Theo Sanders June 2015

The generation of fertile allotretraploid species hybrids by chromosome doubling

For many years, I tried to transform diploid narcissus species and species hybrids from diploid to tetraploid. At first, I used colchicine, without any success, and later on Oryzalin. With this medium, I was effective. This spring I found one plant of Hawera (Fig. 1) and three plants of Fairy Chimes (Fig. 2) within the treated group that produced diploid pollen. Hawera and Fairy Chimes are both crosses of N. jonquilla with N. triandrus.

Figure 1. Transformed Hawera

Figure 2. Transformed Fairy Chimes

Hybrids of genetically different diploid species are commonly infertile. Some plants only generate a few unreduced pollen and / or egg cells. If the hybrid is transformed from diploid to tetraploid or to a special mixoploid (chimera), it becomes fertile because the whole tissue or the tissue for reproduction contains two chromosome sets from each species. In addition, the bigger cell nucleus for tetraploids often induces bigger cells and therefore a bigger plant with more substance.

The normal process of cell division when the plant is growing is that from each chromosome are formed two. The doubled chromosomes are distributed

between the two cell halves, which are separated by a new wall. Colchicine, Oryzalin and some other substances prevent the normal procedure of cell division. The doubled chromosomes are not distributed to two cells, and the new cell wall cannot be built. There remains a cell with the doubled chromosome content.

For producing tetraploids it would be the best to take one diploid totipotent cell, treat it with an effective medium and grow it to a daffodil. This method is complicated and expensive. For meristem tissue consisting of many cells not all the cells may be transformed. The diploid cells can grow faster than the new tetraploid cells and in the worst case, the plant will be diploid again after some time. It should be favourable to take tissue with a minimum number of cells and transform as many as possible. The very little bulbs of twin scales or chips seem to be a good material for doubling chromosomes.

The following procedure has been used: The bulbs for chipping were hot water treated for 4 hours at 45 centigrade. Thereafter they were placed for 15 minutes within a 0,2 % solution of sodium hypochlorite in water. The fabricated chips were covered for 15 minutes with a fungicide solution of 20 mg Maneb, 20 ml Chlorthalonil, and 20 ml Carbendazim per liter. Then the chips were hold for 7 days at 20 centigrade (Fig.3). They were located on paper with wetted vermiculite underneath and covered with a plastic sheet.

