A TETRAPLOID PLANT IN WILD RICE
(*ORYZA LONGISTAMINATA*)

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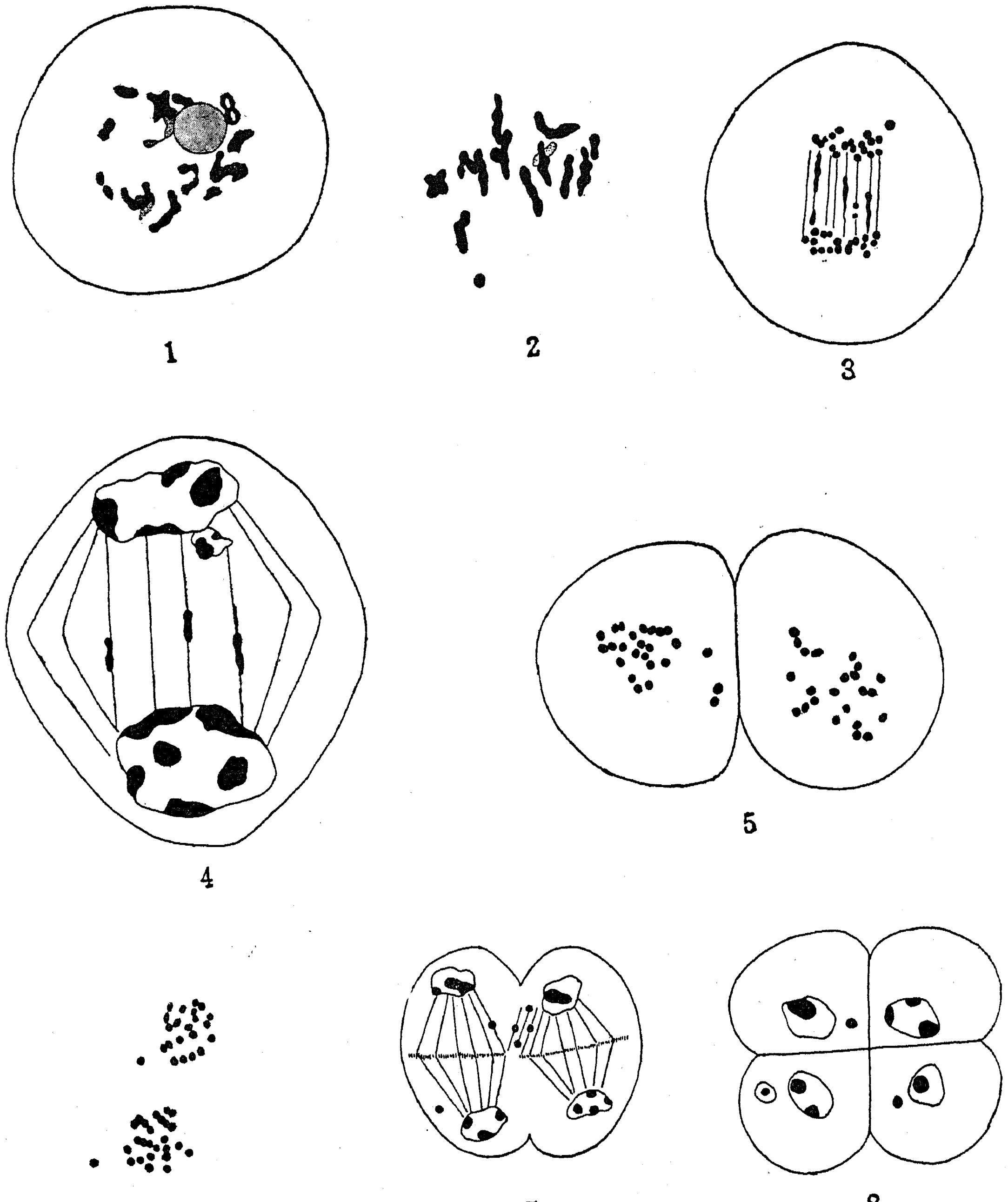
1. *Occurrence and Description.*

