosion. During domestication preferred genotypes were selected, leading to loss of alleles and a decrease in genetic diversity of landraces, compared to wild accessions. Breeding can further reduce genetic diversity by continued selection or, in contrast, may broaden genetic variation through the introgression of alleles from wild relatives. The question remains whether the increase in diversity because of introgression has compensated for the reduction of genetic diversity as a result of inbreeding and selection. We will answer this question, using tomato as an example.

We have studied the diversity of commercial tomato varieties for glasshouses in North West Europe since the 1950s. We randomly selected about 12 varieties per decade and investigated these on two levels: 1. Genetic diversity, so at the DNA level; and 2. Phenotypic diversity of the fruits.

Genetic diversity

We isolated DNA from 90 tomato varieties that were commercially released between 1950 and 2016 in North West Europe. All varieties were for greenhouse production of tomatoes for the fresh market. The DNA was analyzed by means of a SNP array, yielding 7720 SNP-marker scores per variety. These SNP scores allowed us to estimate the genetic diversity between the varieties within a decade. Figure 1A shows that the genetic diversity was very low initially, in the 1950s and the 1960s. However, from the 1970s onwards, the diversity increased markedly. Apparently, the increase in diversity caused by introgressions from wild accessions was far more pronounced than the decrease in diversity by selection. Nowadays, approximately 28% of the genome of modern glasshouse tomato varieties is 'novel', compared to the 1950s and 1960s. That 'novel' DNA consists of chromosomal fragments from wild relatives, introgressed by plant breeders.

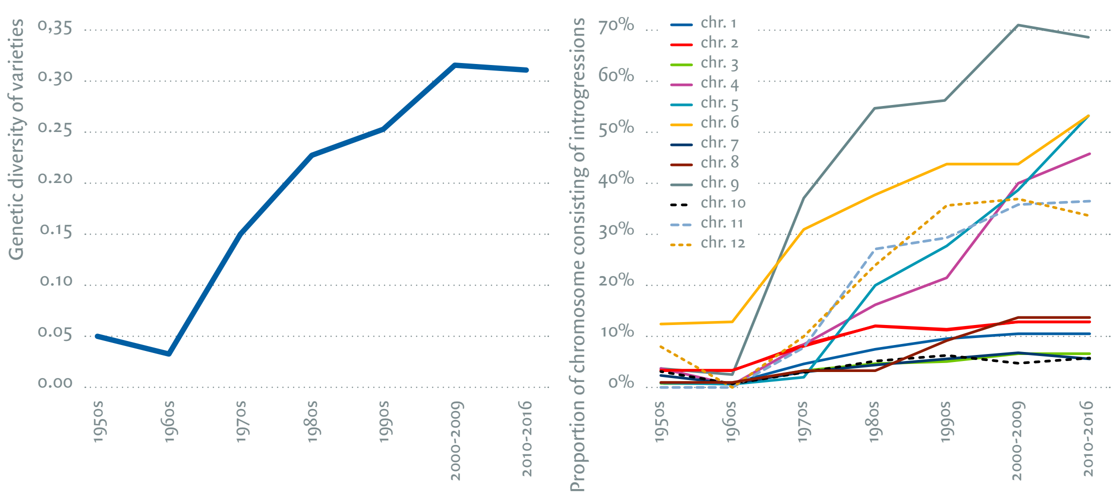

Figure 1. Genetic diversity of tomato varieties that were commercially introduced from 1950 through to 2016. (A) The diversity between varieties within a decade; (B) Proportions of the chromosomes of commercial tomato varieties consisting of introgressions, compared to the prevailing tomato genome in the 1950s and 1960s (modified from https://doi.org/10.3389/fpls.2019.01606)

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There is a tremendous increase in diversity, both at the genotypic and phenotypic level

The introgressions have occurred in all tomato chromosomes, but some chromosomes had far higher proportions of DNA from wild relatives than other chromosomes (Fig. 1B). These differences in quantity of 'wild DNA' between the chromosomes are not caused only by the number of introgressed traits per chromosome, but also by the linkage drag that comes along with the introgressed alleles.

First diversity boost

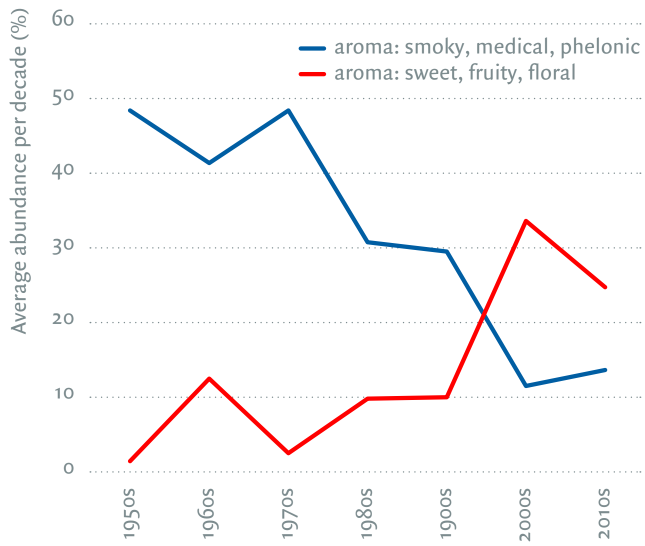
The Dutch Descriptive Variety Lists ('Beschrijvende rassenlijst voor groentegewassen') provide insight into the phenotypic traits of tomato varieties since the 1940s. These lists show that at the phenotypic level, too, the diversity among varieties was very small in the 1950s and 1960s, but from the 1970s onwards, the diversity increased because of an increasing number of resistances to diseases and pests.

