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Spores, germination and apogamy in *Isoetes* of Rajasthan, India

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ABSTRACT

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Four species of *Isoetes* (Isoetaceae) are known from Rajasthan. In all these species, both megaspores and microspores (wherever present) show variations in morphology and nature. The spores start germinating immediately after the first shower of rain (July end) and hundreds of young plants of *Isoetes* are visible in patches during the middle of August. On the other hand, little success has been achieved in spore germination in the laboratory. It is concluded that, in nature, majority of young plants are apogamous.

Key-words: Quillwort, morphology, spores, germination, apogamy, Rajasthan, India.

INTRODUCTION

The genus *Isoetes* occurs throughout the world and is represented by over 200 species (Sharma et al. 2008, Sharma & Purohit 2011). In India, it is represented by 16 species, 3 sub-species and 3 varieties (Shukla et al. 2005). According to Fraser-Jenkins (2009), there are only 2-3 species of *Isoetes* in India and remaining ones are synonyms of *I. coromandelina* L.f. This observation is not acceptable (Sharma et al. 2008, Sharma & Purohit 2011). In Rajasthan, there are four species of *Isoetes*, e.g. *I. coromandelina* L.f. (Sharma & Bohra 1978, Singh et al. 1983, Sharma et al. 1985a, b), *I. tuberculata* Gena & Bhardwaja (1984), *I. reticulata* Gena & Bhardwaja (1984) and *I. rajasthanensis* Gena & Bhardwaja (1984). *Isoetes tuberculata* is aquatic while others are amphibious or terrestrial (Sharma & Harsh 1993). *Isoetes coromandelina* and *I. tuberculata* are large sized plants whereas *I. rajasthanensis* is medium sized and *I. reticulata* is small sized. The stem is reduced to a corm or rhizomorph with green, acicular, ligulate leaves. Roots are forked and arise in regular rows. Sporangia are large, basal, adaxial and heterosporous. In smaller forms (*I. rajasthanensis* and *I. reticulata*), velum is present to cover the sporangium while in larger plants, velum is absent and the upper labium protects the ligule (Sharma & Purohit 2001, 2011). Both mega- and microspores show wide variations in morphology and behaviour (Pant & Srivastava 1962, Bohra et al. 1980, Panigrahi 1981, Singh et al. 1983, Sharma et al. 1985a, b).

Goebel (1897) reported, for the first time, apogamy in *I. lacustris* and *I. echinospora*. In India, Ekambaram and Venkatanathan (1933) studied sporogenesis in *I. coromandelina* and noticed presence of two fertile and two sterile spores in a megaspore tetrad. Ninan (1958) stated that "in *I. coromandelina* all the collections examined from

South India were found to be apogamous". Verma (1960, 1961) published a cytological account of nucleate and enucleate spores of *I. coromandelina*. Pant and Srivastava (1965) observed 50% fertile and 50% sterile spores in a tetrad of *Isoetes*. They also observed apomictic germination in *I. indica* Pant & Sriv. and *I. panchananii* Pant & Sriv. Pant and Srivastava (1962) figured a number of sterile spores of different shapes and sizes. Sharma et al. (1985a) noticed 0 to 100% formation of fertile spores in a tetrad in *I. coromandelina* and *I. rajasthanensis*. In these two species, microsporophylls occur but not necessarily in every plant. On the other hand, male leaves are not yet seen in *I. tuberculata* and *I. reticulata* collected from Atru in Baran District, Rajasthan (Gena & Bhardwaja 1984, Sharma et al. 1985b). Only a few Indian workers could achieve success in germination of *Isoetes* spores in the laboratory (Srivastava et al. 1996b). On the other hand, in nature, a large number of young plants are seen growing after the first shower of rain in the middle of August at places like Daosa, Jhalawar, Atru, Mt. Abu, etc. It is observed that majority of young plants of *Isoetes*, especially at Atru and Jhalawar, are apogamous because of the absence of male sporangia and microspores. Cytological study is needed of the *Isoetes* species, in which microsporangia are yet to be seen.

MATERIAL AND METHOD

The authors collected all the four species of *Isoetes* found in Rajasthan from Atru, Jhalawar, Daosa, Mt. Abu, Menal, Chittorgarh, etc. during the months from July to early October and studied their habit and habitat. Spore morphology was studied by acetolysis technique and microtome sectioning of the sporangia.

SYSTEMATIC DESCRIPTION

Division: Lycopodiophyta

Class: Isoetopsida

Order: Isoetales

Family: Isoetaceae

Genus: *Isoetes* L.

