Effects of Temporary Intergeneric Grafting on the Chromosome Number of Mulberry Tree

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Grafting which is done in many plant species may be classified into the following three categories.

The first category is the grafting that is merely regarded as a method for propagation and the effects of stock on the scion are hardly expected. The grafting prevailing in mulberry trees falls into this category. Mulberry trees being allogamous plants are reproduced by means of vegetative propagation, and about 70% of nursery plants are produced by grafting in Japan. In that grafting, a piece of branch grown in the previous year and a root of one-year-old seedling are usually used as a scion and a stock respectively, and the effects of stock on the scion are hardly considered.

The second category is the grafting in which some useful characteristics of the stock such as disease resistance, dwarfing effect and so on are made use of. For example, we can see the grafting prevailing in some fruit trees and fruit vegetables in which breeding and using of useful varieties for stock are positively performed, and this type of intergeneric grafting has been carried out in many plants, i.e. between *Poncirus trifoliata* Rafin and *Citrus unshiu* Marcov, and *Cucurbita moschata* Duch or *Lagenaria leucantha* Rusby and *Citrullus vulgaris* Schrad or *Cucumis melo* L. In this case no genetic variation is expected, though the physiological effects of stock on the scion can be found.

The third category is the grafting that is performed to induce some genetic variations. Such grafting has been carried out in *Solanum melongena* L.,[1,7] *Lycopersicon esculentum* Mill[2,3] and *Capsicum annuum* L.[6,8] in Japan.

The author is very interested in the grafting belonging to the third category, especially in the grafting between plants which are of being in wide relations each other, because new variations which have not been reported so far may be expected. From this point of view, the author tried some graftings between mulberry and *Broussonetia kazinoki* Sieb. or *Cudrania tricuspidata* Breau., but could see no taking root in the grafts. Therefore, the author developed a new method of grafting with a unique procedure mentioned afterward, and named it temporary or non-taking root- intergeneric grafting.

In this paper, some chromosomal aberrations observed in the mulberry trees obtained by temporary intergeneric grafting and in their progenies are reported.

Repetition of temporary intergeneric grafting

The intergeneric graftings between mulberry and *B. kazinoki* or *C. tricuspidata* using pieces of branches grown in the previous year as scions and roots of one- or two-year-old seedlings as stocks have never been successful, because the scions hardly sprouted and the stocks did not produce new roots. However, when grafting was performed using the roots of *B. kazinoki* or *C. tricuspidata* treated with a rooting accelerator (NAA of 80–180 ppm) as stocks, and mulberry seedlings from a cross between varieties Shirome-keiso ♀ and Shirome-keiso ♂ as scions, and when they were kept under the condition of 28 °C and more than 80% in humidity, the scions grew somewhat well, though they withered after all. Particularly, when mulberry seedlings at the fourth leaf stage without roots were grafted on the roots of *B. kazinoki*, the growth of scions recovered and unfolded about ten leaves at the maximum (Plate 1-a). While, when younger seedlings at the second leaf stage were used as scions, two to three abnormal leaves were unfolded on the scions in both intergeneric graftings (Plate 1-b). Accordingly, the growth of scions seems to be better in the intergeneric grafting using somewhat larger mulberry seedlings like the fourth leaf stage, as scions. However, from a point of view of expecting the effects of stock on the scion, younger seedlings are considered more promising scions.

Accordingly, grafting was performed using roots of *B. kazinoki* or *C. tricuspidata* as stocks and mulberry seedlings at the cotyledonary stage without roots as scions. In this case, the stock, *B. kazinoki* or *C. tricuspidata*, survived with the development of adventitious buds, while the scions, mulberry seedlings, died after unfolding one to two leaves in 70 days after

