POLLEN GRAIN SIZES IN *ORYZA*

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In the course of a study in the cytogenetics of the genus *Oryza*, attempts were made to induce tetraploidy in varieties and hybrids of *Oryza sativa* L. To determine the effectiveness of the treatment applied, the pollen grains from treated and untreated plants were examined, for it is well established that in artificially produced polyploids pollen grains show marked increase in size. Initially, there was some difficulty in deciding the chromosome constitution, for there is considerable variation in pollen grain size within a strain, species and genus. The general principle involved that pollen grain sizes are affected by changes in its chromosome number is fairly well established (Dermen, 1937). The details of normal sizes prevailing in *Oryza sativa*, and the extent of changes genetically caused, has not yet been recorded. The results of measurements in a large number of *Oryza* forms are given below.

Search in literature showed that comparatively little attention has been paid to this genus. Wodehouse (1935) describes the type of pollen grain present in the genus. Jones and Newells (1948) have given the detailed measurements of fluctuations of grain diameters in 40 types of grasses of America. Hector (1936) in dealing with crop anatomy gives dimensions of pollen grains in *Triticum*, but not in *Oryza*. Morinaga and Fukushima (1937) giving an account of autotetraploid forms of *Oryza sativa* give measurements of pollen grain sizes in the varieties studied by them, but do not compare diploids with polyploids. Muntzing (1940) in a detailed study of the genus *Poa* points out the increase in size of pollen grain accompanying increase in chromatin material. The present paper deals with both aspects, namely, the variability as well as enlargement consequent on polypoidy of pollen grains in *Oryza*.

Material and Methods

The pollen grains were collected from the plants maintained in the extensive collection in the Paddy Breeding Station at Coimbatore. Only ripe pollen grains were measured, but without differentiating between naturally shed grains and those teased out of fully ripe anthers, for, detailed measurements in one plant showed that the pollen grain size gradually increased from formation up to the time of maturity of anthers but the diameter did not diminish between this stage and the dry stage found in dehisced anthers.

The fresh pollen grains were mounted in aceto-carmine for observation, and since it was desired to retain the mounts for some days a jelly form of aceto-carmine was used. Following the techniques developed by Zirkle (1937), maltose, agar and pectin was added to the aceto-carmine. For convenience, a medical preparation meant to be a special dietary product called Mead's Pectin-Agar (made by Mead Johnson and Co., of U.S.A.) was used. This preparation contained 88% dextrin and maltose, 6% pectin, 4% agar and 2% sodium chloride. For preparing the jelly, ten grams of the pectin-agar powder was added to 100 ml. of hot 40% acetic acid, dissolved, and 1 gram of carmine powder added. When cold the preparation is a viscous liquid, difficult to filter, and a decanted solution has to be used. The mounting medium did not show swelling or shrinking action on *Oryza* pollen grains.

For measuring the pollen grains, a Spencer microscope with fixed tube length and a combination of 10 × micrometer eyepiece and 44 × dry objective was used. The micrometer divisions gave a calibration of 1 div. = 1·6 micron. In recording the data, all readings were to the nearest micrometer division, and only full or 'perfect' pollen grains were measured. All drawings were made at the bench level with the aid of a Spencer's camera lucida giving a magnification of × 750.

