Next-level orchid breeding

Floricultura

ORCHIDACEAE & ARACEAE

Next level orchid breeding: less is more

Floricultura has been working on the implementation of 'Novel Breeding Tools' for several years now.

'Novel Breeding Tools' is a collection of molecular DNA techniques that allows our breeders to make use of specific genetic knowledge with regard to traits that are of interest to us. Traits such as flower colour, resistance to pests and diseases or inflorescence period are all determined by innumerable genes and the genetic variation present in plants. To gain a clearer understanding of traits at molecular genetic level, the first thing we want to do is identify the location in a genome where the information resides that is responsible for a specific trait.

Unfortunately, the combination of intensive and highly selective breeding and vegetative clonal propagation in the past has made a disaster of the genetic background of many commercial orchids, with deviating chromosome numbers and sizes as well as structural chromosomal abnormalities. The use of what is referred to as a reference genome, which is possible for a number of model crops and consumer crops such as tomatoes and maize, is not an obvious choice when applied to orchids. All of this makes breeding orchids a complex yet extremely interesting undertaking.

Recent innovations in high-throughput sequencing allow us to read and efficiently map the DNA code of individual plants. The entire genome of a plant can be read, for example. This can be compared to reading and analysing all the books in a library. That would be equal to 1,600,000,000 letters in the case of the relatively small *Phalaenopsis equestris* genome. We, however, in conjunction with Spark Genetics, have chosen an alternative approach in which only short fragments distributed across the entire genome are read. This is sort of like reading individual sentences from different books. This technique is called Genotyping-by-Sequencing (GBS) and enables us to systematically analyse the same set of short fragments in every plant.

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GBS in combination with SNP analysis offers unprecedented possibilities for breeding orchids

The question then arises of whether it would be useful to know only the sequence of these small and scattered regions rather than the entire genome. To begin with, the genome of most orchids is of a considerable size, which still makes complete genome analyses a costly and time-consuming procedure. In contrast, GBS allows you to analyse large numbers of plants relatively quickly at the same cost. There is, however, also a biological phenomenon that we can use to our advantage: genetic linkage. This is based on the fact that genes are often inherited in clusters rather than individually. For more information, see the figure on Page 5.

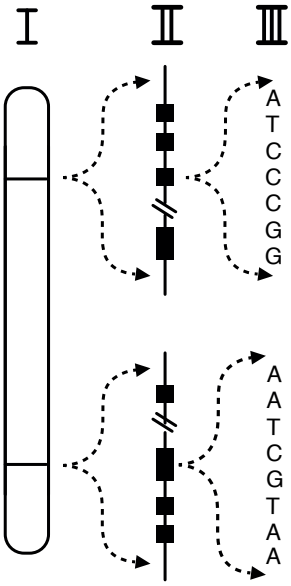
Imagine that we know just a small, unique bit of a sequence of a single gene thanks to GBS, and that gene is always contained in a cluster of 50 genes. This means that when we find our specific piece of DNA in a plant we have actually found the entire cluster of 50 genes! Now things are getting interesting! So, let's suppose that one of these fifty genes is responsible for a specific trait that we are interested in. Without knowing exactly which of these 50 genes is responsible for the trait, we can genetically track our trait in plants and breeding programmes through a single, small piece of a known sequence from the gene cluster.

This means that if we can read only a fraction of the relevant genome sequence, we will be able to trace our trait very efficiently. In this context, less really is more! The true power of molecular genetics is revealed when we combine GBS with another phenomenon called 'single nucleotide polymorphism' (SNP). This is the fact that there is genetic variation between individuals, cultivars or related species. You could compare this to typing errors in a library of books. This is often only a subtle variation, in which only one letter (a 'single nucleotide') is different. These latter variants (SNPs) sometimes impact the function of a gene, although they often have no perceptible effect on the organism concerned. SNPs can also be found in the tiny fragments that we analyse by means of our GBS approach.