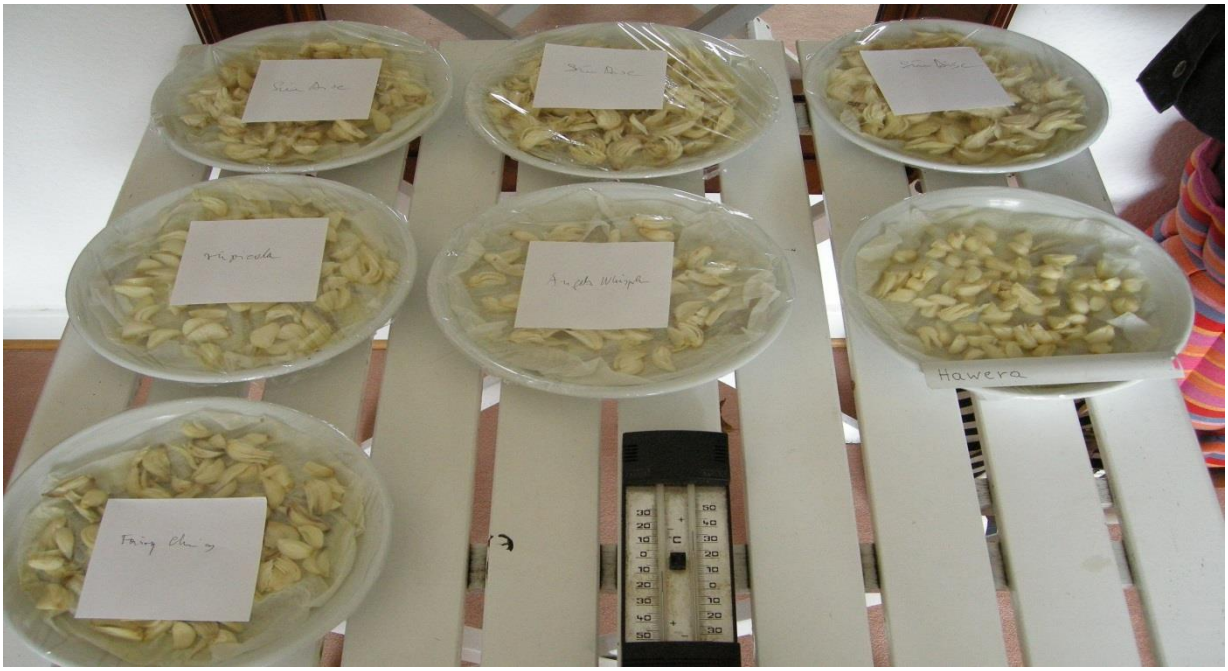

Figure 3. Storing chips for seven days

For chips of Hawera it was found out that they form little bulbs with a size of 0,3 mm within 15 days at 20 centigrade. After 7 days, the bulbs should be much smaller. The chips were washed under flowing water and given in penetrable nylon bags to a solution of 1 l water with 10 mg Oryzalin solved in 2 ml DMSO (Dimethylsuloxide) for 48 h at 20 centigrade. The solution with the chips was agitated with an underwater pump. The container was assembled within a fridge at 17 centigrade, because the pump otherwise heats up the solution to more than 20 centigrade. After this operation, the chips were held in agitated clear water for 24 hours, taken out and treated with the fungicide solution. Thereupon they were filled into plastic bags together with moist vermiculite and kept at 20 centigrade for three months within a heat box. During this time the little bulbs become bigger and in the end (Fig.4) they were planted into a peat compost (called TKS1 in Germany) at a temperature of 15 centigrade for the first month, 10 centigrade for the second, and 8 centigrade for the third. Then in March and later the planting bed under glass has not been heated. The twin scaling or chipping is made during August and the early part of September with the best results. The chips should have a weight of about one gram.

Figure 4: Little bulbs after three months in the heat box

With this technique, I created the four tetraploid or special mixoploid plants. For three of them the chips were treated in 2012 and for one in 2013. They flowered this spring and originated many pollen grains (Fig.5 and 6).

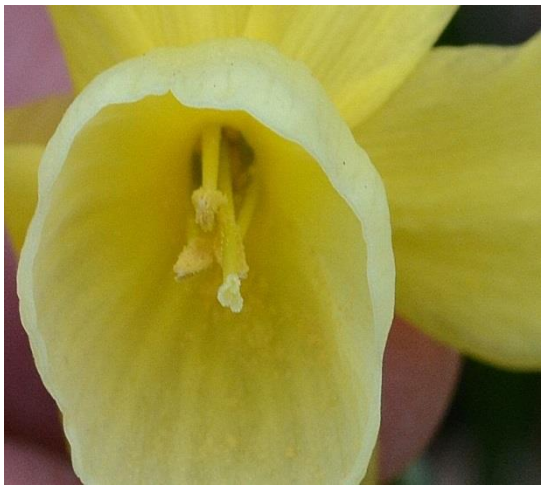

Figure 5. Hawera with pollen

Figure 6. Fairy Chimes with pollen

About 60 to 70 % of the diploid pollen that measures about 46 µm for the short axis sprouted (Fig.7 and Fig. 8). For the normal diploid Hawera I found one flower only of 20 plants with very few unreduced pollen grains (Fig.9) An earlier provisional identification as tetraploid or mixoploid for example by the size of the leaves was not possible because the little bulbs are of different size and generate also plants of different size. The transformation rate was about 1 to 3 %. I am waiting for some further tetraploid or mixoploid plants: Sun Disc, Sun Dial, Angels' Whisper, N. rupicola, N. assoanus, N. cordubensis, and N. henriquesii have been treated for chromosome doubling.

