Cytological analysis for meiotic patterns in wild rice (*Oryza rufipogon* Griff.)

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\textbf{ABSTRACT}

The present report explores the chromosomal patterns during meiosis as a fundamental cell division study in wild rice (*Oryza rufipogon* Griff.). Cytological assays revealed normal meiosis in most cases but in some instances meiotic abnormalities such as weak desynapsis, univalent and quadrivalent formation, translocation, spindle abnormalities and precocious movement of chromosomes were noticed. Interestingly, this wild species also has the bi-nucleoli in first meiotic stages alike the cultivated species of *Oryza* (*O. sativa*). The present investigation emphatically addresses the questions of high adaptability of wild rice supported by high pollen fertility for their potential to strong fitness in nature.

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1. Introduction

*Oryza rufipogon* was often referred to as *O. perennis*, *O. perennis* var. *balunga*, *O. perennis* sub-species *balunga* or as *O. balunga* in different literatures. The species was conclusively identified as *O. rufipogon* [1,8,10]. A number of other synonyms of *rufipogon* such as *formosana*, *paraguayensis* and *cubensis* were also used in several literatures. *O. rufipogon* is a diploid, perennial, aquatic plant having adaptable habit, and erect and lax panicles that stand narrow, oblique, and beaked spikelets with awns. The anthers are around two-third or longer as spikelets [4,11]. The perennial *rufipogon* grows in swamps, channels, marshes, by the boundaries of ponds and lakes with a depth of 1 m of water. Since, the soil in such places often remains moist even throughout summer months, the plants thrive as clumps and rejuvenate in full growth in rainy seasons. *O. rufipogon* is a runner, creeping on the ground and rooting at internodes. The species thus propagates by vegetative means year after year in marshes and low lands [7]. Branches of the culm often (but not always) emerge from nodes piercing the leaf sheath. Leaves are almost at right angle to the main culm. If the culm gets inundated, it remains suspended in the water but the panicles rise above the water level. Panicles are well exerted and branches of the panicle are spreading. Spikelets are long with red awns (5.5–10.6 mm) and apiculus is often pigmented. Anthers are longer than 3 mm and fill the spikelet completely. Spikelets turn black on maturity, shatter and drop off into water. Kernel is red in colour [11].

The present work was undertaken to study the meiosis of *O. rufipogon* (Griffith) collected from *Maricha Bil* (local lake) near Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal, India where it is grown naturally for periods showing great adaptability to grow in that area [6]. Although this species is capable of reproducing vegetatively, we noticed it sets plentiful seeds (but cannot be considered as good yield) and the farmers collect the seeds (*local name uru dhana*) and consume. The reason behind the adaptability of this particular rice species, which exist in that particular area thriving someway during summer but profusely emerged out in monsoon under waterlogged condition, could be interesting to explore. Cytological investigation of such wild rice is important since it may help to explore whether there is any structural heterozygosity or not in connection to its adaptive value. Such study will enhance our knowledge on the nature of speciation and evolutionary aspect of the genus *Oryza* including the feasibility of interspecific gene transfer from wild to cultivated species.

2. Materials and methods

*O. rufipogon* is photosensitive in nature and flowers during short days during the month of November and December in its natural habitat. The spikelets were collected from *Maricha Bil* (local lake), beside Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India. There, it was growing in almost 100 of acre of

