

## Summary

### Studies on Breeding of Seedless Cultivars using Sterility in Citrus

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Seedlessness is a desirable characteristic for both fresh and processed citrus market, thus, it is a major breeding objectives. Therefore, purpose of this study is to develop seedless cultivars using sterility efficiently.

1. It has been suggested that hybrid seedlings with aborted anthers appear from crosses of varieties having cytoplasm of satsuma mandarin (*Citrus unshiu* Marc.) as a seed parent. However, it was revealed that the appearance of hybrid seedlings with aborted anthers by using 'Encore' (*C. nobilis* Lour. X *C. deliciosa* Tan.) as a seed parent.

When 'Encore' was crossed with 'Minneola', 'Murcott' and 'Seminole', 8 out of 61, 10 out of 43, and 7 out of 26 seedlings had aborted anthers, respectively, but 'Encore' X Ponkan crosses were all male fertile. Approximately half of the seedlings from 'Kiyomi' X 'Encore' crosses were male sterile (aborted anthers). These results probably support the concept that the genotype of Ponkan is dominant-homozygous and the genotype of 'Encore', 'Minneola', 'Murcott' and 'Seminole' is heterozygous for the male sterility gene. The results also suggested that the genotype of 'Minneola' differed from other heterozygous varieties because it has a different segregation ratio.

2. The incidence of aborted anthers appears to be a heritable characteristic which is probably due to a gene-cytoplasmic interaction. However, in the absence of detailed crosses, this can not be confirmed. Thus, the inheritance patterns of aborted anthers in citrus were studied for 418 seedlings derived from 18 cross combinations, using five varieties with aborted anthers as seed parents. Number of seeds per fruit in the hybrid seedlings was also investigated under field conditions.

Hybrid seedlings with aborted anthers appeared in all cross

combinations, but the apparent ratio differed with different cross combinations. When 'Minneola' and 'Robinson' were used as pollen parents, many hybrid seedlings with viable pollen were obtained. However, when other varieties were used as pollen parent, approximately half of the hybrid seedlings failed to develop anthers. Number of seeds per fruit also differed among cross combinations because it depends on the choice of the parental material.

In many cross combination, the ratio of aborted anthers (MS) to pollen fertile (MF) seedlings fitted a 1:1 segregation. However, for all of the hybrid seedlings obtained with 'Minneola' and 'Robinson' as pollen parents, the ratio of MS to MF was 1:3 than 1:1. From these results, it is apparent that the pollen parents used in this study were heterozygous for normal anthers and that individuals with aborted anthers a recessive gene is present in a homozygous condition. The results also suggest that the incidence of aborted anthers is controlled by two major genes.

3. Electrofusion was conducted to combine *Citrus unshiu* cv. 'Juman' unshiu which probably shows sterile cytoplasm protoplasts isolated from embryogenic callus with *C. sinensis* cv. 'F. N. Washington' navel orange mesophyll protoplasts. One plant was regenerated from the fusion products, and the plant has 18 chromosomes ( $2n=18$  in each parent). The plant showed the same nuclear rDNA fragment pattern as that of *C. sinensis*. Whereas, chloroplast and mitochondrial DNA analyses showed that the plant contained *C. unshiu* chloroplast and mitochondrial genomes. From these results, the regenerated plant was confirmed as cybrid having *C. sinensis* nuclear genome and *C. unshiu* cytoplasmic genome.

4. One means of breeding new seedless or nearly seedless cultivars is to produce male sterile hybrid seedlings. However, the relationship between male sterility and number of seeds per fruit in hybrid seedlings has never been clear. Furthermore, the inheritance pattern for number of seeds per fruit has not been determined. Thus, the inheritance for number of seeds per fruit and the relationship between number of seeds per fruit and pollen yield in 237 hybrid seedlings resulting from 15 cross

combinations were studied.

A positive correlation ( $r=0.772^{**}$ ) was found between the average number of seeds per fruit in the two parents and that in their hybrid seedlings. A negative correlation ( $r=-0.687^{**}$ ) was found between percentage of seedless hybrid seedlings (average number of seeds per fruit less than 1.0) and the average number of seeds per fruit of the parents.

The average number of seeds per fruit in hybrid seedlings ranged 0.2-0.6, 1.4-21.8, and 4.5-18.6 in male sterile progenies, in seedlings of poor yield of pollen, and in seedlings of medium to good yield of pollen, respectively. In the same cross combination, the average number of seeds per fruit was fewer in male sterile seedlings than in male fertile seedlings. The percentage of seedless hybrid seedlings ranged 0.0-100.0%, 0.0-75.0%, and 0.0-50.0% in male sterile seedlings, in progenies of poor yield of pollen, and in progenies of medium to good yield of pollen, respectively. The percentage of seedless hybrid seedlings was higher in male sterile seedlings than in male fertile seedlings in the same cross combination except for one case.

5. Inheritance pattern of number of seed was studied in 1371 seedlings derived from 76 cross combinations employing 40 parent cultivars. Based on the number of seeds, the plants were classified into four groups: seedless, few seeds, seedy and very seedy. The frequency of appearance of seedless seedlings or seedlings with few seeds in hybrid seedlings generally increased when the number of seeds of the parent materials was small. Thus, the cross between seedless cultivars or containing few seeds produced many seedless seedlings and many seedlings with few seeds. Also, the cross between seedy or very seedy varieties produced many seedy and very seedy seedlings. There was a negative correlation ( $r=-0.574^{**}$ ) between the average number of seed per fruit of the parents and the apparent ratio of seedless hybrid seedlings. Similar results were obtained for the relationship between the average number of seed per fruit of the parents and the apparent ratio of seedless hybrid seedlings and seedlings with few seeds ( $r=-0.675^{**}$ ).

6. The relationship of female and male sterility, and self-

incompatibility to seed content in citrus were investigated using 22 male sterile and male fertile cultivars. The male fertile cultivars were grouped into self-incompatible and self-compatible. Positive correlation ( $r=0.927^{**}$ ,  $r^2=0.859$ ) existed between the average number of seeds per fruit obtained by hand pollination, which indicated the degree of female sterility, and that yielded by open pollination. This result indicates that the degree of female fertility and sterility can be estimated from seeds derived by open pollination. The difference between the average number of seeds per fruit by hand pollination and that by open pollination was greater in self-incompatible cultivars and in male sterile cultivars than it was in self-compatible cultivars. This indicates that self-incompatibility as well as male sterility is effective on the reduction of seediness. In open pollination, self-compatible cultivars produced very few seedless fruits, whereas self-incompatible and male sterile cultivars produced many seedless fruits.