Chromosomal Chimeras and Polyploidy in *Solanum gracile* Link¹⁾

By
Fuyuwo Kagawa

Contents

	Page
1. Introduction	733
2. Chromosomal chimeras of diploid and tetraploid	733
3. Pollen formation in diploid and tetraploid	736
4. Triploid and its pollen formation	739
5. Discussion	741
6. Summary	744
7. Literature cited	744

1. Introduction

In the genus *Solanum*, polyploid plants have hitherto been obtained by a number of investigators (Winkler 1916, Jørgensen 1928, Lindstrom and Koos 1931, Lindstrom 1932, Lindstrom and Humphrey 1933, Noguchi 1933 and others) as adventitious shoots developed from the callus tissue that formed on the cut surface of stems. In these investigations, the cutting was made either at the point of union after grafting the stems of two different species or at the ordinary stems of a species or species hybrids.

In the following pages, the results of decapitation experiments in the writer's materials, in which chromosomal chimeras were produced are reported, together with some other studies in polyploidy in this species.

2. Chromosomal chimeras of diploid and tetraploid

The material plants were grown in 1934 from seeds in pots with soil, and the chromosome number²⁾ was verified in iron aceto carmine to be 12 in haploid. The same number of chromosomes was reported

1) The material plant for the present study has been cultivated in our garden for some years, and is certainly of foreign origin. But, as its exact taxonomic name could not be made out here, the herbaria were sent for identification to the Royal Botanic Gardens, Kew, England, who informed us that the plant has the closest affinity to *Solanum gracile* Link, which is of South American origin. In the present study this name is provisionally used, although a more definite taxonomic position, possibly a new one, might be given to it later. The writer wishes to express his cordial thanks to the Royal Botanic Gardens, Kew, for their courtesy.

2) In the present study the chromosomes of PMC were observed exclusively in iron aceto carmine, applied to fresh anthers or those fixed with Carnoy's solution and preserved in 70% alcohol.

by de Vilmorin and Simonet (1928) in their *Solanum gracile* Link material. In the writer's experiments, the stems were decapitated at a point about 10 cm above the ground when the plants were about 20 cm high. From the callus formed on the cut surface, adventitious shoots developed abundantly, and when they formed flower buds the chromosomes of PMC were observed for each shoot.

Thus, about 80 shoots adventitiously developed were examined in the late spring of 1934, and in two of them, which were obtained respectively from Individuals No. 5 and No. 33, 24 was verified as the reduced number.

In Fig. 1, Ind. No. 5 is shown. This plant, when young, was decapitated at point P, and two adventitious buds were formed each

Fig. 1. Photograph of Individual No. 5 with a chimeral shoot. ca. 1/8 natural size. Photographed in late summer 1934. Explanation in text.

of which developed to a vigorous shoot as may be seen in the photograph. The PMC obtained from shoot A, seen at the right in the photograph, showed 24, the tetraploid number of chromosomes in heterotypic and homotypic metaphases, while those from shoot B at the left showed 12, the diploid number in the same stages.

The shape of the leaves and flowers in shoots A and B was similar, and their size differed more or less within each shoot. Generally speaking, however, those in shoot A were larger

and coarser than those in shoot B, and the green colour of the leaves was darker in the former than in the latter. Fig. 2 shows the leaves and flowers of the largest class from each shoot. In several such leaves from both shoots, the size of stomata was measured, and it was found larger in shoot A than in shoot B. The average size of stomatal apparatus (length of guard cells $\times$ their breadth) as

calculated from 300 stomata was $1269.6 \mu^2$ in the former, and $840.4 \mu^2$ in the latter. The hairs on the leaves and stems were larger in shoot A than in shoot B.

Thus, in shoot A, the sizes of some of the organs and cells are much larger than those in shoot B. Similar conditions have generally been reported by investigators in other examples of autotetraploidy.

The formation of fruits and seeds in shoot A, observed in the fall of 1934, was much less compared with that in shoot B and ordinary diploid plants, although in the shape and size of fruits there was no clear difference.

We shall next turn to the shoot from Ind. No. 33, in which 24 was verified as the reduced number. In this shoot, the leaves

and flowers were rather similar to those of ordinary diploid plants, and the tetraploid characteristics that were found in shoot A of Ind. No. 5 were not markedly observed in these organs. Its fruits and seeds were formed in the fall of 1934 abundantly, contrary to shoot A of Ind. No. 5, its fertility apparently being similar to that in ordinary diploid plants.

