Tulip breeding at PRI

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In this article a survey is presented of the tulip breeding research carried out at the DLO-Centre for Plant Breeding and Reproduction Research (PRI; the former IVT) during the last 30 years. In 1963/1964, research on tulip breeding was started at the instance and with cooperation of the Dutch bulb industry. At that time, Joop P. van Eijk was appointed as tulip breeder at PRI (IVT). Good co-operation was achieved with the Bulb Research Centre (LBO, Lisse) and the Agricultural University Wageningen. Since 1974, seedlings of the PRI breeding programme has been released to the Dutch tulip breeders.

Pre-selection

Because of the long juvenile period and the slow propagation rate of tulips much attention was initially paid to pre-selection. Pre-selection is selection at the juvenile stage from small non-flowering seedlings. The main goals of pre-selection were bulb production, forcing for cut flower production and later also disease resistance. With a good pre-selection system, breeders can save money because of less labour input, and reduction of field and greenhouse space.

Pre-selection based on bulb production

Bulb production can be measured as the yearly increase of the main bulb diameter and the number of daughter bulbs. Research on pre-selection of seedlings with a high bulb production resulted in the following selection procedure.

In the first three years, only the main bulbs from each population are cultivated. By sorting the main bulbs during those years into bulb diameter classes (or weight classes) and by replanting only the larger bulbs, the best growing bulbs within a population are selected. In the fourth year, only plants which produced two or more daughter bulbs are selected. Thus, during these two steps the only seedlings selected are those which grow very well and give enough daughter bulbs. This selection procedure is mainly useful for populations with a minimum of 1500 seedlings, since many seedlings can be discarded at an early stage.

Another selection procedure which gives comparable results involves looking at the number of years from seed to flowering plant. It has been found that the first seedlings to flower in a population grow better and give more daughter bulbs than seedlings which flower for the first time one or two years later. This selection procedure is only useful for small populations as all seedlings have to be grown until the first year of flowering.

It has also been found that pre-selection based on bulb production does not interfere with selection for early forcing.

Pre-selection based on forcing

Forcing for cut flower production is the most important quality for tulip breeders. This will be obvious from the following production figures. Approximately 50 per cent of the bulb growing acreage in the Netherlands is planted with 20 cultivars which are all suitable for forcing. Furthermore, the ten most important cut flower cultivars account for approximately 50 per cent of the tulip cut flower production in the Netherlands.

Research on pre-selection showed that the juvenile seedlings which sprout and die early in comparison with the other juvenile seedlings can be forced as the earliest flowering ones at the adult bulb stage. This knowledge is rarely used by the hybridizers in their breeding programmes because of the administrative effort needed.

Besides earliness, other characteristics such as stem length (lower and upper internode), firmness of leaves and stem, leaf position and leaf number, flower size, bud presentation and the proportion between flower and leaves are also important. Consequently, in practical breeding, seedling populations consisting of bulbs large enough to initiate a flower are all forced and judged for the characteristics mentioned above. This strategy means that many seedlings have to be cultivated for at least five years but in the end will not pass the selection criteria.

In 1978 and 1979, plant material obtained from the pre-selection research was released by PRI to the breeding companies. The cultivars ‘Debutante’ and ‘Silver Dollar’ are the best known results at present, they also have a long vase life.

Shortening of the juvenile period

Besides pre-selection, another approach to speed up tulip breeding is shortening of the juvenile period. By cultivation in greenhouses and storage in climate rooms with standardised conditions the growth cycle could be reduced from one year to eight months. In two years, three growth cycles could be obtained. Despite additional lightning, however, the seedling bulbs from the greenhouse with three cycles of cultivation were not larger than the bulbs from outside with only two cycles of cultivation.

Nowadays, by applying optimal field and storage conditions it is possible to shorten the juvenile period from 4-5 years to 3-4 years.

Flower colour and flower shape

The tulip derived its fame mainly from its diversity in flower colour and flower shape. Almost all consumer wants can be fulfilled by exploring this diversity. Biochemical analyses of flower pigments and crossing experiments were carried out to obtain more knowledge about the inheritance of flower colour and flower shape.