Observations

For determining the degree of accuracy of the observations, the following procedure was used. The same mount was measured under dry lens and under oil immersion lens, and the average of 10 readings were compared. The measurements under dry lens were comparable in accuracy for the present purpose to the detailed measurements made under immersion lens. The same mount was measured by the two authors separately and the average of readings were compared. There were differences in the two estimates, but its extent was well within the standard error. Owing to difficulties in making a large number of micrometer measurements, the size of the sample had to be restricted. A test showed that a sample of 20 measurements gave a reliable data in those paddy varieties where the variation was not large. This size of sample was used in most cases. In polyploids, the sample had to be larger, and 600 grains were measured from triploid Co. 4. The effect of altering the environmental conditions on the pollen grain size was studied in a few instances. Pollen grains were measured from the Russian paddy T. 759 when grown in summer (April), and when grown in the south-west monsoon (August), and from the stubble flowers after harvest (October). The three sets of measurements were in agreement. But in two types, in *Oryza perennis* Moench and in Khasipichodi, environment did have a considerable effect on pollen grain size, thus showing that precautions have to be taken in comparing different plants and types. This factor in pollen grain size was not ignored, but the following procedure was adopted to give reliable data. All the observations given here are those made during the months August to October 1949, on plants growing normally in the main paddy season of the area.

The data collected by measuring pollen grains from different varieties of *Oryza sativa* are given below in a tabular form. The figures refer to the average diameters of at least 20 grains, measured to the nearest unit division of micrometer. Using the factor 10 divisions ≐ 16 microns, the table shows that the pollen grain diameters vary from 32 to 48 microns. Table I refers in turn to Indian cultivated paddies (*a*), to foreign cultivated paddies (*b*), and to naturally occurring 'Spontanea' or wild varieties of paddy (*c*). The letter T. before number refers to the type collection numbers of homozygous strains of the Paddy Breeding Station, Co. refers to selected strains distributed for general cultivation and BAM. refers to selected strains of Berhampore, Orissa.

TABLE I

Diameter of Pollen Grains in Ocular Micrometer Divisions

Type	Source	Mean	S.D.	Source	Type	Mean	S.D.
		(*a*) Indian Cultivated Paddies					
T. 386	Madura	25·3	1·1	T. 945	Punjab	28·7	1·7
T. 652	Punjab	25·5	1·4	Co. 18	Coimbatore	28·9	0·9
T. 522	Tinnevelly	25·7	3·7	BAM. 1	Berhampore	29·1	2·3
T. 920	Salem	25·8	1·3	T. 492	Tinnevelly	29·5	1·1
BAM. 3	Berhampore	27·7	1·0	Co. 13	Coimbatore	29·6	1·1
T. 499	Tinnevelly	28·4	3·6	T. 418	Coimbatore	30·0	0·7
		(*b*) In Exotic Paddies					
T. 600	West Africa	20·1	1·4	T. 980	China	24·9	1·7
T. 364	Japan	21·8	1·9	T. 724	Portuguese China	25·8	1·3
T. 1664	Italy	22·8	1·1	T. 908	Russia	26·5	2·3
T. 1450	Brazil	24·3	1·2	T. 749	Russia	27·2	3·3
T. 363	Japan	24·8	2·0	T. 758	Russia	27·8	1·1
		(*c*) 'Spontanea' Paddies					
	China I	25·4	0·9		Sativa type from Africa	27·1	0·8
	Ganjam	25·7	1·1		Russia	29·2	1·2
	China II	25·8	0·9		Malabar	29·4	1·3
	Godavary Dist.	26·0	1·1				

The table brings out the following features. The pollen grains in Indian forms are between 40 (25 × 1·6) and 48 (30 × 1·6) microns in diameter. Some of the exotic paddies have distinctly smaller grains and none reach 48 microns. In wild paddies the sizes are somewhat less than those of Indian cultivated forms. The variability as revealed by standard deviation is larger than 2 units in T. 522 and T. 499 of Tinnevelly, BAM. I of Berhampore and T. 908, T. 749 of Russia. It is possible that this variability is due to change in the normal environment of the types.