Suppose we have a cross between two Phalaenopsis plants in which a trait such as a red colour (in the flower) is part of the plant we ultimately envision breeding. Now, Parent 1 carries white flowers but has other interesting traits that we want to combine with the parent that bears the red flowers (Parent 2). Using the GBS approach described above, we are able to distinguish the plants that bear red flowers from the ones bearing white ones through a single SNP. Although we do not know exactly which gene is responsible for the red flowers, we can track the trait perfectly in a cross population through genetic linkage. This type of plant selection is particularly interesting for traits that can only be selected in the flowering phase through conventional division. Plants can now be selected on flower colour as early as in the seedling stage. This means that fewer plants need to be grown, which results in more space for other projects.

So, GBS in combination with SNP analysis offers unprecedented possibilities for breeding orchids, in which speed and the targeted selection of the desired traits will further optimise our breeding processes. This allows us to keep serving our customers with the best varieties.

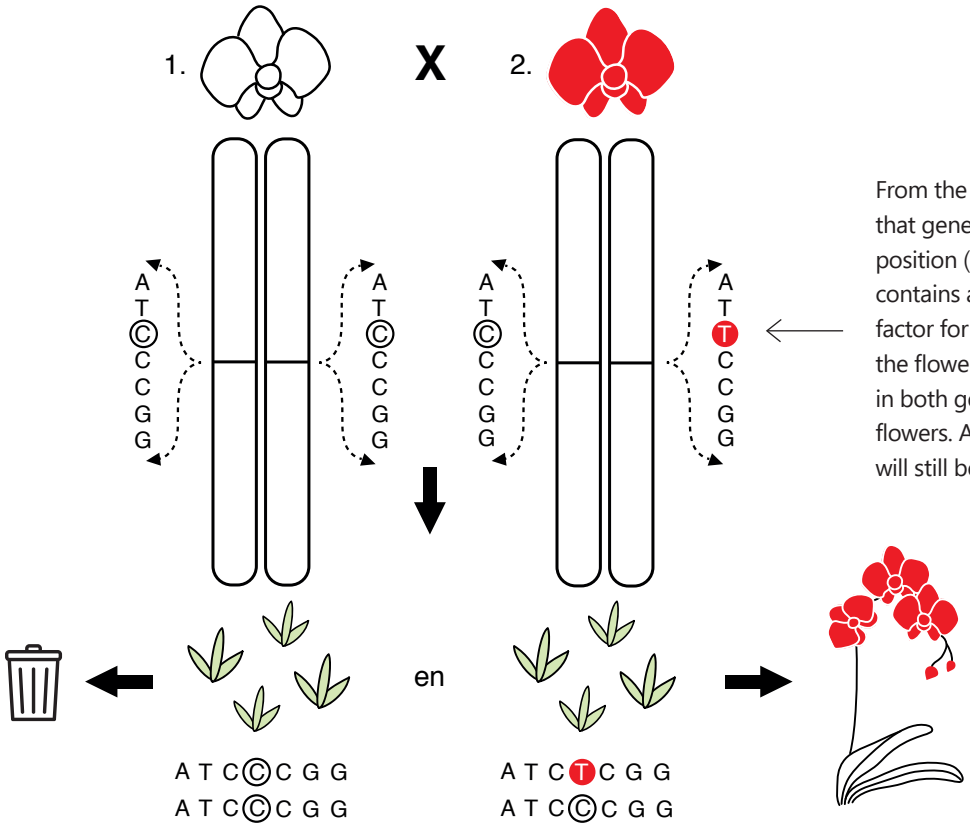
A



This is a schematic diagram of a genome (I) in which the horizontal lines indicate two places that we will zoom in on more closely (II). The individual genes are shown here (as black blocks). Genes that are located between the arrowheads are inherited as a linked cluster. If we zoom in even more closely, we will end up at the local DNA sequence. When you conduct a GBS experiment, you can easily map more than 10 thousand of these local fragments.

B

A cross between a white (1) and a red (2) flower-bearing parent. Both parents carry two genome copies, inherited from their respective parents.



From the GBS analyses, we learned that genetic variation (SNP) at this position (the DNA in this case contains a Ⓒ or a Ⓣ) is a significant factor for determining the colour of the flowers. A plant that carries a Ⓒ in both genome copies bears white flowers. A plant with one or two Ⓣ s will still bear red flowers.

Offspring from this parental cross are analysed for the presence of the relevant SNP Ⓣ, which is linked to the production of red flowers. Seedlings with only a Ⓒ in this position will therefore produce white flowers, and will in this case not be selected.

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