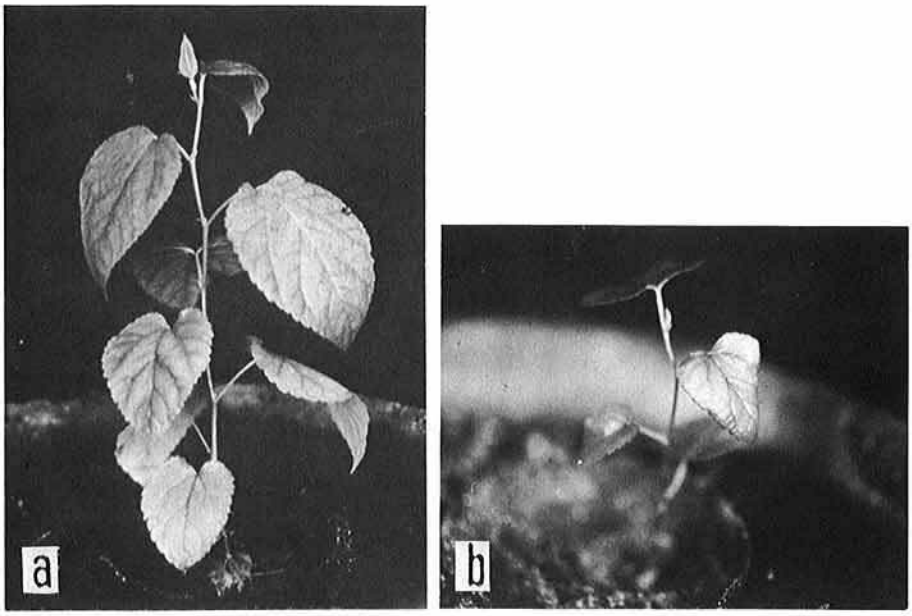

Plate 1. Mulberries grafted on the roots of *B. kazinoki* (stock)
a: Development of 10 leaves from a scion, a mulberry seedling at the fourth leaf stage without roots.
b: Development of 3 abnormal leaves from a scion, a mulberry seedling at the second leaf stage without roots.

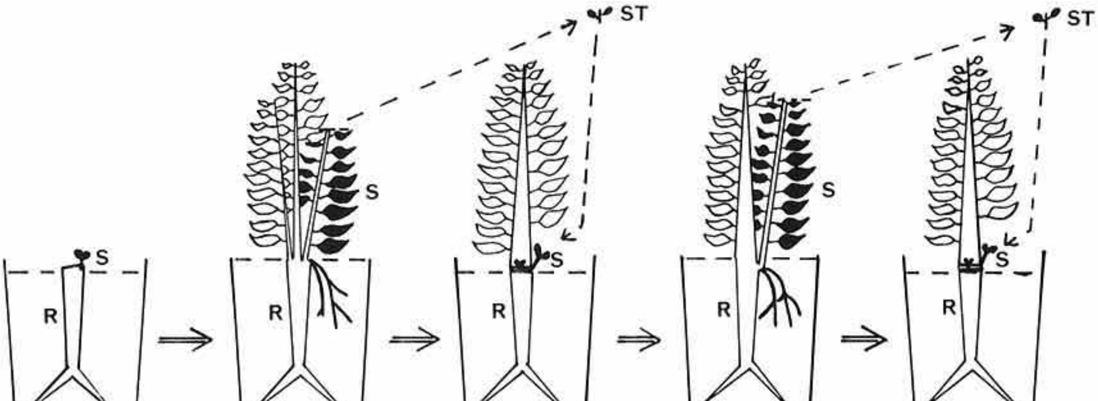

Fig. 1. Scheme showing temporary intergeneric grafting
S: Scion (mulberry), R: Rootstock (*B. kazinoki* or *C. tricuspidata*),
ST: Shoot tip of scion.

grafting. Then, the adventitious buds on the stocks were allowed to grow and a part of graft-union was covered with soil 30 to 60 days after grafting to accelerate root initiation at the base of the scions. With this handling, some scions developed their roots and grew to about 15 cm tall by 6 to 12 months after grafting. Then, the tips with two leaves were taken from the scions and again grafted on *B. kazinoki* or *C. tricuspidata*. After grafting, the scions grew a little, but soon stopped growing. Therefore, rooting of the scions was induced by covering the base of the scion with soil 30 to 60 days after grafting and allowed to grow, and tips of the scions were again grafted on *B. kazinoki* or *C. tricuspidata*. Such a process as "grafting — rooting from scion — growing of scion — grafting", was repeated as many as 11 and 13 times at the maximum in the cases of using *B. kazinoki* and *C. tricuspidata* as stocks, respectively.

Fig. 1 shows a scheme of the grafting, and the author named it temporary intergeneric grafting.

