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MEGASPOROGENESIS IN ORYZA SATIVA L. AND RHYNCHORYZA SUBULATA (NEES) BAILL. INDICATING SOME TAXONOMIC SIGNIFICANCE

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SUMMARY

The megasporogenesis and structure of the embryo sac in *Oryza sativa* L. and *Rhynchoryza subulata* (Nees)Baill. (syn. *Oryza subulata* Nees.) were studied. The embryo sac is of the 8 nucleate *Polygonum* type in both species. The main differences between the two species were in the ratio of length to width of the egg cells and in the distances between egg cells, polar cells and antipodals. *R. subulata* had a smaller, wider egg cell and greater distance between the mega structures. The importance of these differences in classification and for fertility are discussed.

1. INTRODUCTION

Classification of species in the genus *Oryza* has posed a considerable problem for the taxonomist, due to wide variations within the genus. Early classifications recognised 19 (ROSCHEVICZ 1931), 22 (CHEVALIER 1932) or 23 species (CHATTERJEE 1948) while the revisions by TATEOKA (1962, 1963) and SAMPATH (1962) proposed 22 species. In these various classifications, species were separated on the basis of cytogenetical differences, chromosome numbers, shape of spikelets, structure of lemma and palea, genetic differences like purple and green pigmentation on the plant body and geographical distribution.

At a Symposium on Rice Genetics and Cytogenetics held at The International Rice Research Institute in 1963, a committee on taxonomy recommended that 19 distinct and valid species be recognized (CHANG 1964). This committee also recommended that the wild species *O. subulata* should be transferred to the genus *Rhynchoryza*. This recommendation was based on gross differences in spikelet morphology and other genetic features. MORISHIMA & OKA (1960) had previously suggested that *O. subulata* was quite separate from other *Oryza* species, based on their statistical analysis of a range of morphological, physiological and quantitative characters for 16 *Oryza* species. *O. subulata* is now recognized as *R. subulata* (Nees) Baill.

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Because of the special position occupied by *R. subulata* and our lack of knowledge of megasporogenesis in *Oryza* generally, the present project was conceived. The aim of this project was to compare the megasporogenesis of *O. sativa* – the type species of this genus and *R. subulata* to ascertain any characteristics that may be of value in classification.

2. MATERIALS AND METHODS

The two species *O. sativa* and *R. subulata* were grown in pots in a glass house in dark-red soil. One month after planting, 5 g of urea was sprinkled on each pot and reapplied each month until flowering. Spikelets of the two species, at various stages of development from early short blade, late short blade, panicle tip emergence and panicle fully emerged stages were fixed in a mixture of formalin + acetic acid and ethanol (1:1:18) for 24 hours, then transferred to 70% ethanol for storage. A pinch of eosin was introduced in the 70% alcohol to make the ovaries conspicuous in the wax. For paraffin sections, the ovaries were dissected out and dehydrated in an ethanol series, then transferred to an ethanol + chloroform mixture and embedded in 54 °C m.p. paraffin wax. Longitudinal sections were cut at 8–10 μm on a rotary microtome and stained with 0.5% of Heidenhain's iron-haematoxylin and mounted in Euparol. Sections were then examined under a microscope.

For a quantitative study of the mature embryo sacs, 10 plants were selected at random from each species at the panicle fully emerged stage (flowers unopened). After fixation and processing measurements were made, using a calibrated ocular micrometer. The characters measured were length and width of i) embryo sacs ii) egg cells iii) polar cells iv) antipodal cells v) distance between inner integument and egg cell at the micropylar end vi) distance between egg cell and polar cell and vii) distance between polar cell and antipodal. All the measurements were made at a magnification of × 440.

3. RESULTS

3.1. Embryo sac development

Both species have several features in common.

The ovules in the two species are of the anatropous type. The micropyles are formed by both inner and outer integuments. The integumentary cells come in such intimate contact with each other that the micropylar canals are extremely narrow and imperceptible. As the embryo sac matures, the nucellar cells are gradually used up. The archesporial cell originates below the epidermis which becomes more conspicuous than the others owing to its larger size, dense cytoplasm and more prominent nucleus. This functions directly as the megaspore mother cell which undergoes two successive transverse meiotic divisions to form a linear tetrad of four megaspore cells. In spite of the enlarge-

ment of all the megaspores of the tetrad, the distal three degenerate and the proximal one becomes functional (*fig. 1 A*). The functional one enlarges further and the nucleus divides and gives rise to a two- nucleate embryo sac (*fig. 1 B*): the subsequent divisions form four- (*fig. 1 C*) and then eight- nucleate embryo