In the fall of 1934, several branches of shoot A of Ind. No. 5 and the shoot of Ind. No. 33 in which 24 was found as the reduced number, were cut and propagated asexually in soil in pots. These were overwintered in a green house, and in the spring of 1935 were placed out of doors. Numbers of branches came out from every individual during this period, and their PMC were observed in the summer of 1935.

As may be expected, 24 reduced number of chromosomes was found in the PMC in 5 plants from shoot A of Ind. No. 5. But, quite unexpectedly, 12 was clearly certified in diakinesis, in first and second metaphases as the reduced number in some flower buds obtained from two other plants derived asexually from shoot A of Ind. No. 5, and in one plant from the shoot of Ind. No. 33, in which the tetraploid number was found the preceding year.

Fig. 2. Leaves and flowers of tetraploid and diploid. a, tetraploid. b, diploid. ca. 4/5 natural size.

In view of these facts, it must be concluded that shoot A of Ind. No. 5 and the shoot of Ind. No. 33, from both of which 24 was verified in 1934 as the reduced number, were really not the tetraploid shoots, but chromosomal chimeras that constituted of both tetraploid and diploid tissues. To the modes of their arrangement we shall come later.

3. Pollen formation in diploid and tetraploid

The pollen formation in the anthers obtained in 1934 from shoots A and B of Ind. No. 5 will now be described. In shoot B, 12 was shown as the reduced number in the first as well as in the second metaphase (Figs. 3, 4). Meiosis proceeded regularly, and usually 4 microspores were formed from a PMC (Fig. 5), though rarely 5-6 such were formed owing to 1-2 lagging chromosomes appearing rarely in first and second anaphase and telophase. The proportion

Figs. 3-6. Diploid. 3, first metaphase, 12 bivalents. 4, second metaphase, 12 chromosomes. Polar views. $\times 1700$. 5, tetrad. $\times 1250$. 6, pollen grains. $\times 700$.

of PMC in which apparently the normal tetrad was brought about, was 97.0% (609 in 628). The proportion of good pollen grains was 88.2% (7,149 in 8,105), the remainder being empty or shrunken. Mature pollen grains¹⁾ of normal structure were usually provided with 3, occasionally 2, germ pores (Fig. 6), while those having 4 were not detected. The average diameter²⁾ of pollen grains of normal structure calculated from 358 grains was 26.2μ .

1) The pollen grains were observed in lactic acid.

2) The pollen grains may appear, when seen from a certain angle, to be either spindle shaped or triangular according to the number of germ pores, as the surface of the grain somewhat projects at the places where the germ pores are located. But, if they were observed from an other angle, they may appear almost spherical, if the germ pores are seen above or below the grain surface under the microscope. Such pollen grains showing spherical shape were selected, and their diameter was measured.

In tetraploid PMC from shoot A, 24 bivalents were observed at first metaphase (Fig. 7), but occasionally certain numbers of quadrivalents were observed together with bivalents. In Figs. 8 and 9, 2 and 6 quadrivalents and respectively 20 and 12 bivalents are observed.

At late metaphase, 1-3 univalent chromosomes were often observed away from the equatorial plane. At late anaphase, some univalents were often found located at the periphery of the PMC, separated from the anaphasic groups of chromosomes (Fig. 10). The lagging univalents were almost always found at late anaphase and telophase, and were observed split there.

Figs. 7-11. Tetraploid. 7-9. First metaphase. Polar views. 7, 24 bivalents. 8, 2 quadrivalents and 20 bivalents. 9, 6 quadrivalents and 12 bivalents. 10, first late anaphase: 2 chromosomes are apart from two main groups of chromosomes. Only the chromosomes clearly observable are drawn in main groups. Side view. 11, second metaphase, 24 chromosomes. $\times 1700$.

At telophase, the chromosomes, instead of being arranged in two compact groups at the heterotypic poles, were located rather loosely in a comparatively larger spaces in the polar regions. Consequently, some chromosomes located at the periphery of the groups occasionally formed later one or more extra nuclei separated from the two main telophasic nuclei. The extra nuclei were also formed derived from lagging chromosomes.

At second metaphase, 24 chromosomes were found (Fig. 11). But, very frequently a small number of chromosomes appeared

separated from the two metaphasic plates, which was probably brought about by the irregular distribution of chromosomes in the first division. At late anaphase and telophase also, the chromosomes separated from the main groups and the laggards were quite often observed (Fig. 12).

Usually 4 groups of chromosomes were found in a PMC at second telophase. But in most groups, the chromosomes were distributed in comparatively larger spaces and not compactly arranged. And a nuclear membrane did not develop surrounding all the chromosomes of each group, but the chromosomes located at its periphery or somewhat away from it, or those which were lagging behind, occasionally formed one or more extra nuclei (Fig. 13).