Inheritance of flower colour

Flower colour is determined by the composition and proportion of different flower colour pigments. Colour pigments have been determined from approximately 500 modern and older cultivars and *Tulipa* species. Yellow flowers contain only carotenoids, while orange flowers contain at least carotenoids and cyanidin. Red flowers contain cyanidin and mostly also pelargonidin and purple flowers contain a combination of cyanidin and delphinidin. Pink flowers contain the largest variation in flower colour pigments but the level is mostly lower compared to the other colours. White flowers contain almost no colour pigments.

From approximately 1000 seedlings obtained from 21 populations the flower colour was compared with the flower pigment composition. In addition to single-coloured tulips there are also multi-coloured tulips e.g. pink-red and purple-red. Flowers with approximately the same colour differed in the flower pigment composition and flowers with approximately identical flower pigment composition differed in flower colour. Furthermore, in several seedlings flower pigments were found which were not present in either of the parents. Consequently, it is generally not possible to predict the flower colour of the progeny on the basis of the flower pigment composition of the parents. For example, a yellow x yellow cross can result in some red seedlings.

Inheritance of flower shape

Crosses were made between lily-flowered, double-flowered, edge-coloured, single-coloured and base-coloured cultivars to study the inheritance of flower shape and other flower characteristics. As with the inheritance of flower colours, prediction of the inheritance of flower shapes in relation to that of their parents appeared to be difficult.

The lily-flowered cultivar ‘Dyanito’ combined with a non lily-flowered cultivar gave almost no pure lily-flowered seedlings. Populations with *T. accuminata* as a parent, however, gave many beautiful lily-flowered seedlings. The cultivars ‘Novired’ and ‘Talbion’ were released from this programme by PRI.

The double-flowered tulip ‘Abodement’ gave hardly any pure double-flowered seedlings. Cultivars are known, however, which pass on this characteristic much better. Crosses between edged-coloured cultivars gave high percentages of edged-coloured seedlings and when combined together with non-edged cultivars the offspring resulted in approximately 50 per cent of edged-coloured seedlings.

Thus, of the flower colours and flower shapes investigated, we find that the results depends on the parents used. Only when a parent is used several times in crosses can a prediction be given for its progeny.

Parrot tulips

Parrot tulips are famous because of their bizarre flower shape. In the past, this shape was obtained only by spontaneous mutation of cultivars. From an inbreeding programme at PRI a "genetic" parrot was found in a selfing of the cultivar ‘Cordell Hull’. This cultivar, named ‘Amethyst’, and other “genetically” based parrots were released to the breeders in 1978. Crosses with these parrot tulips gave new parrots in their progeny.

Mutation breeding

New cultivars can be obtained by mutation breeding as well as by hybridization. Spontaneous mutations are found regularly in tulips. Mutations can show another flower (edge) colour or flower shape (parrots, fringed and double). Differences exist in the mutation sensitivity of cultivars. Many mutants are known from cultivars, like ‘Bartigon’, ‘William Copland’, ‘Murillo’ and ‘Apeldoorn’.

In order to stimulate mutations artificially, the possibilities of Röntgen (X-rays) for mutation breeding in tulips have been investigated. Main bulbs as well as daughter bulbs can be used for mutation induction; the dosage required varies from 350 to 550 rad. Tri- and tetraploids need a higher dose than diploids. The radiation can be applied early in the planting season (August) or late (November). Using early radiation, many deformed plants are found in the field in spring and sometimes mutations are already visible. Late radiation gives no deformation in the first year, but does do so in the second year of cultivation, when many bulbs will not flower. All plants have to be cultivated three to four years, before selection for mutations can begin. Again, differences between cultivars can be found in the number of mutations obtained. Besides mutations in flower colour and flower shape mutations also occur in the colour of the leaf edge, in plant length and in bulb production.

In 1975, 1977 and 1978 several radiation mutants of ‘Preludium’ and ‘Lustige Witwe’ were released by PRI. One of the mutants was ‘Santina’, a leaf edge mutant of ‘Lustige Witwe’. Many breeders are still using the mutation techniques developed by PRI.

Tetraploids

The number of chromosomes of many cultivars and *Tulipa* species have been counted at PRI. The tulip assortment consists mainly of diploids (2 sets of 12 chromosomes; $2n = 2x = 24$), some triploids (mainly Darwinhybrids) and a rare tetraploid ($2n = 4x = 48$). Since tetraploids are more robust and firm than diploids, research has been carried out to obtain more tetraploids. By making crosses between tetraploids new tetraploids were obtained. Several of these tetraploids were released by PRI in 1974 of which ‘Judith Leyster’ is best known.

