

**Apogamous Isoetes coromandelina L.f. (Isoetaceae) with asynaptic meiosis**

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**Abstract.** This cytological study on *Isoetes coromandelina* L.f (Isoetaceae) from Tirunelveli District, Tamil Nadu, South India shows the presence of twenty two individual chromosomes along with a fragment (n=2n=22+1) during meiosis in megaspore mother cells without the presence of any bivalent. Cytomixis between two megaspores is very common, resulting in the formation of a high percentage of abortive spores. The present material is of an apogamous taxon with asynaptic Meiosis as reported by Abraham and Ninan from Kerala and Karnataka.

**Keywords:** Isoetes coromandelina, asynaptic meiosis, apogamy.

**INTRODUCTION**

Lycophytes and Pteridophytes form the dominant vegetation on the earth next to flowering plants. Fern allies and ferns are a monophyletic group and the closest living relatives to seed plants (Pryer *et al.*, 2001). This group of plants, with homosporous clubmosses, heterosporous spikemosses and quillworts, is the ancestor for Lycopodiopsida and Monilophytes Smith *et al.* (2006). Quillworts are considered by some to be the last remnant of the fossil tree lycopsid with which they share some unusual features including the development of wood and bark, a modified shoot system acting as roots, bipolar growth, and an upright stance (Retallack, 1997). *Isoetes*, one of the extant members of Lycopodiopsida, is a heterosporous fern. They are small aquatic herbs and they are commonly called quillworts or Merlin grass. Many aquatic plant populations including quillworts are under severe threat in the Western Ghats, particularly in Kerala and Maharashtra. For example, the endangered *Isoetes panchganiensis* has been reported from temporary ponds and pools on the high altitude plateaus of Panchgani table lands in Maharashtra and Kemmangundi Hills in Karnataka (Molur *et al.*, 2011). Some aquatic species, often found in mixed populations containing taxa of different ploidy, appear to have evolved abruptly via interspecific hybridiza-
tion and chromosome doubling. Evidence from distribution patterns, megaspore morphology and viability, chromosome numbers, and electrophoretic profiles of leaf enzymes supports a hypothesis of allopolyploid speciation (Taylor & Hickey, 1992).

*Isoetes* is not only a very complicated genus but also a remarkable genus with lot of things yet to be studied and understood. From India, large number of species under *Isoetes* has been reported as new species. After Fraser-Jenkins et al. (2017) it is clear that there are only four different species (*I. coromandelina* L.f., *I. dixitii* Shende, *I. sahyadrii* Magab. ex L. N. Rao, *I. udipiensis* P. K. Shukla, G. K. Srivast., S. K. Shukla and P. K. Rajagopal) of *Isoetes* in India. As the species of *Isoetes* are mostly aquatic and Tamil Nadu can have low rainfall for several years with rare occurrence of normal rainfall, the occurrence of *Isoetes* species is rare and seasonal. But once there is enough rainfall, they flourish immediately and cover large area in several acres as mono-dominant species which is the characteristic features of several related fossil lycophytes like *Pleuromeia rossica*, which grew in wet shore side habitats and formed mono-dominant communities. Such ecological type of a mono-dominant plant community is quite typical of such hygrophilous vegetation in both the geological past and the present-day world, but it is inhabited by taxonomically different plants. The occurrence of mono-dominant *Isoetes coromandelina* L. f. has been reported from Kallidaikurichy, Tirunelveli District by Manickam and Irudayaraj (1992) and from a lake near Bharathidasan University, Tiruchirapalli, Tamil Nadu on 1999. They have mentioned that although the species is common throughout India, it is a rare species in Tamil Nadu. With the presence of rainfall above the average amount during the year 2014, this aquatic weed started to grow in and around several ponds and water pits in several localities, including the same locality Kallidaikurichy of Manickam and Irudayaraj (1992). Thanks to the availability of this seasonal aquatic weed, we aimed to make detailed cytological studies on the quillwort *Isoetes coromandelina* L. f.

**MATERIALS AND METHODS**

Materials for the present study were collected from Kallidaikurichy, Tirunelveli District, Tamil Nadu, India during 2014 (Figure 1 A, B). Sporangia (Figure 1 C, D) were fixed in the mixture of Absolute alcohol, Chloroform and Acetic acid (6:3:1). Acetocarmine squash technique was followed to study the chromosomes behaviour in megaspore mother cells. Voucher has been deposited in St. Xavier’s College Herbarium, Palayamkottai.