Figs. 12-15. Tetraploid. 12, second late anaphase: some chromosomes are lagged behind or located apart from the main groups of chromosomes in cytoplasm. Side view. 13, second telophase showing extra nuclei. $\times 1700$. 14, polyspory. $\times 1250$. 15, pollen grains. $\times 700$.

Owing to the foregoing irregularities in chromosome behaviours in both divisions, polyspory resulted in almost all PMC (Fig. 14). The proportion of PMC in which polyspory was observed was 96.3% (881 in 915), the highest number of microspores formed in a PMC

being 13. The proportion of good pollen grains was only 12.8% (1,281 in 10,005). The mature pollen grains of normal structures were usually provided with 4 germ pores, sometimes 3 and, rarely, 5 (Fig. 15). Those having 2 germ pores were not met with. Their average diameter calculated from 358 grains was 31.9μ .

4. Triploid and its pollen formation

In the spring of 1935, the seeds obtained by open pollination from the shoot of Ind. No. 33 in which the tetraploid number of chromosomes was observed in 1934, were sown and the young plants that emerged decapitated. In one individual, only an adventitious shoot had formed showing tetraploid characteristics in the external characters of the leaves and flowers. The tetraploid number 24 was also observed in their PMC. It is not certain, however, whether the shoot of this individual had formed adventitiously on the tetraploid plant without duplication of chromosomes, or whether it had formed on the diploid plant as a tetraploid shoot in which the chromosomes were doubled. Both these possibilities may be conceivable in view of the constitutional nature of the shoot of Ind. No. 33, from which this individual was derived.

This individual was overwintered in a green house, and many branches came out until the summer of 1936. A number of chromosome countings were made on different branches, and the tetraploid number 24 was always detected as far as the observation up to present is concerned. This plant may probably be a true tetraploid.

In 1935, this individual was used as the maternal plant and the pollen of diploid plants was pollinated, and four F_1 plants were obtained together with several offsprings in which 24, the tetraploid number was observed.¹⁾ In F_1 the external characters of the leaves and flowers and the size of stomata were mainly intermediate between diploid and tetraploid.

The chromosome number of F_1 should be 36 in a somatic count. In the first meiotic metaphase of the PMC, 12 trivalents were sometimes clearly observed (Fig. 16), but more frequently, different numbers of bivalent and univalent chromosomes were observed together with trivalents. These three kinds of chromosomes were distinguishable in many cases from each other by their size. In Fig. 17, 10 trivalents, 2 bivalents and 2 univalents are observable, while in Fig. 18, 3 trivalents, 9 bivalents and 9 univalents are shown.

1) As to the cause of the formation of tetraploid progeny, which was not the self fertilization in the mother plant, we hope to deal with it in a following opportunity.

At the first anaphase, the components of trivalents passed on to poles as univalents, but some bivalents which were made probably of the two component univalents of trivalents were also observed on their way to the poles. In Fig. 19, which is a polar view of this stage, 1 bivalent plus 12 univalents and 2 bivalents plus 18 univalents are observed on different optical planes passing to poles. The numbers of chromosome elements going to a pole that were counted in polar views of first anaphase were 12-19. At first telophase 1-4 univalent chromosomes were frequently found lagging behind and split there.

At the second metaphase, 12-18 chromosomes were observed (Fig. 20), and occasionally some of the chromosomes were located separated from the main metaphasic groups. At the second late telophase, we frequently found extra nuclei derived from some chromosomes that were lagging behind or were located away from the main

Figs. 16-23. Triploid. 16-18, first metaphase. Polar views. 16, 12 trivalents. 17, 10 trivalents, 2 bivalents and 2 univalents. 18, 3 trivalents, 9 bivalents and 9 univalents. 19, first anaphase. Polar view. 20, second metaphase with 18 chromosomes. Polar view. 21, second telophase, extra nuclei. $\times 1700$. 22, polyspory. $\times 1250$. 23, pollen grains. $\times 700$.

telophasic groups of chromosomes (Fig. 21). So that polyspory resulted in the majority of PMC (Fig. 22), the highest number of microspores formed in a PMC being 11. The proportion of PMC showing polyspory was 92.4% (501 in 542). Dyad cells were never met with at tetrad stage.