A problem with the existing tetraploids was that their progeny flowered late in the season, which made them unsuitable for early forcing. Consequently, other techniques for obtaining tetraploids were used. By placing young seed buds, which were pollinated a week before, for one day in a cylinder with laughing gas (N_2O) with five to six atmospheric pressure, tetraploid seedlings were obtained. To improve the fertility of these tetraploids they were crossed mutually. Highly fertile tetraploid cultivars of *T. gesneriana* and tetraploids of *T. fosteriana* and *T. kaufmanniana* were released in 1989. In 1991, tetraploid lily-flowered *T. gesneriana* tulips were released by PRI.

Crossing tetraploids with diploids can result in strong growing triploids. Unfortunately, these triploids are mostly sterile and can not be used in further breeding. Tetraploid *T. gesneriana* cultivars can also be used in combination with diploid *T. fosteriana* to obtain Darwinhybrids with a better vase life and suitable for early forcing. In 1988, the cultivar ‘World's Favourite’ was released, bred from a tetraploid *T. gesneriana* seedling (‘Denbola’ x ‘Lustige Witwe’) crossed with a diploid *T. fosteriana* seedling.

Interspecific hybridization

Much attention has always been paid to hybridization between different *Tulipa* species (interspecific hybridization) in combination with the taxonomy of the genus *Tulipa*. Several characteristics are almost not available in the *T. gesneriana* assortment (e.g. extreme earliness), or not available at all (e.g. resistance against tulip breaking virus). In this situation, *Tulipa* species can sometimes be used for introducing these characteristics. Furthermore, many *Tulipa* species or interspecific hybrids can be used to obtain a larger garden tulip assortment.

PRI has probably the largest *Tulipa* species collection in the world. A recent re-examination of this collection by taxonomists has resulted in a revision of the nomenclature of the genus *Tulipa*. The genus consists of the two sub-genera *Eriostemones* and *Tulipa* (formerly *Leiostemones*). The sub-genus *Eriostemones* and *Tulipa* consist respectively of three and five sections (see figure).

Many cultivars of *T. gesneriana* were pollinated with most of the species in our collection. In this way a good view was obtained of which species can be used for interspecific hybridization. Crosses between *T. gesneriana* and species from the *Eriostemones*, like *T. tarda*, *T. pulchella* and *T. turkestanica* were not successful at all. Crosses within the sub-genus *Tulipa* are possible, like the hybrids obtained between *T. gesneriana* and *T. fosteriana* (Darwinhybrids), *T. kaufmanniana*, *T. greigii*, *T. eichleri*, *T. ingens*, *T. albertii* (formerly *T. vvedenskyi*) and *T. didieri* (see figure). Recently, new in vitro techniques were developed by which interspecific hybrids can be obtained which were not possible by normal (in vivo) hybridization.

Interspecific hybrids exhibiting characteristics like earliness and resistance against tulip breaking virus and *Fusarium* and several garden tulips were released by PRI. Some well known cultivars are ‘Beau Monde’, ‘Come Back’, ‘Explosion’, ‘Lefeber's Memory’, ‘Pink Impression, ‘Purple World’ and ‘Spryng’.

Vase life

The vase life of the cut flower is one of the most import characteristics for the consumer. Research showed us that a large genetic variation is available in the cultivar assortment. Screening tests were developed by which the vase life of a genotype could be estimated. A good correlation was found between the keepability of the flower still attached to the bulb and the vase life of the flower. This means that breeders can select for vase life during the forcing period of the best genotypes. When a clone of a genotype has been obtained, the vase life can exactly be determined using a cut-flower test in water. The time between colouring of the flower bud and the moment that the ornamental value of the flower drops below a certain level, can be taken as a selection criteria. The vase life has been determined for many cultivars and the results made available to breeders and forcers.