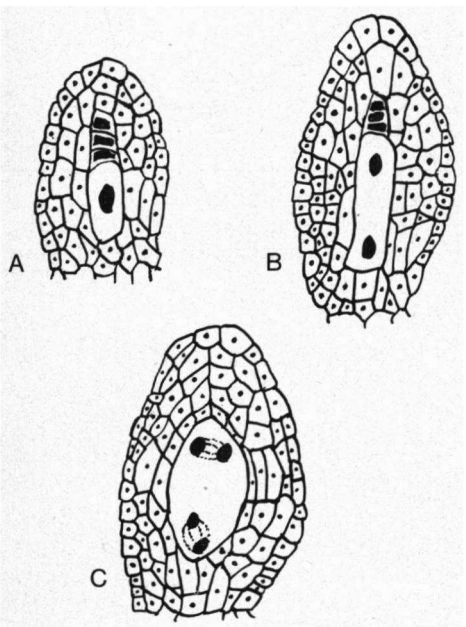

Fig. 1. Longitudinal sections of ovules, × 440.
A. Tetrad of megaspores with the proximal cell functioning.
B and C. Two-nucleate and four-nucleate embryo sacs.
A, B and C represent early short blade stage, late short blade stage and panicle tip emergence stage respectively.

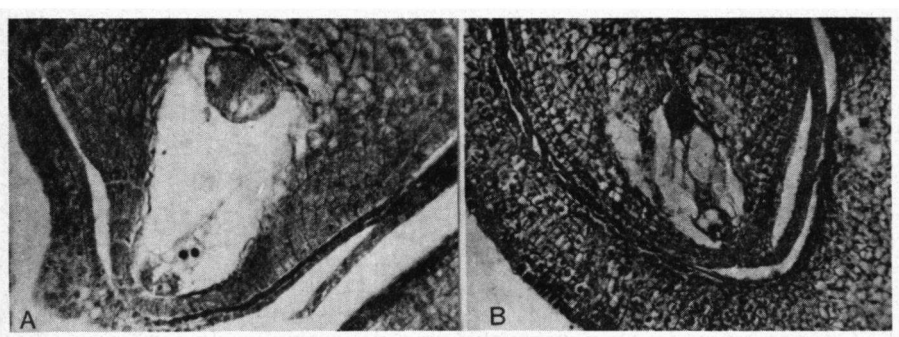

Fig. 2. Photomicrographs of L.S. of embryo sacs, × 440.
A. *O. sativa*, and B. *R. subulata*. Identification of parts can be made from the line drawings of *fig. 3*. This figure represents the panicle fully emerged stage (flowers unopened).

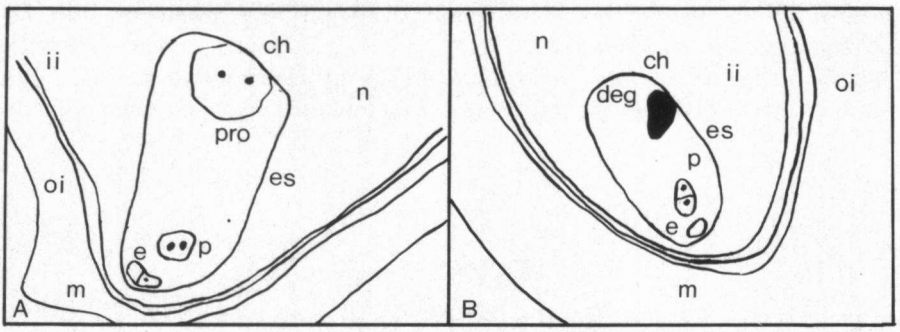

Fig. 3. Outline drawings of the L.S. of *fig. 2*. A. *O. sativa*, B. *R. subulata*.
ch. chalazal end; deg. degenerated antipodals; e. egg cell; es. embryo sac; ii. inner integument; m. micropylar end; n. nucellus cells; oi. outer integument; p. polar nuclei; pro. proliferation of antipodals.