Development of 2x-4x mixoploid

The chromosome number and morphological traits were investigated in the mulberry trees

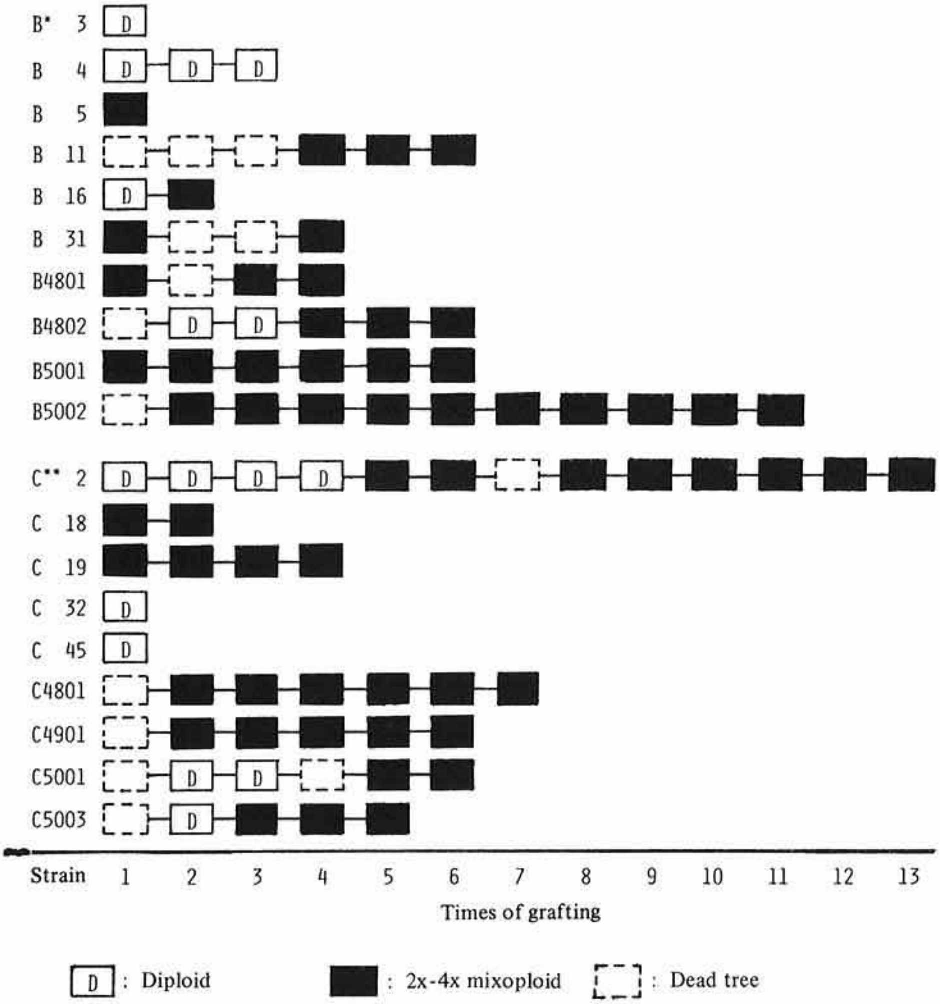

Fig. 2. Ploidy in the mulberry trees grafted temporarily on *B. kazinoki* or *C. tricuspidata*
* Grafted on *B. kazinoki*, ** Grafted on *C. tricuspidata*.

grafted temporarily on *B. kazinoki* or *C. tricuspidata*.

As a result of examination of chromosome number of young leaf tips by Feulgen's squash method, it was found that most of the mulberry trees after temporary intergeneric grafting were mixoploids with the somatic chromosome number of 28 and 56,

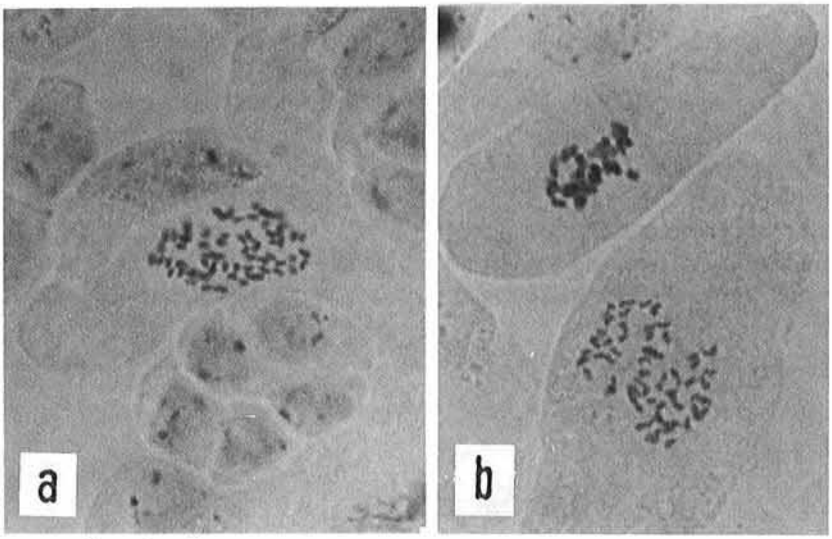

Plate 2. Chromosomes in leaf tips of 2x–4x mixoploids
a: 4x cell (2n=56)
b: 2x (upper) and 4x cells (lower) observed in a mixoploid