THIS species of rice was originally obtained from Ceylon and has been growing in the Paddy Breeding Station, Coimbatore, for the last twelve years. It is a perennial plant, spreads rapidly by rhizomes and the natural setting of seed is greatly hindered by its peculiar blooming habit (Ramiah, Parthasarathy and Saravayya, 1925). As in any other wild rice the few grains that do occasionally set shatter badly even before they are fully mature. Although it was originally raised from seed, subsequent propagation has been mainly from rhizomes which lie dormant during the off-season, February to July, and shoot up again with the receipt of irrigation water in July. In the field where this species has been growing, during 1934-1935 season, a clump of shoots was noticed which resembled generally other plants of the species, but was characterised by thicker stems, darker pigment on internodes, broader, darker and thicker leaves and larger spikelets (Plate LIX). The pollen on examination was generally bigger than in the normal plant and showed considerable amount of abortion. This plant on cytological examination turned out to be a *tetraploid*. There is no record, as far as the authors are aware, of a tetraploid in this species, although recently Nakomori (1933) in Japan has reported the appearance of tetraploid in the F_1 progeny of an intervarietal cross in *Oryza sativa*.

2. *Meiosis in Pollen Mother-Cells.*

Aceto Carmine smears (Belling, 1926) and permanent sections stained with Iodine-Gentian-Violet (La Cour, 1931) were prepared of the pollen mother-cells for study of meiosis. Stages earlier to diakinesis were not investigated. At diakinesis, the chromosomes were found largely in tetravalent groups in the form of rings, rods, Xs and Ss, although bivalents and occasionally univalents were also noted (Fig. 1). The usual number of quadrivalents noted at diakinesis and first metaphase (Fig. 2) was ten or eleven although



1 2 3 4 5 6 7 8

FIGS. 1-8.

occasionally cells with as few as seven were also found. Lagging chromosomes were frequently seen at anaphase and some of the univalents were also seen to divide late on the spindle. In many instances, at telophase, some chromosomes were seen drawn out, extending through a major part of the spindle even from pole to pole occasionally (Fig. 3). Such tardy and incomplete disjunction is probably the result of incomplete terminalisation of interstitial chiasmata, which persist up to metaphase. By actual counts at first anaphase it was found that out of eighty cells as many as sixty showed laggards. At times the chromosomes were seen lying off the spindle free in the cytoplasm. These and the laggards either collected themselves to form additional nuclei at interphase or lay free in the cytoplasm. As a result of the irregular reduction division, the distribution of chromosomes at second metaphase was greatly affected and the cells were observed to contain chromosomes ranging from 20-28. Straying chromosomes not previously included in the divided nuclei, were also met with at this stage. One remarkable feature of the second metaphase is the tendency of the chromosomes to appear in groups of four, three and two exhibiting the phenomenon known as secondary association. This aspect of the work has received considerable attention on the part of the workers at the John Innes Horticultural Institution and has helped in throwing light on the basic number of chromosomes in several genera. No counts were taken of the different groups in this tetraploid rice plant but the existence of the association was very evident (Figs. 5 and 6). The homotypic division in this tetraploid was not as regular as is reported of other tetraploids. Chromosomes either lagging on the spindles or remaining free in the cytoplasm were not uncommon features of the second division. Only rarely cells were met with where additional nuclei divided on supernumerary spindles (Fig. 7). Of 100 cells examined at second anaphase 80 showed either laggards or straying chromosomes. The microspores formed were usually in tetrads but a majority of them contained either micronuclei or straying chromosomes embedded in the cytoplasm (Fig. 8). A considerable number of pollen grains, as compared with the diploid, was shrunken and aborted, only about five per cent. of them being apparently fertile with contents. They were generally bigger though variable in size and stainability. From observations made already the percentage of fertility of pollen would appear to vary with the season.

3. *Discussion.*

Tetraploid plants are known to arise by the duplication of chromosomes in cells either by the two nuclei failing to separate or by the failure of the formation of cell wall between them after division. Such a condition is particularly likely in the rather irregular growth of callus and this fact has been made use of by Jorgensen (1928) in *Solanum lycopersicum* and *Solanum nigrum*, Lesley and Lesley (1930), Lindstrom and Koos (1931) in tomatoes to produce tetraploids. Randolph (1934) has also produced tetraploids in maize by subjecting the proembryo to intermittent heat. Application of other external agencies like cold, X-rays, and even injury to growing regions are known to produce duplication of chromosomes leading to the production of tetraploids. In the present case it is probable that a similar chromosome duplication occurred in one of the buds either on account of seasonal factors or injury imparted in the course of cultural operations, and this might account for the appearance of the tetraploid.