The majority of pollen grains were empty and shrunken, and the proportion of good pollen grains was 13.0% (1,447 in 11,166). The normally developed pollen grains were provided with 3-4 germ pores (Fig. 23), a number intermediate between those observed in diploid and tetraploid. Pollen grains showing 2 or 5 germ pores were not detected. The average size of normally developed pollen grains calculated from 358 grains was 27.7μ in diameter, a size intermediate between those of diploid and tetraploid.

5. Discussion

In the decapitation experiments hitherto reported by investigators, either the diploid or the tetraploid number of chromosomes was, or seems to have been, exclusively found in an adventitious shoot developed from the callus tissue on the cut surface. Some, however, have reported the examples in which the organs of diploid aspects appeared in a tetraploid shoot formed in this way, although a chromosome counting in such regions that showed a diploid nature was not made.

Winkler (1916) obtained tetraploid individuals of *Solanum nigrum*, that were asexually derived from adventitious shoots developed from the callus tissue on a cut surface, made at the point of union after grafting the stems of *S. Lycopersicum* and *S. nigrum*. He observed in such tetraploid *S. nigrum* that, in certain leaves there appeared the shape, colour and anatomical structures that were characteristic of diploid *S. nigrum*, from which he inferred that the diploid cells were contained in such leaves, although the chromosome number in them was not observed. He further considered that the reduction in chromosome number might have occurred in the original somatic cells, and that by the diploid cells derived from these cells such diploid regions were formed. He considered it possible that a part of a flower, an anther for example, may become diploid and produce pollen grains containing the haploid number of chromosomes.

According to Jørgensen (1928), a triploid tomato shoot was formed as a somatic sport on a plant that was regarded from its morphological aspects to be diploid, though on its formative cause no conclusion was made. In this connection, a possibility was stated that, trinucleate somatic cells may be formed in a diploid plant resulting in hexaploid somatic cells, and that the triploid cells may be

formed through reduction of chromosome number occurring in such cells, from which a triploid shoot may be derived.

Jørgensen (1928) obtained in *S. nigrum* by the method of decapitation a periclinal chimera of diploid and tetraploid tissues, together with tetraploid plants. It was reported that in this chimera, the diploid core was covered by at least two layers of tetraploid skins, although the precise descriptions of this chimera, especially of its chromosomes, were not given. He also pointed out the possibility of the formation of chromosomal chimera in tomatoes. Lindstrom and Koos (1931) also obtained through the decapitation method applied to diploid plants, some tomato plants that were apparently sectorial chimeras of diploid and tetraploid tissues, although the chromosomes were not examined.

It seems quite natural that the growing point of an adventitious shoot developing from the callus may consist of diploid and tetraploid tissues, located side by side sectorially, or arranged periclinally, or mixed together in a complex manner. The cases of adventitious shoots formed by tetraploid cells alone might be special occurrences.¹⁾

A discussion of reduction of chromosome number in somatic cells, which may play a certain rôle in the formation of chromosomal chimeras, is outside the purpose of the present paper. However, the appearance of diploid tissues or organs in the so-called tetraploid shoots might naturally be expected if such shoots were not really the tetraploid, but of the chimeral nature developed adventitiously from diploid and tetraploid cells in the callus.

In the late spring of 1934 when the plants were young and small, shoot A of Ind. No. 5 showed in their leaves and flowers morphological characteristics of tetraploid plants, while in the shoot of Ind. No. 33 in which 24 was found as the reduced number, the leaves and flowers were rather similar to those of the diploid plants, in marked contrast to shoot A of Ind. No. 5. This seemed odd at that time. In this connection, it might be supposed that, the leaves and flowers of shoot

1) In our laboratory, the analogous chimera shoots were obtained in this plant *S. gracile* Link by S. Sakamoto and Y. Araki during the years 1935-1936 (unpublished). In the spring 1935, several adventitious shoots of tetraploid outlook were obtained, by the decapitation callus method, on diploid stocks, and 24 the tetraploid number of chromosomes was observed in PMC from each of these shoots. These plants were overwintered in a greenhouse without being propagated by cutting, and in the summer of 1936, the chromosomes were again observed in PMC from the same shoots. In two shoots on different stocks, the tetraploid number was exclusively found in a number of countings, while in one other plant 12 the diploid number was counted in 9 flower buds obtained from different positions on the shoot, in which the tetraploid number was detected the preceding year. This shoot must evidently be a chromosomal chimera.

A of Ind. No. 5 were formed at that period mostly of the tetraploid tissue, while the diploid tissue was not yet properly developed, though such was contained in some parts of the shoot. On the other hand, the shoot of Ind. No. 33 from which the tetraploid number was ascertained might have possibly been constituted at that time as a periclinal chimera, the subepidermal layer of which was made of the tetraploid cells. In periclinal chimeras the external characters may be more or less intermediate between tetraploid and diploid.

