Introducing DNA Finger Printing in Breeding of Kalanchoë©

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Knud Jepsen A/S has carried out plant breeding and clonal selection in *Kalanchoe blossfeldiana* and other *Kalanchoe* species for a very long time. Commercial breeding has produced many cultivars that conform to the broad phenotype of, for example, flower colour, size, and form as well as leaf morphology. The selection and identification of cultivars has until now been based on morphological and physiological characteristics only. However, the phenotype is only partially determined by the genetic information. The impact of the genotype can be masked by environmental factors. By using molecular identification techniques it becomes possible to detect directly specific fragments of genetic information and to follow these fragments in crossing experiments. Also the control of patent rights may be more efficient.

Several important traits are controlled by a relatively large number of loci; each makes a contribution to produce the final phenotype value of the trait. Such loci are called quantitative trait loci (QTL). The ability to find an association between an important phenotype and a marker depends upon the size of the population being studied and the effect of the QTL on the trait and as well as the recombination frequency between the marker and the QTL. If the marker and the QTL are located far apart; the possibility that they will be transmitted together to the progeny individuals will be reduced. In general, the likelihood of identifying a marker linked to a gene or QTL is inversely proportional to the distance between the marker and the gene or QTL.

Molecular DNA marker technology makes it possible to characterise and identify individual *Kalanchoe* cultivars at the genotypic level. The heritable information of each cultivar is present in its genes. These are distributed over the chromosomes. All genetically determined differences among individuals are a reflections of changes in DNA sequences. These changes can be used as markers and give a specific fingerprint unique for each plant.

The technique used for DNA finger printing at Knud Jepsen A/S is called AFLP (Amplified Fragment Length Polymorphism) technique (Fig. 1). AFLP is a technique in which a genome is cut into a vast amount of different pieces by enzymatic digestion. A very small portion of these pieces is selected using a selective priming method. This results is in about 100 pieces of DNA of varying lengths. These pieces are separated electrophoretically and stained to form a pattern of bands (Fig. 2), which becomes the , the DNA fingerprint, and where the bands are formed by the markers. If the procedure and the *Kalanchoe* cultivars are the same, the markers will always fall in the same place.

The first thing to analyse is whether the combination of primers is unique in order to separate the different traits between *Kalanchoe* cultivars. Because the priming is selective, every possible marker will not be visualised on the fingerprint. In that case, it will be too difficult to read the print and too many uninteresting markers with no individuality will be visualised. By use of mathematic algorithm, the DNA fingerprint can be modified to a dendrogram (Fig. 3), showing the relation between the different *Kalanchoe* cultivars. In the example shown in Fig. 3, the cultivars can

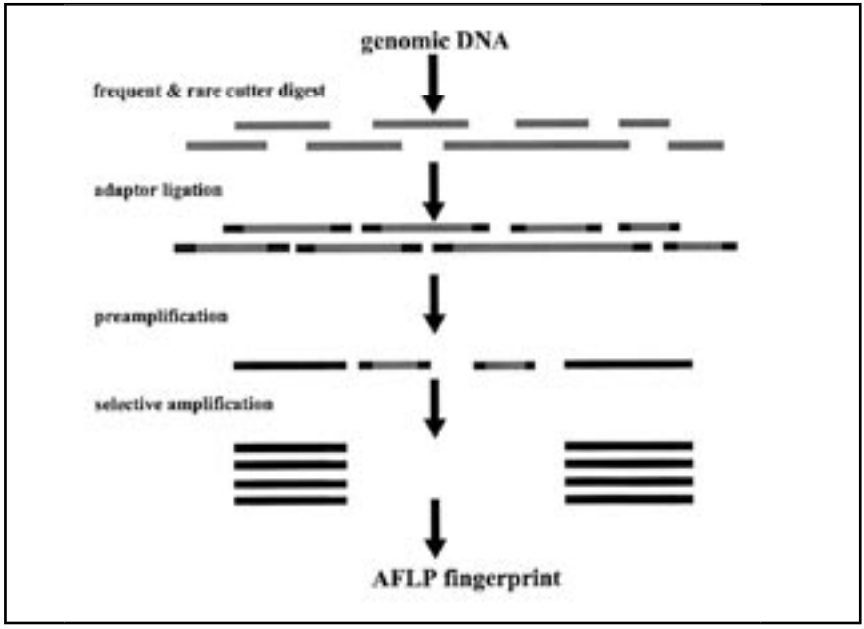

Figure 1. Overview of AFLP technique. The restriction of the DNA with two restriction enzymes; the ligation of double-stranded adapters to the ends of the restriction fragments; the amplification of a subset of the restriction fragments using two primers complementary to the adapter and restriction site sequences, and extended at their 3' ends. (Keygene, 2004)

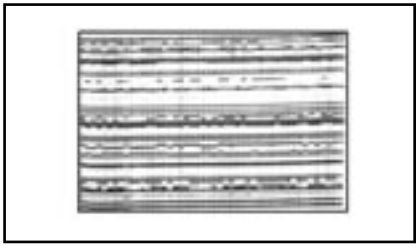

Figure 2. An example of a DNA fingerprint (Keygene, 2004). Gel electrophoresis of the amplified restriction fragments (described at Figure 1) on denaturing polyacrylamide gels ("sequence gels"); and the visualization of the DNA fingerprints. The different *Kalanchoe* cultivars can be followed horizontally. The pattern of markers connected to the *Kalanchoe* cultivars are visualized vertically.

be separated into two different families, which again can be divided into five groups. Within the groups the DNA differs within 1% to 15%.

