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## Meiotic Events in a Diploid and Polyploid *Abrus* and Possible Reasons for Fruit Abortion and Low Seed Set in *Abrus pulchellus*

I.O. Agbagwa

Department of Plant Science and Biotechnology, Faculty of Biological Sciences, University of Port Harcourt, P.M.B 5323, Port Harcourt, Rivers State, Nigeria

### ABSTRACT

Meiosis and pollen viability in two species of *Abrus* (*Abrus precatorius* and *Abrus pulchellus*) were investigated. Anthers from young flower buds collected from both species and fixed in 95% ethanol-acetic acid were dissected on slides stained with FLP-Orcein. These were examined under a Leitz Diaplan microscope together with pollen grains stained in lactophenol cotton blue. Pollen grains in both species were observed to be highly viable with viability ranging from 78.70-85.16% in *A. pulchellus* and from 97.50-99.21% in *A. precatorius*. Eleven bivalents at diakinesis, regular metaphase pairing and clear disjunction at anaphase were observed in *A. precatorius*. Meiotic observations in *A. pulchellus*, however, revealed high incidence of bivalent and multivalent configurations at diakinesis, lagging chromosomes and non-disjunction at anaphase. Notwithstanding the high pollen viability values recorded, these meiotic irregularities resulted in the low fruit and seed set observed in *A. pulchellus*.

**Key words:** *Abrus*, anaphase, chromosomes, diakinesis, meiosis, pollens

### INTRODUCTION

*Abrus* Adanson is a lesser known genus of the family Papilionaceae. Three species, *A. precatorius* Linn., *A. pulchellus* Wall. and *A. canescens* Welw. ex Bak. occur in Nigeria (Hutchinson and Dalziel, 1958). These lesser known species have been shown through scientific investigations to possess ethnomedical, ethnopharmacological and toxicological properties (El-Shabrawy *et al.*, 1987; Amer *et al.*, 1989; Reda *et al.*, 1989; Dimetry *et al.*, 1990; Sinha, 1990; Burkill, 1995). They are however, underexploited and threatened with extinction (Agbagwa *et al.*, 2007; Agbagwa and Obute, 2007). Recollection of *A. canescens* from areas of previous occurrence in Nigeria has not been possible.

Cytological studies on this genus revealed gametophytic and sporophytic chromosome numbers of 11 and 22 respectively for *A. precatorius* (Borgen, 1980; Gill and Husaini, 1986; Yeh *et al.*, 1986; Kumari and Bir, 1990; Agbagwa, 2011). Agbagwa and Okoli (2005) observed 44 chromosomes in *A. pulchellus* which is double the number previously reported for *A. precatorius* alluding to the fact that *A. pulchellus* is a natural polyploid. The karyotypes of *A. precatorius* and *A. pulchellus* which were published by Agbagwa (2011), showed that both species possess three cytomorphological groups (metacentrics, submetacentrics and acrocentrics). The number of chromosomes in each group in *A. precatorius* were, however, observed to be an exact double of that in *A. pulchellus*. Through breeding system and pollination ecology studies, Agbagwa and Obute (2007) established obligate and facultative cleistogamy in *A. precatorius* and *A. pulchellus*, respectively. They also noted that low outcrossing rate occurs in both species despite the predominant cleistogamous characteristics

in the genus. These previous studies notwithstanding, the actual behavior of the chromosomes of both species at meiosis is unknown. The present study which centers on meiosis, gives insight on the behavior of chromosomes of both species and provides empirical genetic reasons for fruit abortion and poor seed set in *A. pulchellus*.

## MATERIALS AND METHODS

Seed samples of both species were collected from the wild from different parts of southern Nigeria. The localities where the seeds were collected are Owerri, Imo State (05°29"N, 07°05" E), Onne, Rivers State (04°51"N, 07°03"E), Ibadan, Oyo State (07°23"N, 03°54"E), Kwale/Okpai, Delta State (05°42"N, 06°29"E) and Kolo Creek, Bayelsa State (04°54"N, 06°22"E). The seeds were planted out in the University of Port Harcourt Botanic Garden and monitored from germination to flowering. For each species, five different stands were maintained according to the collection locality. Stakes were provided for the plants to climb as they would under natural conditions.

Meiotic studies were made from young flower buds collected from these stands before noon and fixed immediately in 95% ethanol-acetic acid fixative (3:1 v/v) (Okoli, 1992). During examination, anthers were dissected on slides, stained with a drop of FLP-Orcein and gently squashed. Slides were examined under a Leitz Diaplan microscope and good meiotic chromosome plates photographed with Leica WILD MPS 52 microscope camera on Leitz Diaplan microscope.

Viability of pollen grains was determined by their stainability in cotton blue in lactophenol, deeply stained and round pollen grains were considered fertile. Ten randomly chosen microscopic field views at objective lens×20 were scored for the proportion of stained and unstained pollen grains for each species. Percentage viability was thereafter computed.