Further data were collected from pollen grains of polyploids of cultivated paddies. Two auto-tetraploids were being grown, one derived from the selected strain Co. 24, and another from the introduced slender grained Khasipichodi of Hyderabad. Of these two, tetraploid Co. 24 has been grown for five generations without reversion to diploidy and shows about 50% sterility in the ear. The tetraploid Khasipichodi is in the second generation and shows a high degree of sterility. In addition, an auto-triploid Co. 4 was studied. This triploid is completely sterile and is being maintained by vegetative propagation. The pollen grains are abundantly formed in all these types and the mean and standard deviations calculated from 100 grains of each is given in Table IV. This table shows that polypoids have markedly larger pollen grains than the corresponding diploids. In the diploid Khasipichodi the pollen grains are small and variable, while in its auto-tetraploid the size increase is accompanied by reduced variability as shown by its standard deviation. In triploid Co. 4 the variability is greater than in the diploid. To illustrate these features, two graphs are given (Figs. 1 and 2) of the 'Frequency Distribution Curve', of the sampled

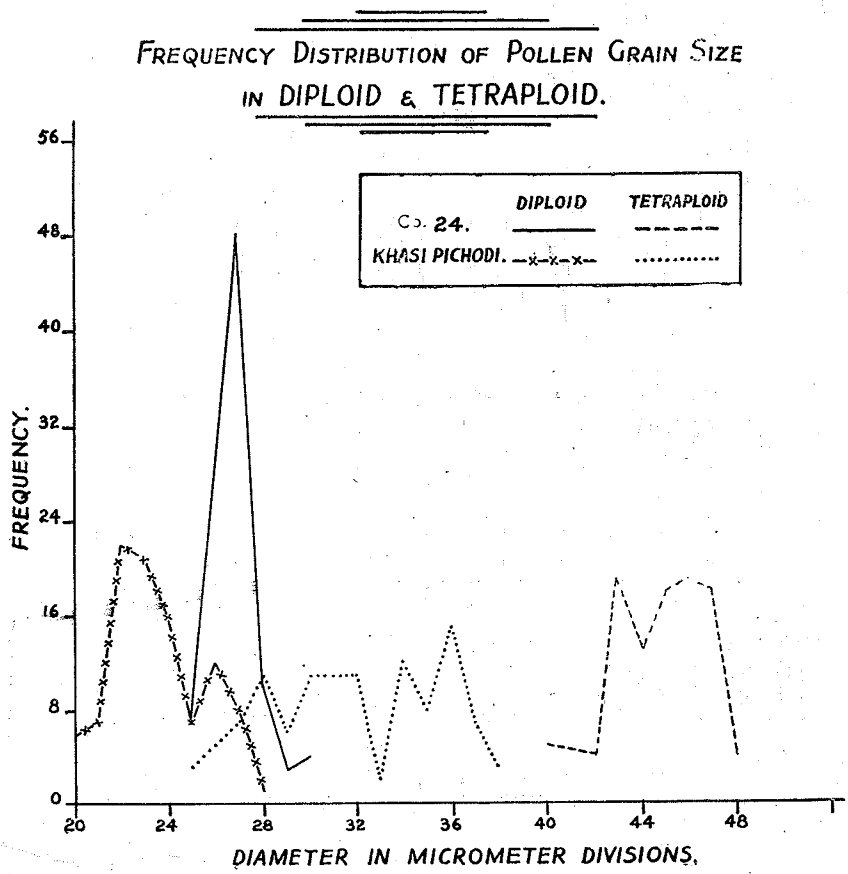

FIG. 1

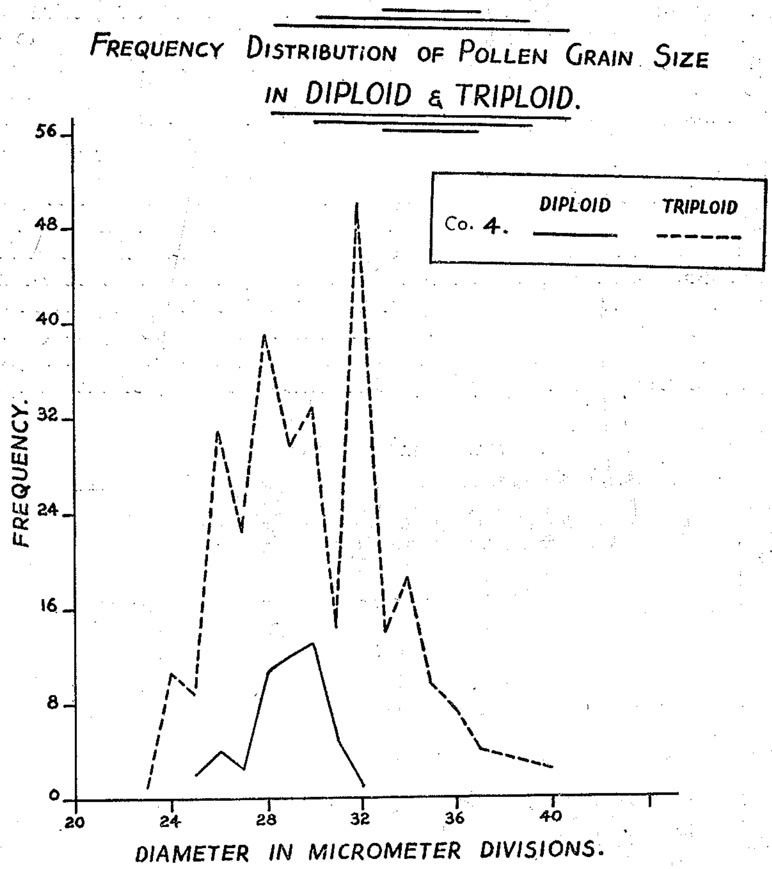

FIG. 2

population. Curves are used instead of histograms suited to the topic, in order to compare the diploids with polyploids.

The graphs bring out details in size and variability. In two polyploid plants some pollen grains can be found which are equalled in size by those of corresponding diploids. However if a sample of 50 grains are taken, the average size will show whether the plant being examined is a diploid or not. For such determination it is assumed that the sizes of pollen grains in the corresponding diploid are known.