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lowlands during rainy season when the land became fallow due to 1–1.5 m waterlogging. The spikelets were collected when the juncture of the flag leaf is about 1 cm out of the boot. Spikelets of proper stage (where all the meiotic stages are happening) were fixed in farmers fluid fixative i.e. acetic alcohol (3:1:ethyalcohol: glacial acetic acid). Traces (10 mg/100 ml) of ferric chloride were added to the fixative to improve the staining of chromosomes. Fixation was done in between 7 and 10 O’clock in the morning. After maintaining the materials in the fixative for 24 h, they were transferred into 75% (v/v) alcohol and stored until further use. Anthers, removed from the spikelet of proper stage, were transferred to a drop of 1% acetocarmine on a slide and covered with cover glass. The slide was warmed gently and then smeared with cover glass by a needle tip. Based on requirement, a few drops of 45% (v/v) acetic acid were added to the side of the cover glass to reduce the staining of cytoplasm. The additional stain was soaked by filter paper. Initial cytological analysis was done using temporary slides under compound microscope. The preferred and selected slides were sealed with paraffin and left for 48 h for development of stain. Later, paraffin was removed and wiped with xylene. Temporary slides were prepared permanent by normal butyl alcohol method. The slides were inverted in 1:1 mixture of glacial acetic acid and butanol solution. After detachment of cover glasses from the slide, they were transferred into butanol solution. Then, the slides and cover glasses were taken out from the butanol solution and were mounted separately with a synthetic resin (DPX) by using fresh cover glass and a fresh slide respectively. A microscope with photomicrographic attachment was used for capturing photomicrographs of desired cells from permanent slides at a magnification of 1000× using oil immersion lens. The negatives were suitably enlarged at the time of printing to observe and analyze the identified meiotic stages.

3. Results and discussion

Cytological observations of the microsporocytes in O. rufipogon (Griff.) were made from pachytene to pollen formation stage and most of the cells showed normal meiosis. A total of 1777 cells of pachytene stage were analyzed. In most of the cells, chromosomes showed normal and complete pairing except in some cells where small-unpaired segment was observed (Fig. 1a). This unpaired segment might be due to structural differences in chromosome homology. This desynaptic behaviour may vary based on the duration in different plants, probably due to several reasons both intrinsic and extrinsic. According to Praaken [5], desynapsis might occur due to (1) gene action, (2) loss of chromosome-pair, (3) apomixis and (4) structural or numeral changes of chromosome. Further, he distinguished desynapsis in three types – (i) weak desynapsis – few univalents in some cell, (ii) medium strong desynapsis – many univalents in most cells, (iii) complete desynapsis – most of the cells reveal the presence of univalents with some rare bivalents. In the presents study, only weak desynapsis was observed and the univalents might have originated due to early disjunction of the bivalents and/or due to lack of pairing. Translocation was present in some of the cells as substantiated by cross (X) configuration at pachytene (Fig. 1b). This was further verified by the presence of chain of four configurations at diakinesis and metaphase-I. The nucleolus was

Fig. 1. a Pachytene stage showing two nucleoli and arrow indicates unpaired segment, b: Pachytene stage showing one nucleolus and arrow indicates translocation, c: Diakinesis stage; arrow indicates quadrivalent, d: Diakinesis stage; arrows indicate univalent, e: Metaphase-I: two chromosomes (indicated by arrows) having precocious movement.
conspicuous at pachytene stage. There were one to two nucleoli that might or might not be attached to specific regions of pachytene bivalents. Among the 1777 studied cells, 424 (23.86%) cells had two nucleoli of different size. The rest cells had single nucleolus. In two cells, duplication loops were observed. Data on number of different types of nucleoli have been presented in the Table 1. Shastry et al. [9] for the first time reported supernumerary nucleoli in O. sativa at pachytene. Supernumerary nucleoli appear constantly at pachytene in all the species of Oryza with the exception of O. australiensis and O. meyeriana [3, 4]. Considering the morphological similarity of supernumerary and major nucleoli, it is possible to predict that species, which are evolutionary, advanced and consequently subject to a greater degree of chromosomes structural changes might exhibit supernumerary nucleoli more frequently.