## RESULTS AND DISCUSSION

No previous description of the meiotic behaviour of *A. precatorius* and *A. pulchellus* is known to the author. However, Agbagwa and Okoli (2005) and Agbagwa (2011) observed and reported somatic chromosome numbers of 22 and 44 for *A. precatorius* and *A. pulchellus*, respectively. These observations imply that *A. precatorius* is diploid while *A. pulchellus* is polyploidy. Karyotype of both species as reported by Agbagwa (2011) comprises three morphological types as follows; 4 metacentrics, 4 submetacentrics and 3 acrocentrics in *A. precatorius*, 8 metacentrics, 8 submetacentrics and 6 acrocentrics in *A. pulchellus*. Observations made in this present study show that regular bivalent formation during diakinesis ( $n = 11$ ), regular metaphase pairing and disjunction at anaphase occur in *A. precatorius* as shown in Fig. 1a-d. The diakinesis observation of 11 chromosomes agrees with an earlier meiotic (gametophytic) chromosome count in *A. precatorius* (Gill and Husaini, 1986). Figure 1a shows the pachytene stage in *A. precatorius* with attraction of homologous pairs. The Nucleolar Organizer Region (NOR) is clearly visible at this stage. Figure 1b shows the diplotene stage in this same species with 11 bivalents. Figure 1c shows a diakinesis stage with 10 bivalents and one ring chromosome while Fig. 1d shows regular metaphase pairing in *A. precatorius*.

The meiotic behaviour observed in *A. pulchellus* ( $2n = 44$ ) is quite different from that of *A. precatorius* (Fig. 2a, b). There is high incidence of bivalent and multivalent configurations at diakinesis in *A. pulchellus*. Thus, lagging chromosomes, anaphase and non-disjunction bridges were observed to be characteristic with the anaphase stage. Though, the meiotic stages observed in *A. precatorius* were regular, the several aberrations observed at diakinesis and anaphase in *A. pulchellus* are linked to the different sizes of chromosomes (double the number in *A. precatorius*)

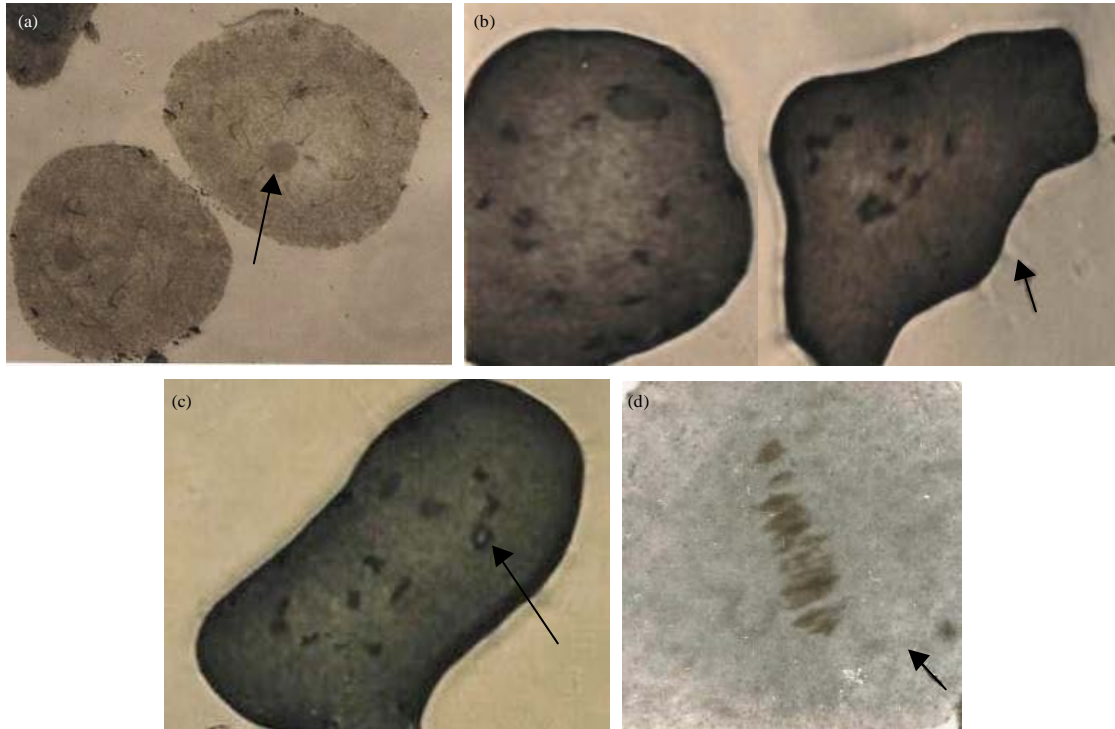


Fig. 1(a-d): Different meiotic stages in *Abrus precatorius*, (a) Pachytene stage with arrow pointing to NOR, (b) Diplotene stage, (c) Diakinesis with arrow showing ring chromosome and (d) Metaphase