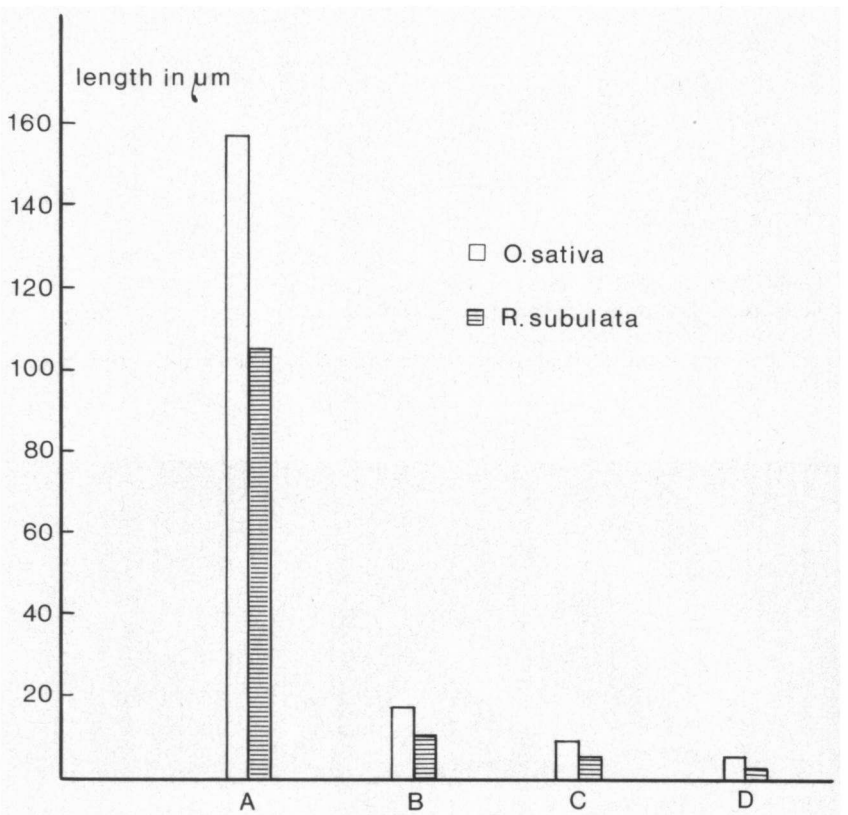

Fig. 4. Histogram showing the variations in the components of embryo sacs of *O. sativa* and *R. subulata*. A. embryo sac; B. polar cell; C. egg cell; D. antipodal.

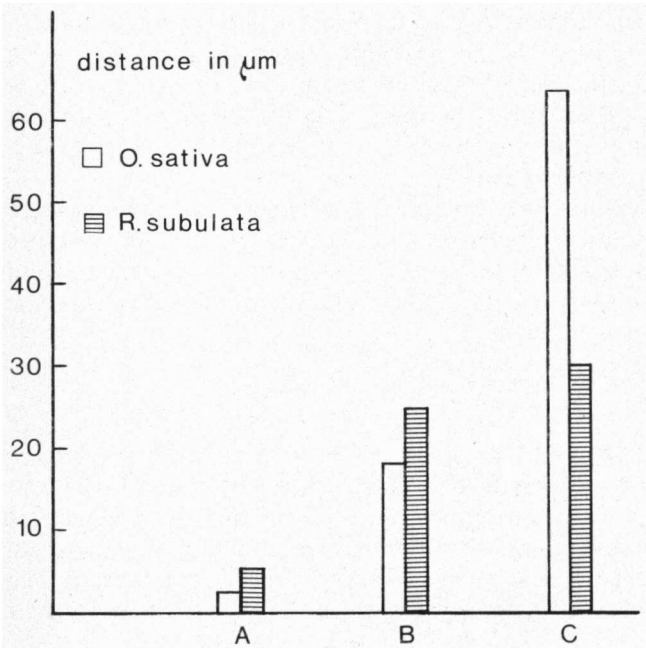

Fig. 5. Variation in the distance between mega structures. A. inner integument and egg cell; B. egg cell and polar cell; C. polar cell and antipodals.

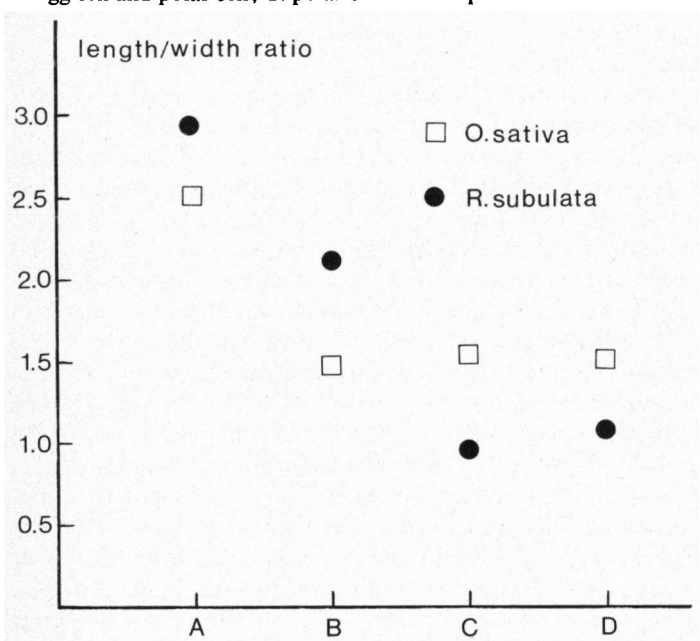

Fig. 6. Length/width ratio for the characters. A. embryo sac; B. polar cell; C. egg cell; D. antipodals.

sacs. Embryo sacs at maturity have 8 nuclei comprising 2 synergids, an egg, 2 polar nuclei and 3 antipodals. The synergids degenerate after fertilization and sometimes remnants of one of them persist up to a few divisions of the endosperm. *Figs. 2* and *3* show details of embryo sacs in the two species studied.