In the case of an adventitious shoot that started from the callus tissue on the decapitated surface, it is possible that the diploid and the tetraploid tissues are arranged in various ways, sectorially, periclinally, or in some other complex manner. It is also probable that the modes of arrangement of different kinds of tissues change in some branches or branchlets during cultivation for long periods. Jørgensen and Crane (1927) obtained a number of *Solanum* chimeras as the adventitious shoots started from the callus tissue on a cut surface, that was made at the point of union after grafting the stems of two different species. According to these authors, the periclinal chimeras which were obtained in this way, and which were constituted of two kinds of tissues from different species, produced pure shoots constituted exclusively of either one or the other kind of the component tissues, when propagated asexually and during cultivation for two years.

The fertility in the shoot of Ind. No. 33 which showed the tetraploid number at late spring in 1934, was much higher than that in shoot A of Ind. No. 5, at the fall of the same year. It is possible that in this individual, the diploid branches or the chimera branches having diploid cells in the subepidermal layer, grew abundantly after the chromosomes were examined, resulting in greater fertility, which was owing to the regular meiosis in diploid PMC and the subsequent formation of good pollen grains.

In the branches or branchlets, from which the PMC showing the diploid number was detected in 1935, the subepidermal layer must naturally have been composed of diploid cells, although the branches or branchlets themselves might have been constituted exclusively of diploid cells or of diploid and tetraploid cells in chimera arrangement of different modes.

In order to ascertain how the branches or branchlets of different chromosome contents were distributed in a plant in 1935, it might be useful to compare the external characters of the leaves and flowers. But, unfortunately this was not possible, as the plants grew poorly and were stunted, which was largely the result of lack of sufficient temperature and illumination in the green house, malnutrition in

small pots, and frequent spraying of insecticides. Ind. No. 5 itself was also overwintered under the same conditions, and later placed outdoors in 1935. And its shoots A and B did not show, owing mainly to the same reasons, any distinct difference in the characters of leaves and flowers in the green house and outdoors in 1935, although it was clearly observed the preceding year.

In view of the foregoing results, we can not always regard the adventitious shoots with large flowers and large and dark green leaves as true tetraploid plants, even should the meiosis in some anthers obtained from such shoots show the tetraploid number of chromosomes. On the other hand, if a plant grown from seed which had formed on such adventitious shoot shows the tetraploid number of chromosomes, it may then be concluded that it is a real tetraploid plant. This must be noticed in the course of genetical and breeding works which may be undertaken in connection with the decapitation callus method.

6. Summary

1) In *Solanum gracile* Link, the chromosomal chimeras composed of diploid ($n = 12$) and tetraploid ($n = 24$) tissues were obtained by the decapitation callus method applied to diploid plants, and the occurrence of such chimeras and the modes of arrangement of the two kinds of tissues were discussed.

2) Triploid plants were obtained by crossing an individual, which was probably a true tetraploid, with the pollen of diploid plants. In tetraploid and triploid, the course of pollen formation resulting in a high degree of pollen abortion was studied.

Sept. 1936, Utsunomiya Agricultural College

7. Literature cited

- Jørgensen, C. A. 1928. The experimental formation of heteroploid plants in the genus *Solanum*. Jour. Gen. Vol. 19.
 — and Crane, M. B. 1927. Formation and morphology of *Solanum* chimeras. Jour. Gen. Vol. 18.
 Lindstrom, E. 1932. A fertile tetraploid tomato. Jour. Hered. Vol. 23.
 Lindstrom, E. W. and Koos, K. 1931. Cyto-genetic investigations of a haploid tomato and its diploid and tetraploid progeny. Amer. Jour. Bot. Vol. 18.
 — and Humphrey, L. M. 1933. Comparative cyto-genetic studies of tetraploid tomatoes from different origins. Genetics, Vol. 18.
 Noguchi, Y. 1933. Formation of tetraploid tomatoes by the decapitation-callus method. (Japanese). Bot. and Zoolog. (Tokyo) Vol. 1.
 Vilmorin, R. de et Simonet, M. 1928. Recherches sur le nombre des chromosomes chez les Solanées. Verhandl. V. Intern. Congr. f. Vererb.-wiss.
 Winkler, H. 1916. Über die experimentelle Erzeugung von Pflanzen mit abweichenden Chromosomenzahlen. Zeitschr. f. Bot. Bd. 8.