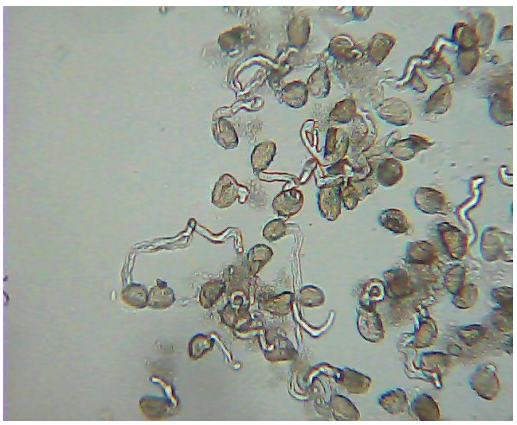

Figure 7. Pollen of the new Hawera

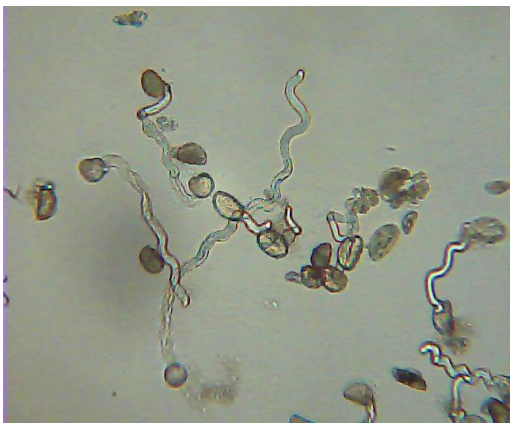

Figure 8. Pollen of the new Fairy Chimes

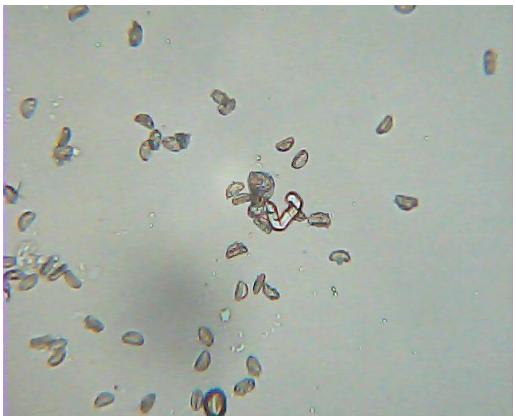

Figure 9. Unreduced sprouting pollen grain of the normal diploid Hawera

In 2014, I thought the method would not function and developed another process which I shall use in the future and which may have higher transformation rates. Two obvious variations of the described technique can be considered: The concentration of oryzalin within the solution and/or the time of the treatment can be increased. It is useless to take the first way because the maximal solubility of oryzalin in water is 2,5 mg/l; the chosen 10 mg/l are more than enough. Increasing the time means that the chips are within the solution for more than two days. This surely is no optimal condition for growing the little bulbs and producing a great number of new cells. It seems to be better to hold the chips at the open air for a longer time wetted with the solution. I tried this in 2014 for Hawera for three, five and seven days. The chips were wetted with the solution from the first method for one minute and then for ten minutes they are environed by air. This is possible by a simple method: The chips are stored in a bucket at a greater height than the bowl with the solution that is boosted every 10 minutes by a pump steered by an interval switch. When the pump stops after one minute the solution flows back to the lower positioned bowl. For a treatment of seven days the number of the bulbs after three months is a little reduced, but for five days there are produced nearly as many bulbs as without using the oryzalin treatment. The new system (Fig. 10 and 11) will be applied this year to transform April Tears, Wood Star, Pixies' Sister, Kidling and some species. I hope that I can produce tetraploid daffodils as a routine operation in the future.

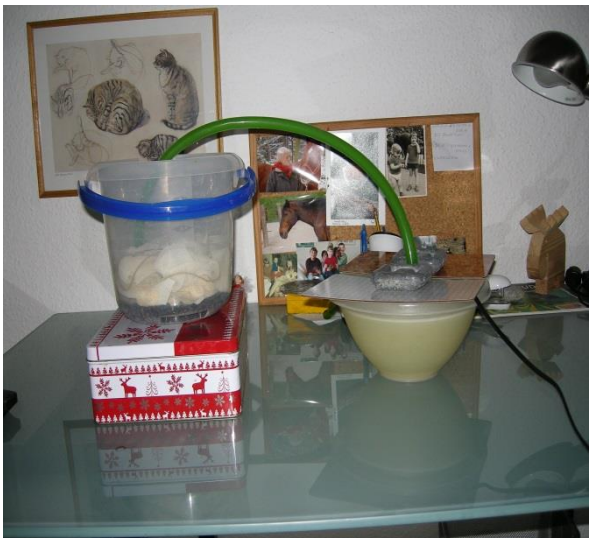

Figure 10. Wetted chips in air

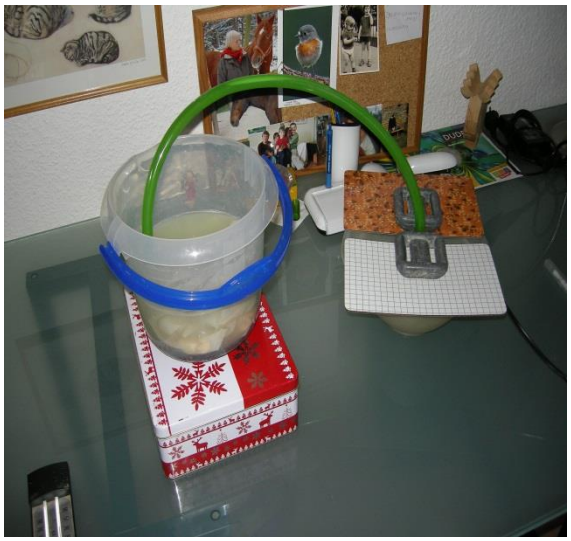

Figure 11. Chips in oryzalin solution

In the best case, the new tetraploid or special mixoploid Hawera and Fairy Chimes are not only fertile but look better and have more vigour than the originals. Selfed or crossed with each other they should generate three types of beautiful plants either of them should be uniform for the first generation. The second generation for Hawera x Fairy Chimes should show some difference in appearance similar to that which can be observed for narcissus species in the wild. If the commerce concentrates on propagation by seed and not by bulb multiplication, it is possible to generate many plants in a short time.