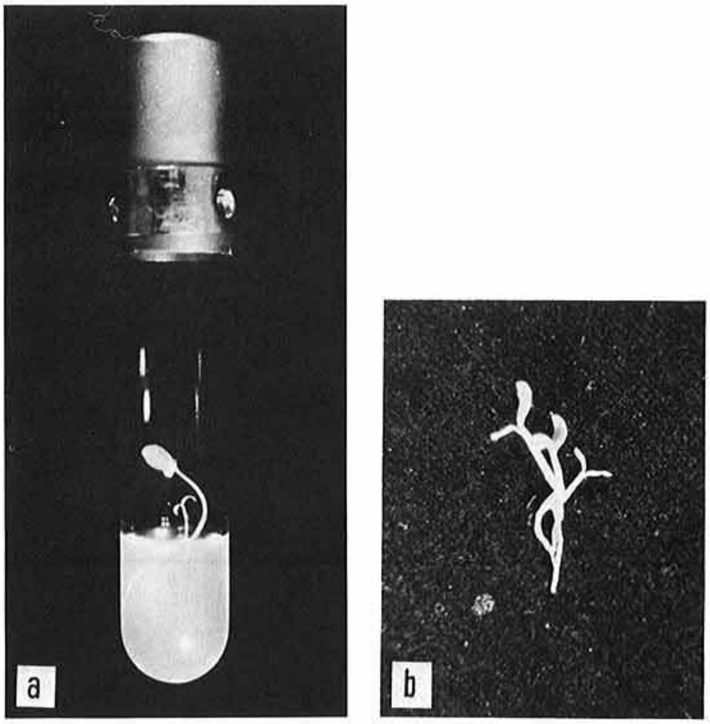

Plate 3. Mulberry seedlings from a twin seed (a) and a triplet seed (b)

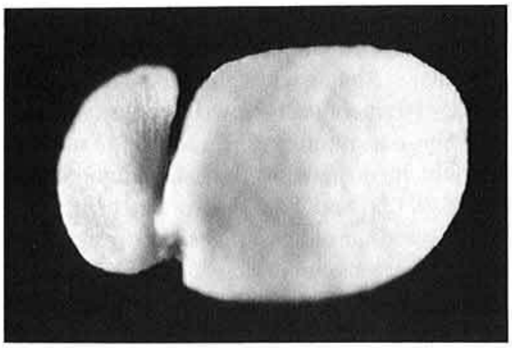

Plate 4. Malformed seed gathered from 2x–4x mixoploids

while all plants of control, seedlings from a cross Shirome-keiso ♀ × Shirome-keiso ♂, were diploids with 2n=28.

Fig. 2 shows the ploidy of mulberry trees after temporary intergeneric grafting. As shown in Fig. 2, the 2x-4x mixoploids were detected in the mulberry trees grafted temporarily on both *B. kazinoki* and *C. tricuspidata*, and the frequencies were very high. Moreover, the frequency of mixoploids increased with an increase of the number of times of grafting.

Plate 2 shows the chromosomes of 2x-4x mixoploid. As shown in Plate 2-b, 4x cells in the mixoploid were generally larger than 2x cells, and the chromosomes of 4x cells were thinner and longer than those of 2x cells and stained rather pale with the Feulgen's staining. The ratio of 4x cells in a mixoploid was estimated less than 5%, though it also increased with an increase of the number of times of grafting. And, doubling of chromosomes was found in some pollen grains of a mixoploid with the frequency of less than 0.1%.