At Knud Jepsen A/S, DNA fingerprinting will be used as a tool to:

- Plan a more precise breeding strategy
- Increase the efficiency of the selection
- Reduce the selection time in developing new cultivars
- Identify the relationship of cultivars

When knowing the parental lines, it becomes easier to design a breeding strategy and select the correct parents for the wanted combinations of traits. It will, to some extent, be possible to predict the individuals of the progeny, and thereby avoid waste of work and money. In order to combine a marker with a phenotypic trait, a population with this specific character must be studied. Hereby it is possible to combine the unique pattern of markers with the known pattern of population. A

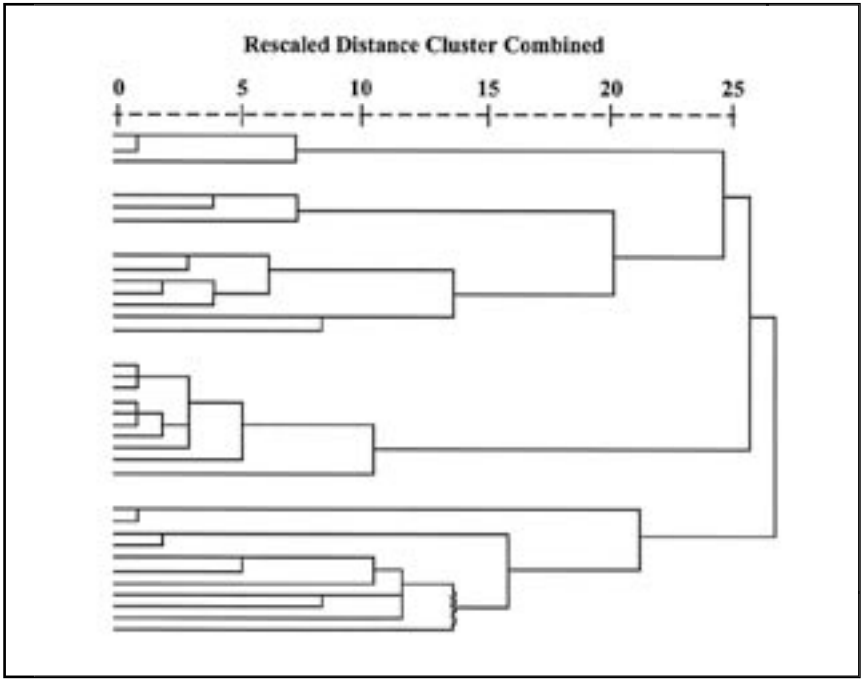

Figure 3. Dendrogram visualizing the relation among *Kalanchoe* cultivars.

marker found to visualise a specific gene or QTL makes it possible to select or avoid this trait.

Some of the different traits (Fig. 4), Knud Jepsen A/S is focusing on, are:

- The colour, size, and form of the flowers
- The colour, size, and form of the leaf
- The sensitivity to ethylene
- The scent
- The durability
- The photomorphogentic reaction time
- Fading of the colour of the flower
- The ability to reproduce (sterility)

These different traits do all have a potential in developing marker assistant breeding by coupling a DNA marker to the trait.

By now a full range of different *Kalanchoe* cultivars has been analysed. This large amount of data has made it possible to estimate the total genetic variation in *Kalanchoe* as visualised by a dendrogram. Hereby we are able to identify a plant parental background and show how and how closely plants are related. The method could also become an important tool when dealing with infringement of breeders rights and protection of new cultivars of kalanchoë.

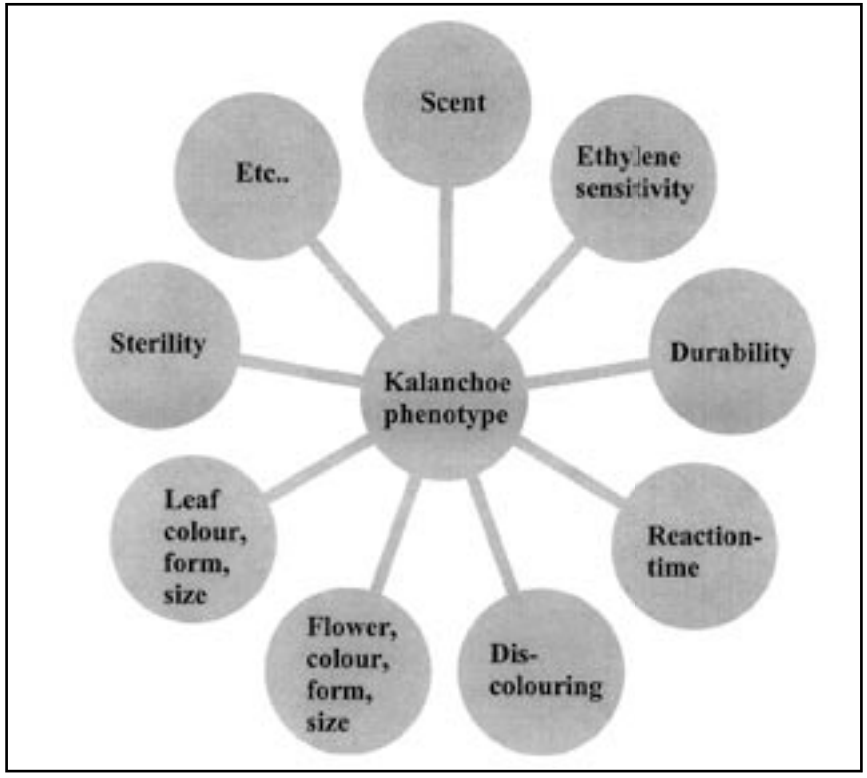

Figure 4. Different traits relevant to get an excellent *Kalanchoe* phenotype at Knud Jepsen A/S.