At diakinesis, out of 76 cells studied, different association of univalents and quadrivalent with bivalents were observed (Table 2 and Fig. 1c and d). At metaphase I, out of the 157 cells analyzed, 152 cells showed twelve bivalents and only five cells showed ten bivalents along with one quadrivalent. Darlington [2] reported a number of meiotic abnormalities, which are controlled genetically. One of these abnormalities, widely designated as desynapsis, is characterized by regular pairing at pachytene followed by the formation of univalents at later stages. In our opinion, the desynaptic nature of homologous chromosomes might prevent recombination and as a consequence the species somehow kept its intrinsic characters, one of which is persistence i.e., without any human intervention this particular rufipogon rice species of that particular region (Maricha bil) grows year after year. The same area experiences regular sativa rice cultivation in the boro season by the local farmers of Maricha bil, but astonishingly, in the kharif when that land becomes uncultivable, only rufipogon wins the race of resurrection, which is nothing but the character of persistency and the sativa genome probably does not carry it. Though rufipogon can grow vegetatively, we noticed no single plant of this species during summer; the monsoon somehow influences genes in its living parts (fertile seeds, roots or stems) buried under the soil during summer, since in each year we notice luxurious greenery of O. rufipogon covering the whole Maricha bil which has already become a lake in the monsoon.

A sum of 158 cells of anaphase-I was analyzed and, all the cells were fully separated with two groups of chromosomes at poles; in most of the cells, 12 chromosomes per pole. Interestingly, very high pollen fertility (93.69%) was observed in the present study. Reciprocal translocation usually leads to a reduction in fertility; however, the high fertility revealed in our study is presumably due to alternate segregation. High pollen fertility of this species of wild rice indicated that metaphase-I orientations of chromosomes involved in translocation were mostly of alternate type, yet, would not be identified in the present study due to smallness of the chromosomes. Precocious movement or early movement of one or two chromosomes towards pole at metaphase-I might be due to spindle abnormalities (Fig. 1e). Nevertheless, it was not confirmed whether those few chromosomes with precocious movement were actually the univalent(s) from the homologues involved in desynapsis during the previous phase (zygotene).

### 4. Conclusion

It may be comprehended from the present study that, the desynaptic behaviour can be genetically controlled that involves non-homologous chromosome and other structural re-arrangement may be due to environmental factors like temperature, humidity and soil conditions which make this wild rice (O. rufipogon Griff.) adaptive and evolutionary advantageous. Such fundamental research will help to understand meiotic cell division behavior for such a greater fitness. Crossing of this wild species with cultivated species and analysis of F1 hybrid will help to understand the extent of homology between wild species and cultivated species. As O. rufipogon is highly resistant to different

### Table 1

| Total number of cells studied | Frequency of cells having different types of nucleoli |
|------------------------------|--------------------------------------------------------|
|                              | One nucleolus | Two nucleoli with almost equal size | One large and one small nucleolus |
| 156                          | 150          | 6                             | 0                             |
| 258                          | 236          | 39                            | 3                             |
| 252                          | 210          | 36                            | 6                             |
| 242                          | 204          | 37                            | 1                             |
| 176                          | 88           | 71                            | 17                            |
| 107                          | 55           | 43                            | 9                             |
| 280                          | 237          | 40                            | 3                             |
| 116                          | 109          | 3                             | 4                             |
| 190                          | 64           | 103                           | 23                            |

### Table 2

| Stage                        | Number of cells observed and their percentage | Type of Association of chromosomes |
|------------------------------|-----------------------------------------------|------------------------------------|
|                              |                                               | Quadrivalent | Bivalent | Univalent |
| Diakinesis                   | (76 cells studied)                            | 1            | 10        | –          |
| 6(7.89%)                     |                                               | –            | 11        | 2          |
| 4(5.26%)                     |                                               | –            | 10        | 4          |
| 1(1.32%)                     |                                               | 2            | 8         | –          |
| 3(3.95%)                     |                                               | –            | 12        | –          |
| 62(81.58%)                   |                                               | –            | 12        | –          |
| Metaphase-I                  | (157 cells studied)                           | 152(96.82%)  | –         | –          |
| 5(3.18%)                     |                                               | –            | 12        | –          |
biotic stresses, the transfer of these resistance genes into the cultivars by hybridization will be beneficial.

**Conflict of interest**

We, the authors of this article, declare that there is no conflict of interest and we do not have any financial gain from it.

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