**RESULTS AND DISCUSSION**

Meiosis was observed in megaspore mother cells. Thin, network chromosomes, without the identity of individual chromosomes were observed in the leptotene stage and long, thick, rod like overlapping chromosomes were observed in zygotene stage (Figure 2, A-C). Pachytene and diplotene stages were not observed. After zygotene stage, each and every chromosome remains as such without pairing of homologous chromosomes. Instead of bivalents formation, they all remain as univalents. Thus all the chromosomes were like that of mitotic ones. There are 22 stout and thick chromosomes of more or less uniform size along with a very short chromosome. The chromosomes are of variable in length and type (Figure 2, D-E). The meiosis is more or less regular from I metaphase to II telophase except the irregular behavior of the extra chromosome (22 + 1) which either forms Chromosome Bridge or lies little away from others during first anaphase and telophase (Figure 2 F-I). There is no indication for the presence of this extra chromosome, in the form of micronucleus, in tetrads or young spores (Figure 2 L-Q). The mature megaspores are in different size and the surface of the megaspores is with prominent tubercles (Figure 1, E-H).

The careful observation on number of chromosomes in each group of Anaphase I, clearly shows the unreduction of chromosome number during first meiosis (Figure 2, F, G, H). From the present study on meiosis in megaspore mother cells of *Isoetes coromandelina* L.f from Kallidaikurichy, Tirunelveli District, Tamil Nadu, it is concluded that the chromosome number is n=22 + 1. This is the first count for *I. coromandelina* L. f from Tamil Nadu and it is in agreement with Abraham and Ninan (1958), who have observed the presence of complete asynapsis in this species in populations from Kerala and Karnatara. They have concluded that this species from South India is of asynaptic apogamous form.

Cytological reports on Indian Quillworts are available. Plants of *I. panchanani*, *I. indica* and Varanasi plants of *I. coromandelina* are with forty-four chromosomes and a fragment at mitosis in root tip smears but the plants of *I. coromandelina* growing in Lohgarha and Konark are with only thirty-three chromosomes and a fragment (Pant & Srivastava, 1965). As a rule, the first meiotic division shows almost complete asynapsis, most of the chromosomes being univalents although a few are bivalents and multivalents. The association of bivalent and multivalent chromosomes takes place in various ways. About 50% of the spores are nucleate and the remaining 50% are enucleate, which again suggests that a second nuclear division is normally absent in the spore mother cells of
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**Figure 1.** A. Large colony of *Isoetes coromandelina* L.f. in Kallidaidurichi, Tirunelveli District, Tamil Nadu, India, B. Habit, C-D. Sporo-phylls, E-H. Megaspores under light microscope (E, G) and Electron Microscope (F-H).
Figure 2. Meiosis in megaspore mother cells of *Isoetes coromandelina* L. f. A-J Meiosis I. K-N. Diads and tetrads; O,P,Q- Young spores.
Indian species of *Isoetes* and that their tetrads are formed by cytokinesis of the two dyads. The young larger mega-
spores of *I. coromandelina*, *I. indica* and *I. panchananii*
usually show one nucleus and one “centrosomic plastid”
while the smaller ones in the same species are enucleate
with only one “centrosomic plastid”. Karyomorphology
on triploid *I. coromandelina* from South India shows Tri-
somy and translocations (Kuriachan and Ninan, 1974a, b).
In general, *I. coromandelina* L.f. in India is a larger
species complex with varied rhizome morphology, orna-
mentation of megaspore and cytology with the presence of
diploid apomictic, triploid apomictic and triploid
hybrid, tetraploid apomictic, pentaploid hybrid and hexa-
& P.K.Rajagopal is diploid sexual (Fraser-Jenkins et al.
2017). *I. dixitii* Shende is with 4x sexual and 3x, 6x sterile
hybrids (Bir and Verma 2010).