The morphological traits and growth of mixoploids were hardly different from those of diploids. However, polyembryonic seeds were obtained from the 2x-4x mixoploids with high frequencies ranging from 2.2 to 9.7%, and many malformed seeds were also produced from them (Plates 3 and 4). Moreover, some plants bearing white soroses developed.[4]

Development of tetraploid in the progeny

To make clear the effects of temporary interge-

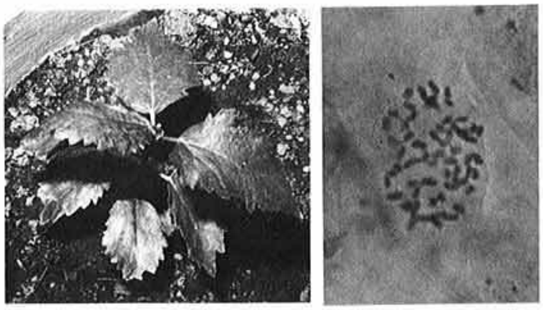

Plate 5. Tetraploid 6 months after sowing and its chromosomes

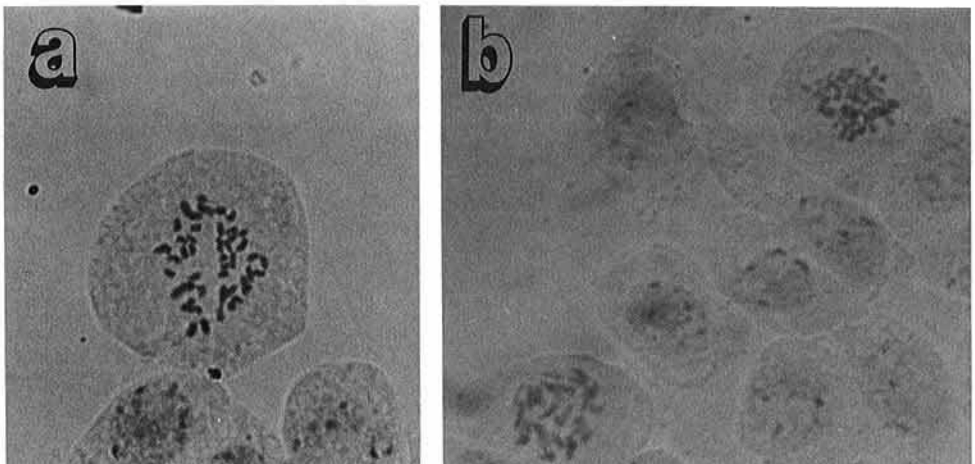

Plate 6. Chromosomes in leaf tips of 2x–3x mixoploids
a: 3x cell (2n=42)
b: 2x (lower) and 3x cells (upper) observed in a mixoploid

neric grafting on the progeny, some investigations were conducted on the morphological traits and chromosome number of the seedlings from crosses between the mulberry trees after temporary intergeneric grafting. Many malformed seedlings were found among the seedlings from a cross between B31-G_4 and B5-G_1 which were confirmed to be 2x-4x mixoploids. After the examination of chromosome number of them, two plants were confirmed to be tetraploids (2n=56), suggesting that each of them developed from a zygote consisting of two reproductive cells with doubling chromosomes (2x) which were generated in the 2x-4x mixoploid parents.[5] One of the tetraploids six months after sowing is shown in Plate 5. The growth of tetraploids was extremely poor and they bore many mottled and malformed leaves.

Development of 2x-3x mixoploid in the progeny

Many twin and triplet plants developed from polyembryonic seeds gathered from eight crosses among the mulberry trees after temporary intergeneric grafting which were confirmed to be 2x-4x mixoploids. Among these plants, the seedlings showing extremely poor growth were examined on the chromosome number, and a total of 17 mixoploid plants which consisted of the cells having chromosomes of 28 (2x) and 42 (3x) were detected.

The chromosomes in younger leaf tips of a 2x-3x mixoploid were shown in Plate 6. The proportion of 3x cells in a 2x-3x mixoploid was different each other, ranging from more than 90 to less than 10%. The 2x-3x mixoploids were detected in the seedlings from mulberry trees grafted temporarily on both *B. kazinoki* and *C. tricuspidata*, i.e. 14 mixoploids in the former and three in the latter, and all of them were the plants showing poorer growth in a pair of seedlings germinated from a twin seed.

Although growth of these mixoploids was very poor until about two months after germination, the growth became considerably vigorous after that, and any differences were hardly admitted in the morphological traits by comparison with diploids.