Another set of observations were made on other species of *Oryza*, namely *Oryza perennis* Moench (Syn. *O. longistaminata*, *O. barthii*), *O. latifolia* Desv., *O. eichengeri* Peter., *O. minuta* Presl., *O. australiensis* Domin., *O. stapfii* Roschev., *O. officinalis* Wall., and *O. glaberrima* Steud. Of all these species, only varieties of *O. glaberrima* are cultivated (in West Africa) and eight types are being maintained in Coimbatore. Data from all the eight are given. Of the species listed, some are diploid ($2n = 24$) and rest are tetraploid ($2n = 48$), and the measurements are presented in Table II along with *O. sativa* data for comparison.

TABLE II

Diameter of Pollen Grains in Micrometer Divisions

Type Diploids	Mean	S.D.	Type Polyploids	Mean	S.D.
	(*a*) Cultivated *O. sativa*				
Co. 24	26·9	3·2	Co. 24 Tetra	44·8	6·5
Khasipichodi	23·5	7·7	K. Pichodi Tetra	31·9	3·5
Co. 4	28·8	4·4	Co. 4 Triploid	31·7	..
	(*b*) *Oryza* wild species				
O. officinalis	20·6	1·1	*O. minuta*	19·8	1·7
O. perennis	26·3	1·8	*O. eichengeri*	20·8	1·1
O. australiensis	26·7	1·2	*O. latifolia*	24·7	0·7
O. stapfii	29·3	1·1	*O. coarctata*	26·3	2·6
O. glaberrima :					
Ogl.104	25·2	0·8			
Ogl. 8	25·5	1·6			
Ogl. 3	26·3	2·6			
T. 732	26·6	1·2			
T. 845	27·1	1·0			
Ogl. 5	27·1	2·0			
T. 746	30·1	1·6			
T. 868	31·5	1·9			

Table II (*b*) shows that in all the tetraploids except *O. coarctata* the pollen grain size is smaller than in cultivated paddies. Pollen grains from cultivated *O. glaberrima* types alone are similar to those of *O. sativa*. The species *O. perennis*, *O. australiensis*, *O. stapfii* and *O. coarctata* have large seeds, approaching or equalling those of *O. sativa*. It is seen that pollen grains of these large seeded species exceed 40 microns and indicate that there may be a correlation between seed size and pollen size. The spikelets of *O. minuta* are the smallest amongst the species and the pollen grains are also the smallest in the types measured.