This tetraploid in common with others, shows gigantism in many of its parts, like the stem, leaf, spikelets and pollen grains. In point of the tendency to quadrivalent formation at diakinesis, it resembles the tetraploids of *Datura* (Belling, 1924), *Primula* (Darlington, 1931), Tomato (Lesley and Lesley, 1930 and Lindstrom and Koos, 1931). In the *Solanum* tetraploids of Jorgensen, however, the chromosomes are found to occur mostly as bivalents rather than as quadrivalents in the majority of cells at diakinesis. Unequal disjunction of chromosomes and the presence of laggards at first division noted in many of the other tetraploids were found to a greater degree in this rice tetraploid which, probably, in addition to other causes, account for such a large percentage of pollen abortion. While the homotypic division of other tetraploids were found to be comparatively free from irregularities, that of the rice tetraploid was marked by the presence of laggards. The pollen sterility was in this case very high and the nearest approach to it is the tetraploid of *Solanum nigrum* which, in spite of fairly regular divisions, showed as high a percentage of sterility as 82. Lindstrom and Koos record that fertility of pollen in some of their tomato tetraploids varied with season and a similar condition appears to exist in the present case. It may be mentioned here that the authors have observed a similar occurrence, namely, variation in fertility of pollen with the season, in the triploid plant of rice reported previously (1933).

The observations of secondary association at second metaphase in the tetraploid of this species, when considered with similar observations in the diploid and the haploid, afford sufficient ground for assuming that the basic number of chromosomes in *Oryza* might be less than twelve. Kuwada (1910) found in the diploid plants of *Oryza sativa* a considerable tendency for association of chromosomes in the metaphase of the homotypic division. We have observed in our own material both in *Oryza sativa* and *Oryza longistaminata* a tendency for the chromosomes to appear in pairs at second metaphase. In the rice haploid (1934) we have observed this tendency for secondary association of chromosomes in many pollen mother-cells. Occasionally cells were observed which showed the twelve chromosomes coming out in six pairs. The Japanese authors, Morinaga and Fukushima, have also observed this tendency in their rice haploid (1934) but not to the same extent as in our case. Already Chao, Lawrence and Yamura, as quoted by Morinaga (1934), regard *Oryza sativa* as a tetraploid species for various reasons. Our observations of secondary associations, which are under investigation in greater detail in other materials as well, point out strongly that the basic number of chromosomes for the genus *Oryza* might be less than twelve.

4. *Summary.*

A tetraploid plant of *Oryza longistaminata* is described for the first time. This arose spontaneously in a field where the diploid plants were growing, mainly by rhizomes, for the last twelve years and is believed to be the result of chromosomal duplication in one of the buds. This plant has thicker culms with deeper pigmentation, broader, thicker and darker leaves, and larger spikelets as compared to the diploid. The pollen of this plant is generally of slightly bigger size than in the diploid and mostly abortive.

Studies of meiosis by smears and permanent sections reveal several irregularities like unequal disjunction, the presence of lagging and straying chromosomes at first and second division. One important feature of the second division is the presence of secondary association at metaphase. This fact together with the observations of similar nature in the diploid and haploid rices point to the assumption that the basic number of chromosomes for the genus *Oryza* might be less than twelve.

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EXPLANATION OF PLATE AND FIGURES.

PLATE LIX.—Drawings showing the internode, earhead and spikelet of the diploid and tetraploid.

TEXT:— Fig. 1.—Diakinesis in the tetraploid.

Fig. 2.—First metaphase.

Fig. 3.—Telophase showing elongated chromosomes on the spindle and a dividing univalent.

Fig. 4.—Late telophase showing an additional nucleus and laggards on the spindle.

Figs. 5 and 6.—Second metaphase showing distribution and secondary association of chromosomes.

Fig. 7.—Second telophase showing supernumerary spindle and straying chromosomes.

Fig. 8.—Tetrad showing microspores containing micronucleus and odd chromosomes in the cytoplasm.

Figs. 1, 2, 6, 7, and 8 are from permanent sections stained with gentian violet. × 3,000

Figs. 3 and 5 are from aceto carmine smears. × 1,500.

Fig. 4 from aceto carmine smears. × 2,000.

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