In the 1940s, 1950s and early 1960s, minor differences in fruit sizes were mentioned, being associated with the number of locules per fruit, however, no beef or cherry types were mentioned yet. No other differences were mentioned for the commercial tomatoes in these decades. As the diversity in traits was very low, one would expect that the number of varieties would be low too. However, that was not the case. In the Descriptive List of 1951, 66 greenhouse tomato varieties were mentioned. This List mentioned that, sometimes, hardly any or no differences could be observed between varieties. In spite of that, these varieties were still released as separate commercial varieties or selections. In these decades, all varieties were still 'open pollinated' inbred lines, so no hybrids yet. No disease resistances were mentioned in the Descriptive Variety Lists until the late 1960s.

Remarkably, in the Descriptive List of 1951, ornamental tomato types were also mentioned ('siertomaten'), with special colours (yellow), sizes (cherry, currant) or shapes (pear shape). These tomatoes were not meant for commercial tomato production, nor for consumption, but just as ornamentals, in gardens, for example. In later Descriptive Lists, these ornamental tomatoes were no longer mentioned. However, the special fruit colours, sizes and shapes would become very important at the end of the 20th century and in the 21st century, albeit not for ornamental purposes, but rather for consumption!

From the late 1960s onwards, more and more

Figure 2. Average abundance (%) of guaiacol (smoky, medical, phenolic aroma) and 2-phenylethanol (sweet, fruity, floral aroma) over the seven decades since 1950s



resistances to pests and diseases were introgressed from wild relatives, leading to a sharp increase in the genetic diversity of commercial varieties. These introgressed resistances are the reason for the increase in diversity in the 1970s and 1980s, shown in Fig. 1. An additional factor was the replacement of homozygous tomatoes by F1 hybrids during the 1970s, but that was not the main reason.

Sometimes, resistance genes were accompanied by large chromosomal fragments that were genetically tightly linked to the resistance genes, leading to linkage drag. A rather extreme example is a huge introgression fragment from Solanum peruvianum in chromosome 9. This introgression carries the tomato mosaic virus (ToMV) resistance gene Tm2 (derived from S. peruvianum PI 126926) or its allele Tm22 (derived from S. peruvianum PI 18650). The exotic fragment encompasses about 79% (53 Mb) of Chr 9 in this modern variety, leading to the enormous change in the composition of Chr 9, shown in Fig. 1B. Although breeding companies already started selling tomato varieties with this introgression in the 1970s, and although more than 90% of the modern greenhouse varieties carry one of these S. peruvianum introgressions, the introgression size has remained very large.

From the 1970s onwards, the genes Cf-genes were introgressed to provide resistance to leaf mould disease, caused by Cladosporium fulvum. Another resistance gene introgressed during the same period is the Mi-1 gene from S. peruvianum, conferring resistance to southern root-knot nematode (Meloidogyne incognita). This introgression fragment has also remained very large since its introgression, occupying nearly 60% of chromosome 6. Many more resistance genes have been introgressed since the 1970s, on nearly all chromosomes of tomato.

Therefore, we can conclude that from the 1970s onwards, there has been a pronounced boost in diversity, caused by introgression of resistances to pests and diseases. The introgression of new resistances has not stopped since the 1970s, and is still ongoing.

'Wasserbomben' crisis

In the meantime, the area occupied by glasshouses for tomato production in the Netherlands increased threefold from 713 ha in 1955 to 2150 ha in 1968. The older open-pollinated tomato types were replaced by new hybrid cultivars. However, the large beef tomatoes increased in importance. In the Descriptive Lists, relevant traits were yield, fruit size, fruit shape, disease resistances and suitability for different growing seasons, with or without heating. Remarkably, taste and sweetness were not mentioned as relevant traits...

But in the late 1980s and early 1990s, German popular news media described Dutch tomatoes as watery-tasting, naming them 'Wasserbomben' (German for water bombs). In 1985, 80% to 85% of these Dutch tomatoes were still being exported to Germany. However, because of the serious damage to their reputation, the Dutch tomato exports to Germany collapsed shortly after, reducing the area occupied by round tomatoes dramatically from ~1,700 ha in 1985 to ~275 ha ten years later. This became an important milestone in tomato breeding in North West Europe, marking the need for consumer quality traits, such as flavour. The 'Wasserbomben' crisis fuelled the second boost of diversity, namely a diversity in fruit types and improved flavours.

Second diversity boost

The most obvious phenotypic diversification that occurred from the 1980s onwards has been fruit size. In the 1980s, the large beef tomatoes emerged already, but since the 1990s, the small cherry and cocktail tomatoes became more and more important. Analysis of fruit traits showed that the cherry tomatoes were sweeter and contained more soluble acids, compared to the standard round tomatoes. Moreover, they were firmer and juicier, all leading to a higher fruit quality. Ripe tomato fruits produce a few hundred different volatile organic compounds, which are responsible for what we recognize as tomato fruit aroma. Different compounds have different sensorial characteristics, or odours. We observed that the average abundance of phenolic volatiles, such as guaiacol,