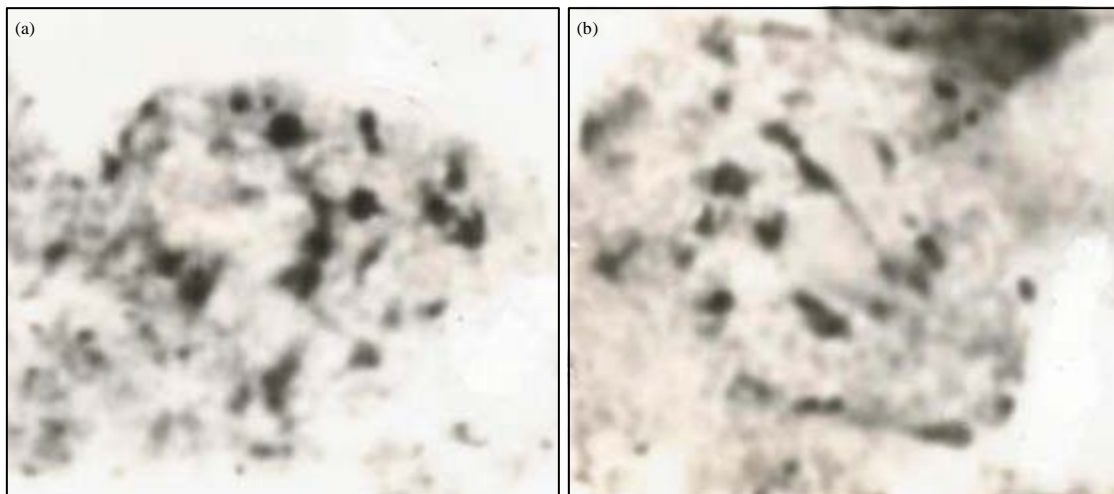


Fig. 2(a-b): Observed meiosis in *Abrus pulchellus*, (a) Multivalent configurations at diakinesis and (b) Lagging chromosomes and non-disjunction at anaphase

reported in the species (Agbagwa and Okoli, 2005; Agbagwa, 2011) which result in meiotic multivalencies. Okoli (1983) and Schulz-Schaeffer (1985) opined that multivalencies arise due to

Table 1: Pollen viability characteristics of *Abrus precatorius* and *Abrus pulchellus*

| <i>Abrus</i> species and locality | Total pollen count | No. of stained pollen | Viability (%) |
|-----------------------------------|--------------------|-----------------------|---------------|
| <b><i>A. precatorius</i></b>      |                    |                       |               |
| Owerri                            | 264                | 257                   | 97.54         |
| Onne                              | 243                | 239                   | 98.35         |
| Ibadan                            | 252                | 250                   | 99.21         |
| Kwale/Okpai                       | 302                | 297                   | 98.34         |
| Kolo Creek                        | 240                | 234                   | 97.50         |
| <b><i>A. pulchellus</i></b>       |                    |                       |               |
| Owerri                            | 283                | 241                   | 85.16         |
| Onne                              | 291                | 239                   | 82.13         |
| Ibadan                            | 254                | 204                   | 80.30         |
| Kwale/Okpai                       | 249                | 196                   | 78.70         |
| Kolo Creek                        | 297                | 244                   | 82.20         |

the presence of structural heterozygosity involving segmental interchanges. Sarbhoy (1977) observed this in *Bongamia pinnata* (L.) and implicated translocation of short segments of non-homologous chromosomes as the cause. These are similar to the meiotic observations in *A. pulchellus* and accounts for the irregularities observed in the meiosis of the species.

The several multivalent associations featuring at diakinesis and anaphase of *A. pulchellus* support its polyploid nature which was previously reported by Agbagwa and Okoli (2005). As stated, multivalent association in a tetraploid might be due to heterozygosis for translocation or segmental homology. These situations according to Okoli (1983), are expected to lead to high sterility or loss of fertility. Notwithstanding the occurrence of these abnormalities in pairing at diakinesis and anaphase, the pollen grains of *A. pulchellus* in present study showed high viability ranging from 78.70-85.16% (Table 1). The percentage viability in *A. precatorius* (97.50-99.21%) is expectedly higher. Pollen viability is generally considered to indicate the ability of the pollen grain to perform its function of delivering the sperm cells to the embryo sac following compatible pollination (Shivanna *et al.*, 1991). It is, therefore, noteworthy that there exist some innate genetic recovery mechanisms in *A. pulchellus* which counter the effect of the meiotic abnormalities. In spite of this high pollen viability, low seed set and fruit abortion were reported to be common in *A. pulchellus* (Agbagwa and Obute, 2007). This implies that the diakinesis and anaphase irregularities in *A. pulchellus* manifest at the seed and fruit stages.

## CONCLUSION

It is plausible to conclude that there are some micro-environmental differences (including variation in soil types and even pollinators) between the original seed localities and the experimental site (University of Port Harcourt Botanic Garden) where the seeds were finally planted. These though allow the normal high pollen fertility, affects seed and fruit set.

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