Certain other observations were also made in this study. The pollen grain of Khasipichodi appeared to show variation in size with the season of growth. The pollen grains of *O. perennis* are somewhat oval in shape, unlike any other *Oryza* type. In this *Oryza* the environment also appears to have marked effect on pollen grain size. This *Oryza* shows sterility when grown in pots. Field cultures are being raised. In *O. minuta*, 50% of the spikelets fail to set seed and there is a good percentage of aborted grains in the pollen. In the tetraploid Co. 24 there is 50% sterility in the panicle, but aborted pollen grains were between 10 and 15%.

Camera lucida drawings of ten selected pollen grains of *Oryza* are given in Fig. 3. It may be seen that the pollen walls are not appreciably thickened in the larger grains. A different situation is recorded by Newcomer (1941) in *Cosmos*, where the increase in pollen grain size in the tetraploid was in the cell walls alone.

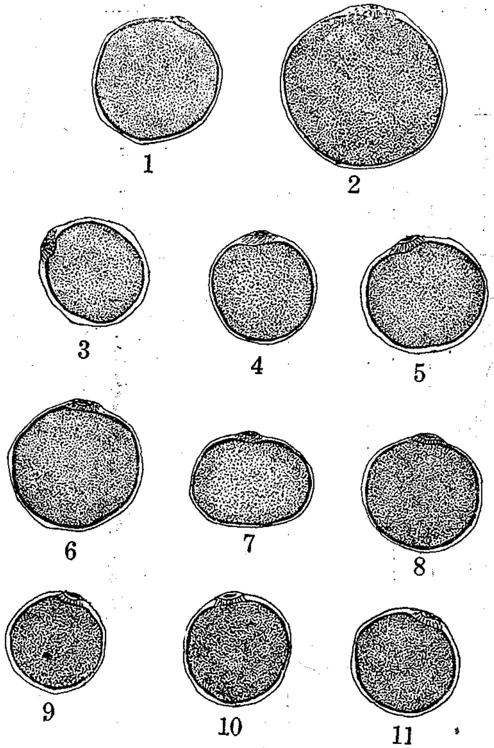

FIG. 3. Camera lucida drawings of pollen grains of different types of *Oryza*. 1. Co. 24 Diploid; 2. Co. 24 Tetraploid; 3. *Oryza anstraliensis*; 4. *O. officinalis*; 5. *O. stapfii*; 6. *O. glaberrima*; 7. *O. perennis*; 8. *O. sativa* var. '*spontanea*' from Andhra Circars; 9. *O. latifolia*; 10. *O. eichengeri*; and 11. *O. minuta*.

DISCUSSION

The main object in this study was to measure the increase in pollen grain diameter consequent on auto-polyploidy. From the data in Table II the conclusions are obvious. In spite of inherent fluctuations in size within a strain, a tetraploid can be identified by comparing its pollen with the diploid. In Co. 24 the average diameter is 27 divisions in diploid and 45 divisions in the tetraploid. Treating the pollen grain as a simple sphere, it can be shown that the volume change is in the ratio 27^3 to 45^3, that is the cell volume has increased 4 to 5 times in the tetraploid plant. This amount of increase is large, compared to the changes in the genus *Poa* as given by Muntzing (1940). In *Poa* *alpina*, Muntzing records that the pollen grains of 22 to 26 chromosomed plants and 42 to 46 chromosomed plants have the volume ratio of 1 : 1·7. In *Cucumis sativus* Shifriss (1942) has compared the diploid with the auto-tetraploid. The pollen grain volume ratio betwen the two types is 1 : 2·4, if the calculation is from unselected pollen and 1 : 2·9, if it is calculated from 'balanced' grains of diploid pollen. Comparison is not possible with the allo-tetraploid *Oryza sativa* studied by Morinaga and Fukushima (1937), because the authors do not give the size of pollen from diploid plants. In their tetraploid No. 4 plant the mean size of pollen was 51 microns, which is considerably below the 72 microns of tetraploid Co. 24 pollen. The reason for such difference is not clear. In Khasipichodi the proportion between pollen grains of diploid and tetraploid plants is as 24^3 to 32^3, that is 1 : 2·37. Even this constitutes a marked enlargement.