3.2. Quantitative comparisons

For the characters measured the two species have significant differences ($P < 0.05$) in length and width of embryo sacs, polar cells, antipodal cells, distance between inner integument and egg cell, egg cell and polar cell and polar cell and antipodal. The only non-significant difference is width of the egg cells. These results are shown in *Figs. 4, 5* and *6*.

4. DISCUSSION

In the present investigation with the cultivated species *O. sativa* and the wild species *R. subulata*, it was noticed that the structure and development of matured embryo sacs were more or less similar. The structure of embryo sac conformed to that of the typical *Polygonum* type found in Gramineae. The embryo sac showed presence of the eight nucleate, 7 or 8 celled type that is characteristic of all sexually reproducing (reduced by meiosis) grasses of Pooideae and Panicoideae as detailed by BROWN & EMERY (1957, 1958). As far as the developmental studies are concerned, there was no fundamental divergence or variation in the basic structures between the two species.

However, quantitative measurements revealed some differences which are possibly of taxonomic significance. Differences in dimensions of the egg cells of *O. sativa* and *R. subulata* are due to the egg cell of *O. sativa* being longer with rich cytoplasm but small and wider in *R. subulata*. The bigger size of embryo sac and in the mega structures may be a consequence of man's selection for larger grains in the cultivated species *O. sativa* (*fig. 4*).

For effective fertilization and development of the pro-embryo, it is vital that the egg and polar cells be as close as possible. The greater distance in *R. subulata* (*fig. 5*) may explain the occurrence of some sterility and lower number of grains formed in that species compared with *O. sativa*. On the contrary, the distance between polar cell and antipodals gave the reverse picture in both the species. In *O. sativa*, the antipodals are unlikely to be fertilized by a pollen tube as the pollen tube will invariably collapse before reaching them due to the long distance. But, in *R. subulata* there is equal chance for fertilization of egg, polar cell and antipodals as they are almost equidistanced (*fig. 5*). This shows that *R. subulata* is more primitive in nature. For natural adaptation either the egg or antipodal cell should be fertilized as both are haploid nuclei and either one can function like an egg and this gives more survival value under selection pressure. SHATTUCK (1905), DERSCHAU (1918) and EKDAHL (1941) have observed fertilization of antipodal cells in some of the crop plants.

The length/width ratios (*fig. 6*) for the four characters reveal that the two

species are quite distinct and could be divided into 2 separate natural groups. TATEOKA & PANCHO (1963) have found that the ratio of length to width is an important character in classifying the species of *Oryza*. In *O. sativa* there is an equal balance ratio between the polar cell, egg cell and antipodals whereas in *R. subulata*, there is a disproportionate balance ratio between the 3 structures. The balance between the 3 is more important for natural perpetuation of a species. When this natural balance ratio is not proportionate, it affects the gene dosage of the developing embryo resulting in genic sterility. MÜNTZING (1933) has stated that alteration in the balance between embryo and endosperm ratio might be of significance for sterility and poor fertility.

The species *R. subulata* occurred as a wild form in south America carrying a diploid chromosome complement of 24 (HOROWITZ & POGLIAGA 1934) and remained distinct from all other diploid and tetraploid species of the genus *Oryza*. This enabled some research workers to set up a separate section *Rhynchoryza* with a single species (ROSCHEVICZ 1931) and later assigning generic status to *Rhynchoryza* (CHANG 1964) so that it can constitute an allied genus of rice like *Hygroryza*, *Leersia* and *Potamophila*. The negative association in morphological features of *R. subulata* from the rest of the species of *Oryza* might be primarily out of adaptation and divergence in the morphogenetic characters and this is in accordance with the view of DOBZHANSKY (1951) in pattern of evolution. The taxonomic differences shown by the species *R. subulata* can be attributed to genetic segregations, natural mutations due to selection pressure and diversities in phytogeographical conditions. A similar view was expressed by TSCHETWERIKOFF (1926, 1927) that a genetic analysis of natural populations would reveal the concealed (mutations), or potential variability.

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