Further crosses with the pollen of Hawera and Fairy Chimes should be possible with standard daffodils for example with white or yellow perianths and pink or red crowns. They contain two chromosome sets of the standard daffodil, one of N. jonquilla and one of N. triandrus. I think there is a high probability that plants with red or pink crowns can be yielded. Their vigour and substance should be excellent. There exist already some varieties of this type that originated from crosses of jonquilla hybrids like Quick Step and Hillstar with pollen from Silver Bells and Chipper. However to get coloured cups is impossible with these parents and Silver Bells and Chipper as triploids generate only few pollen.

Other groups of daffodils which can be combined with the fertile Hawera and Fairy Chimes are the fertile jonquilla- , triandrus- and tazetta-, and viridiflorus-hybrids with two chromosome sets of the standard daffodils and two of the

different species. Tetraploid plants come into being with great vigour and very different form, but it's unlikely that the colour of the crown is red or pink.

The combination of tetraploid standard daffodils with a diploid species gives in most cases infertile NNX- plants. However sometimes they have a few NX-pollen with one chromosome set of the standard daffodil (N) and one of the species (X). This is the same pollen that is generated by the comparable allotetraploids (NNXX). However, from these exist only few, which are mentioned in the preceding paragraph. I have for example a cross of Actaea with N. tazetta .The plant is NNT (with T for N. tazetta) whereas the pollen is NT. If this is combined with the new Hawera you get a tetraploid plant in which each time one quarter of the chromosomes is from Actaea, N. tazetta, N. jonqilla, and N. triandrus. It should have vigour, substance, many white flowers per stem, intense fragrance and sufficient frost resistance. In this way the two chromosome sets of the new Hawera or Fairy Chimes can be associated with one chrome set of a standard daffodil and one chromosome set of nearly all diploid species. You only must have the partially fertile NNX-plants (1).

A further possibility is to use the unreduced pollen of diploid species hybrids (2). I found for example many of these pollen grains on the anthers of N. x christoperi. If the new Hawera is pollinated with this pollen, a tetraploid plant with four different chromosome sets arises: One of N. jonquilla, one of N. triandrus one of N. panizzianus and one of N. assoanus.

It can be assumed that the combination of the transformed Hawera and Fairy Chimes with nearly all diploid species is possible. The descendants are triploid plants with three different chromosome sets, good hybrid vigour and great differences in form. With N. cyclamineus, N. assoanus, N. hedraeanthus, N. bulbocodium ectandrus and N. poeticus as one parent it should be possible to breed many different miniatures.

Probably not all of the transformed four plants are tetraploid for all tissue layers (L-1, L-2, and L-3). If not all cells of the daffodil are converted to tetraploid some tissue will be there which is tetraploid and some which is diploid and the plant is called a chimera or a mixoploid. Because the pollen for all four plants is diploid it must have been generated by a tetraploid plant or by a mixoploid with tetraploid tissue for L-2.From this layer pollen and egg cells are generated. For the proposed crosses, it is important only that pollen and

egg cells are from tetraploid tissue. The descendants of intercrossing or selfing the tetraploids or the special chimeras will be tetraploid plants. Whether the whole oryzalin treated plant is tetraploid or a chimera can be found out only by looking at the chromosomes with a special microscopic technique or measuring the nuclear DNA content by flow cytometry.

As soon as I saw that one plant of Hawera and three plants of Fairy Chimes produced a large quantity of viable diploid pollen I tried the pollen of the new Hawera on Lauren, Altruist, and Compton Court and harvested hundred seed grains. The pollen of the new Fairy Chimes I gave on the stigma of Allrounder and Solar System and got nine seeds. The number of seeds per capsule was about three. The combination of Fairy Chimes with one of my 7Y-Y fertile jonquilla hybrids generated seventy seeds with ten per capsule. I think both types of crosses were successful but open pollination cannot be completely excluded. For the next spring, I plan to repeat some of these crosses protecting the seed parents against open pollination.

Fourteen seeds developed from selfing and mixing Hawera and Fairy Chimes. These seeds must be tetraploid like the plants that later on grow from them.

Literature

(1) Sanders, T.: Pollen Volume and Chromosome Content of Daffodils – Possibilities for hybridizing 2 (January 2013), www. theo-sanders-daffodils.de

(2) Sanders, T.: Hybrids of different narcissus species can be fertile by generating unreduced pollen (October 2014), www.theo-sanders-daffodils.de