In the strain Khasipichodi, some pollen grains from the tetraploid plants are of the same size as some from the diploid plant. The reason for this may be as follows. The pollen grains produced by a diploid plant are haploid and have 12 chromosomes. Correspondingly a tetraploid produces diploid pollen grains. In Khasipichodi tetraploid which is less stable than tetraploid Co. 24, the meiosis may give rise to haploid gametes, diploid gametes as well as aneuploid ones by irregular segregation. The inference is being tested by growing selfed progeny. The plants available were too few for meiotic studies.

A similar production of haploid, diploid and aneuploid gametes is inferred in the tripolid Co. 4. The complete sterility of this form shows that irregular segregation must be occurring as in other triploids. Occurrence of pollen grains with different chromosome numbers caused by irregularity in meiosis may account for the nature of the 'distribution curve' in Fig. 2, where a few of the grains from triploid are smaller than the normal and many of them are of the same size as normal haploid grains. This inference is being verified by using pollen from this plant for hybridising normal paddy.

In interpreting the distribution curves, it has been borne in mind that using different units of measurement and more data, the shape of the curve would be altered. The trend, as well as the variability in size, are the features which have been used in inference.

The factors influencing pollen grain size are many, *e.g.*, environmental, genetic and chromosomal. A complete interpretation of the interactions is at present impossible. As regards environmental factors, the observation is that some strains are more responsive than others, and this agrees with the detailed observations of Jones and Newell (*loc. cit.*). The procedure adopted, namely, to measure pollen grains from types grown in the same season, under similar conditions, reduces environmentally caused variation to a minimum. As regards the chromosomal factors which had been discussed hitherto, the principle used is that the increase of chromosome number increases the nuclear volume, and that there is a definite proportion between nuclear volume and cytoplasmic volume in the pollen cells. The validity of the principle is greatly restricted. The diploid pollen grains of *Oryza minuta*

(Table II *b*) are smaller than the smallest haploid grain of *O. sativa*. Therefore genetic factors cannot be ignored and valid comparisons are between a diploid and its auto-polyploid. To analyse the genetic factors, the hybrids and progenies between cultivated paddies are being studied and results will be published in due course.

The smallness of pollen grains of wild *Oryza*, as compared to *Oryza sativa* varieties is significant. This observation suggests the line of inquiry whether the small size of pollen grain in T. 364 of Japan, and T. 1664 of Italy is correlated with reduced stomatal size and indirectly with physiological characters.

SUMMARY

Pollen grain diameters were measured in *Oryza* species and varieties. The pollen grains of cultivated species of *Oryza sativa* and *O. glaberrima* are larger than those of wild species. In the cultivated types the pollen grain diameters range from 40 to 48 microns. In the wild species the range is from 32 to 48 microns.

Three auto-polyploid types of *Oryza sativa* were compared with their corresponding diploids. The increase in volume consequent on polyploidy is more than double when average diameters are considered.

The pollen grains of polyploid species of *Oryza*, *Oryza minuta* and *O. eichengeri* are even smaller than diploid varieties of *O. sativa*. The significance of this variability in the size of pollen grains is discussed.

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