Disease resistance

Host resistance is the best approach to preventing diseases. The use of resistant cultivars gives better bulb production, less rejection of infected bulb lots, lower costs for chemical disinfection and needs less labour. Resistance is not only important for production, but also for forcing since bulbs infected with, for example, tulip breaking virus will give visible symptoms resulting in a low price at auction. Resistant cultivars are also important for bulb exports, since bulbs infected with *Fusarium* cause extra efforts to clean the stock and give rise to complaints from buyers. Furthermore, resistant cultivars can reduce the amount of chemical disinfection of bulbs.

***Fusarium* resistance**

The *Fusarium* research, carried out in the eighties, has resulted in reliable screening tests for clones as well as for juvenile seedlings at the pre-selection stage. In these tests, tulip bulbs are planted in *Fusarium*-infested soil and grown under standardized conditions during the season. After harvest, bulbs are examined for *Fusarium* infection. Selection of one year old seedlings can reduce the number of plants in populations considerably at an early stage of the selection programme. Since seedling selection give some susceptible plants (escapes), selected plants have to be re-tested at the clonal level. Direct sowing of tulip seeds in *Fusarium* infested soil was less successful.

The inheritance of *Fusarium* resistance was investigated. It was found that the use of one resistant parent resulted in resistant descendants.

Resistant cultivars and *Tulipa* species have been traced. In 1988, *Fusarium* resistant *T. gesneriana* material was released. Several of these genotypes are now becoming successful as new cultivars: examples include ‘Furand’, ‘Furanel’, ‘Fusarino’ and ‘Fusor’. Some plant material released from the interspecific hybridization programme was also resistant.

Tulip breaking virus resistance

The tulip breaking virus (TBV) research also resulted in reliable screening tests at clonal and seedling level. Leaves of flowering plants are inoculated by viruliferous aphids. Flower breaking was observed one year after inoculation. Absolute TBV resistance was found in several *T. fosteriana* cultivars such as ‘Cantata’ and ‘Princeps’. In 1989 and 1991 TBV resistant *T. gesneriana* x *T. fosteriana* hybrids were released. The restoration of the F1-sterility of these hybrids makes further research necessary.

New breeding techniques

Haploidization techniques, to obtain plants with only one set of 12 chromosomes, has been investigated. Embryo-like structures from young pollen (microspores) were obtained in tissue culture. Doubling of haploids (spontaneously or by chemicals) result in homozygous plants, which can simplify inheritance studies. New research is started recently.

In vitro propagation of tulip on a large scale is still a problem. The induction of somatic embryos in cell suspensions can perhaps result in higher production rates. This is now under investigation at LBO and PRI.

Genetic modification of tulips has also been investigated. Using a particle gun, additional DNA with reporter and selection genes, has been shot into regenerating tulip tissue in vitro. Hundreds of tulip bulbs were obtained, but it still takes at least 2 years before these bulbs will flower and stable integration can be proved.

Identification of tulip cultivars, based on protein markers, is now possible at the dry bulb stage. Using bulb extract, cultivars can be uniquely identified after electrophoresis in combination with staining for the isozyme esterase. This technique is also useful to check the hybrid identity of the progeny after an interspecific cross, at an early stage.

Future

Due to the long juvenile period and the slow propagation rate of tulip many new genotypes from different programmes are still under investigation at PRI. If genotypes with useful characteristics are found they will be released to Dutch breeders. The PRI *Tulipa* collection will also be made more accessible to the breeders.

Fertility problems after interspecific hybridization, makes further research necessary, for example to introduce TBV resistance in the *T. gesneriana* assortment. New techniques to prevent sterility or to restore fertility will be developed in near future.

Besides *Fusarium* and TBV, *Botrytis tulipae* is one of the most important pathogens in tulips. Resistant cultivars can play an important role, especially by reducing environmental pollution caused by spraying fungicides. Consequently screening tests have to be developed at clonal and at seedling levels and resistance has to be traced in cultivars or wild species. This research started in 1995.

Pre-selection was the most important goal in tulip breeding research 30 years ago. New techniques using molecular (DNA) markers makes pre-selection again a topic of serious interest. This work will start in the near future.

Publications

Scientific or informative (in Dutch) articles have been published on most of the subjects mentioned in this article. For further information please contact one of the authors.

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Figure: Taxonomic presentation of the genus *Tulipa*. Filled dots are species of which interspecific hybrids with *T. gesneriana* are obtained (for explanation, see text).

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