In the present study, considerable percentage
of microspores and megaspores are abortive ones with high
reduction in size and irregular in shape. Another kind
of abnormality in both microspores and megaspores is the
presence of cytomixis. Mixing of chromatin mate-
rials of two spores through a tubular connection of the
spore wall is a common phenomenon in the presently
studied quillwort. The tubular connection is of differ-
ent size. It is very thin and narrow, or very thick and
short. Sometimes two spores are compactly and closely
joined together without any tubular connection (Figure
3 A-S). Chromosomal abnormalities including cytomixis
occur in meiotic process of pollen mother cells of many
angiosperm species at high frequency, but similar phe-
omenon was rarely reported in lower vascular plants.
Irregular chromosomal behaviour, especially cytomix-
is, is present in *Isoetes sinensis* Palmer (Heng-Chang et
al., 2007). It appeared in each stage of meiotic division
from prophase I to telophase II. The cytoplasmic chan-
nels between microspore mother cells are narrow and
stretched. The genetic material connection is maintained
mainly by visible chromatin fiber. Cytomixis occurs
between two adjacent microspore mother cells of same
stage. A variety of other abnormal chromosomal behav-
iors, such as chromosomal stickiness, fragmentation,
bridge, laggards, micronuclei are also present but with
low frequency. Indeed, the cause of cytomixis forma-
tion and the evolutionary significance of it are still source of
huge controversy. Bobak and Herich (1978) and Morisset
(1978) believed it was just a pathological phenomenon
by pharmaceuticals or mechanical pressure. Ghanima
and Talat (2003) stressed the impacts of extreme envi-
cell fusion and chromatin degeneration were probably
caused by both environmental and genetic factors.

*Isoetes* species are cytologically much variable with
the presence of diploid, triploid, tetraploid and hexa-
ploid species/cytotypes based on the base number x=11.
Majority of them are sexual with few apogamous taxa.
In *Isoetes* reticulate evolution by complex allopolyploid
speciation has well established. So it is necessary to
increase the sampling of *Isoetes* looking for undescribed,
missing species and additional intraspecific variation
(Hoot et al., 2004). Apogamy is not common among fern
allies. In the present case of the apogamous *Isoetes coro-
mandelina* L.f., it is peculiar in forming diplospores by
avoiding normal reduction division during first meiosis
in spore mother cells. Usually, the first meiosis is hete-
rotypic division by the reduction of chromosome num-
ber and the second meiosis is homotypic division with
normal mitosis. In the present case, both first and sec-
ond meiosis are homotypic division without the reduc-
tion of chromosome number resulting in the forma-
tion of diplospores. Formation of diplospores with total
asynapsis has been reported in several cases and the
genetic control of total asynapsis in apogamous taxa
has also been explained. The level and mode of diploid
megaspore formation has been studied in full-sib diploid
potato clones with either normal or desynaptic meiosis
(Jongedijk et al., 1991). The present observation of diplo-
spory in the apogamous *I. coromandelina* L.f is similar
one of pseudohomotypic division during first meiosis.

Apomixis has been repeatedly observed in fern lin-
egages that experienced frequent reticulate evolution
combined with polyploidization. The apomorphic line-
egages showed no increase in speciation rate. Instead, all
apomictic lineages appeared to be short lived despite
some evidence for post origin diversification. In general,
apomictic ferns are evolutionary dead ends in the long
term but maintain the short-term potential to be highly
successful in particular ecological conditions such as cli-
mates with strong seasonality (Liu et al., 2012).

There are several reports of asynaptic meiosis both
in ferns and angiosperms. Manton and Sledge (1954)
reported complete absence of chromosome pairing at
meiosis in two separate wild collections of filmy fern
*Hymenophyllum javanicum* Sprengel from Ceylon.
In another filmy fern *Trichomanes insigne* v.d.B forma α
is with normal meiosis, while forma β is with asynaptic
meiosis (Mehra & Singh, 1957). The filmy fern, *Tricho-
manes proliferum* Bl. from Sarawak, is with asynaptic
diakinesis with 108 univalents in spore mother cell and
32 spores in sporangia (Bell, 1960). Braithwaite (1964)
described a new type of apogamy in *Asplenium aethi-
opicum* complex from Africa with complete asynapsis at
diakinesis resulting in restitution nucleus which further
mitotically divide to form 16 diplospores in each spo-
Figure 3. Isoetes coromandelina L.f. Cytomixis in microspores and megaspores. A,B- Mixture of spores with normal, aborted and twin spores; C-H- Cytomixis in microspores. I-Completely mingled twin microspores, J-S- Cytomixis in megaspores.
rangement. Asynaptic meiosis with the formation of unreduced gamete has been reported in interspecific hybrid of *Trifolium* (Ansari *et al.* 2022).

**REFERENCES**