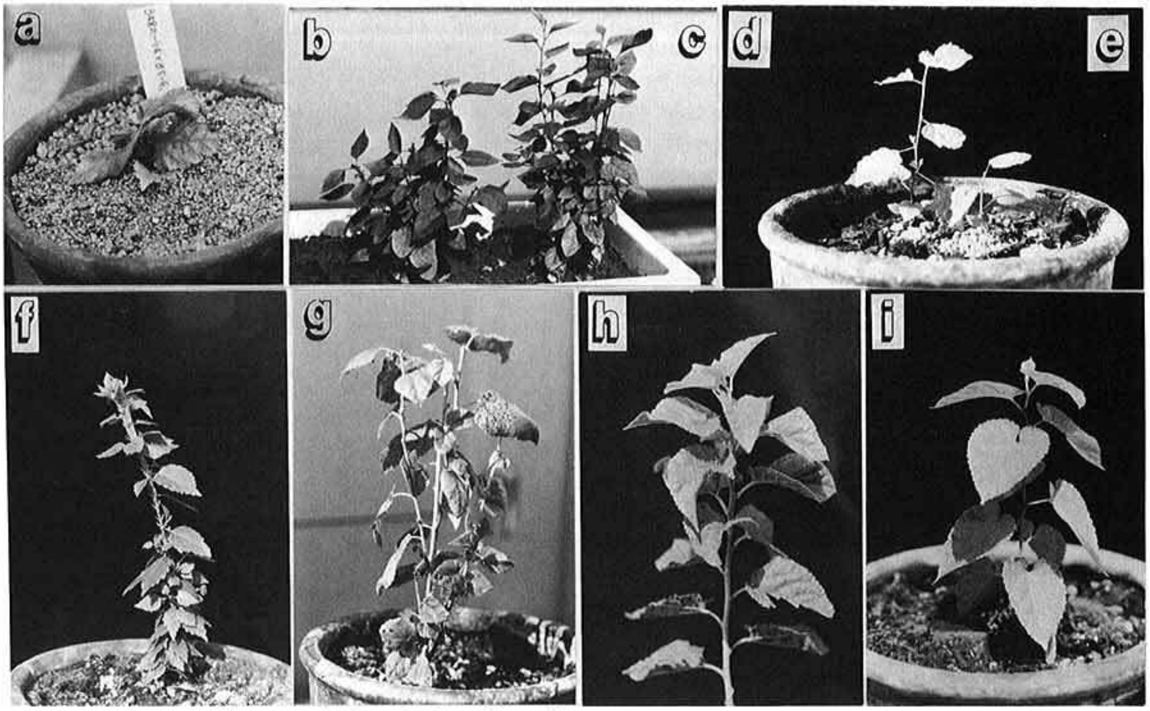

Plate 7. Six types of trisomic plants in mulberry
a: *Rosette*, b and c: *Bush*, d and e: *Pale*, f: *Fasciated*, g and h: *Robust*, i: *Pseudonormal*.

Development of trisomics in the progeny

Among the seedlings from monogerm seeds gathered from six crosses of the mulberry trees after temporary intergeneric grafting, many deformed and/or stunted seedlings were observed. As a result of investigation of their chromosome number, a total of nine plants having chromosomes of 29 (2x+1) were detected. The frequencies of trisomic plants were 0.2 to 0.3%, and they were detected in the seedlings from crosses of the mulberry trees grafted temporarily on both *B. kazinoki* and *C. tricuspidata*, i.e. seven trisomic plants in the former and two in the latter.

The nine trisomic plants could be classified into six types through an investigation about their morphological traits such as stems, leaves, buds and so on, and they were named as *Rosette, Fasciated, Bush, Robust, Pale* and *Pseudonormal* according to their major external characteristics (Plate 7). There were two trisomic plants for every *Bush, Robust* and *Pale*, and each of the two plants belonging to *Bush* and *Robust* developed from different crosses.

The nine trisomic plants could be distingushed from diploids in the early growth stage, and each of the types could easily be discriminated from others due to their external traits.

Conclusion

As mentioned above, some chromosomal aberrations were observed in the mulberry trees obtained after the temporary intergeneric grafting using mulberry seedlings at the cotyledonary stage as scions and the roots of *B. kazinoki* or *C. tricuspidata* treated with a rooting accelerator as stocks, and their progeny. The 2x-4x mixoploids detected in the mulberry trees after temporary intergeneric grafting seem to have developed not by natural crossing, spontaneous mutation or chimera formation, but by chromosomal aberration which was induced as the effects of stock on the scion in temporary intergeneric grafting. And, the 2x-3x mixoploids and trisomic plants detected in the progenies of 2x-4x mixoploids seem to have been developed by the gene or genes inducing chromosomal aberration,but not by other causes. Therefore, it may be concluded that the character of disordering nuclear division inherited from the mulberry trees after temporary intergeneric grafting to their progenies, though further studies are needed to clarify the mechanism responsible for the occurrence of chromosomal aberration.

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