***Isoetes coromandelina* L.f.**

Plate 1, figures 5-6, Text-figures 2-3, 5, 7-9, 16-19

Description: Plants amphibious to terrestrial, rhizomorph 2-5 lobed with 15-40 acicular, green, ligulate leaves ranging in length from 30-70 cm and are produced in close spirals in acropetal succession. Roots are produced in rows and are generally forked. Each leaf has a large oval sporangium on basal adaxial portion. The outer whorls of leaves bear megasporangia while some of the inner leaves (1-3) may have microsporangia. Each megasporangium contains 200-400, or sometimes more, trilete megaspores (Plate 1, figure 5) while in a microsporangium, large number of monolete or trilete microspores are produced. Sometimes, megaspores are also produced in a microsporangium and make it a mixed sporangium (Plate 1, figure 6, Text-figures 2-3, 5) (Singh et al. 1983). In addition to monolete microspores (Text-figure 2), trilete microspores are also known to occur in *I. coromandelina* (Plate 1, figure 6, Text-figures 7-9) (Srivastava 1995, Srivastava et al. 1996a). Both mega and microspores show abnormalities like smaller and bigger spores, fused spores (Text-figure 19), circular spores, jointed spores (Plate 1, figure 5, Text-figures 16-18), nucleate spores and enucleate spores (Verma 1960, 1961, Pant & Srivastava 1962, Bohra et al. 1980, Panigrahi 1981, Sharma et al. 1985a, b).

Plate 1

1, 4. *Isoetes tuberculata*. 1. Partially submerged plants in a small pond at Atru during rainy season. 4. Megaspores, x40. 2-3. *Isoetes rajasthanensis*. 2. Terrestrial, medium sized plant at Mt. Abu during rainy season. 3. Megaspores, x40. 5-6. *Isoetes coromandelina*. 5. Two jointed and one large sized megaspores, x40. 6. A sporangium with small, trilete microspores and three large, trilete megaspores, x60.

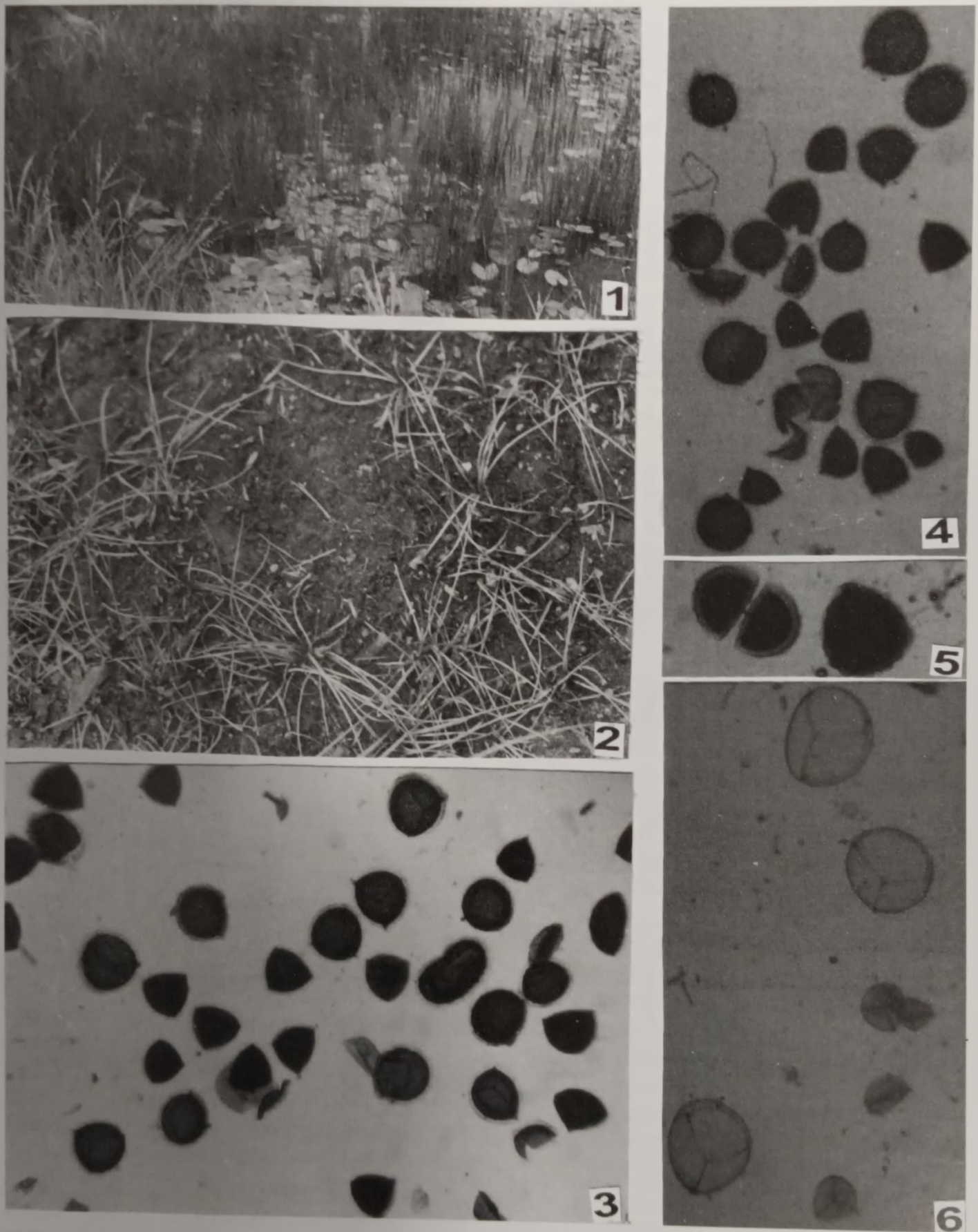
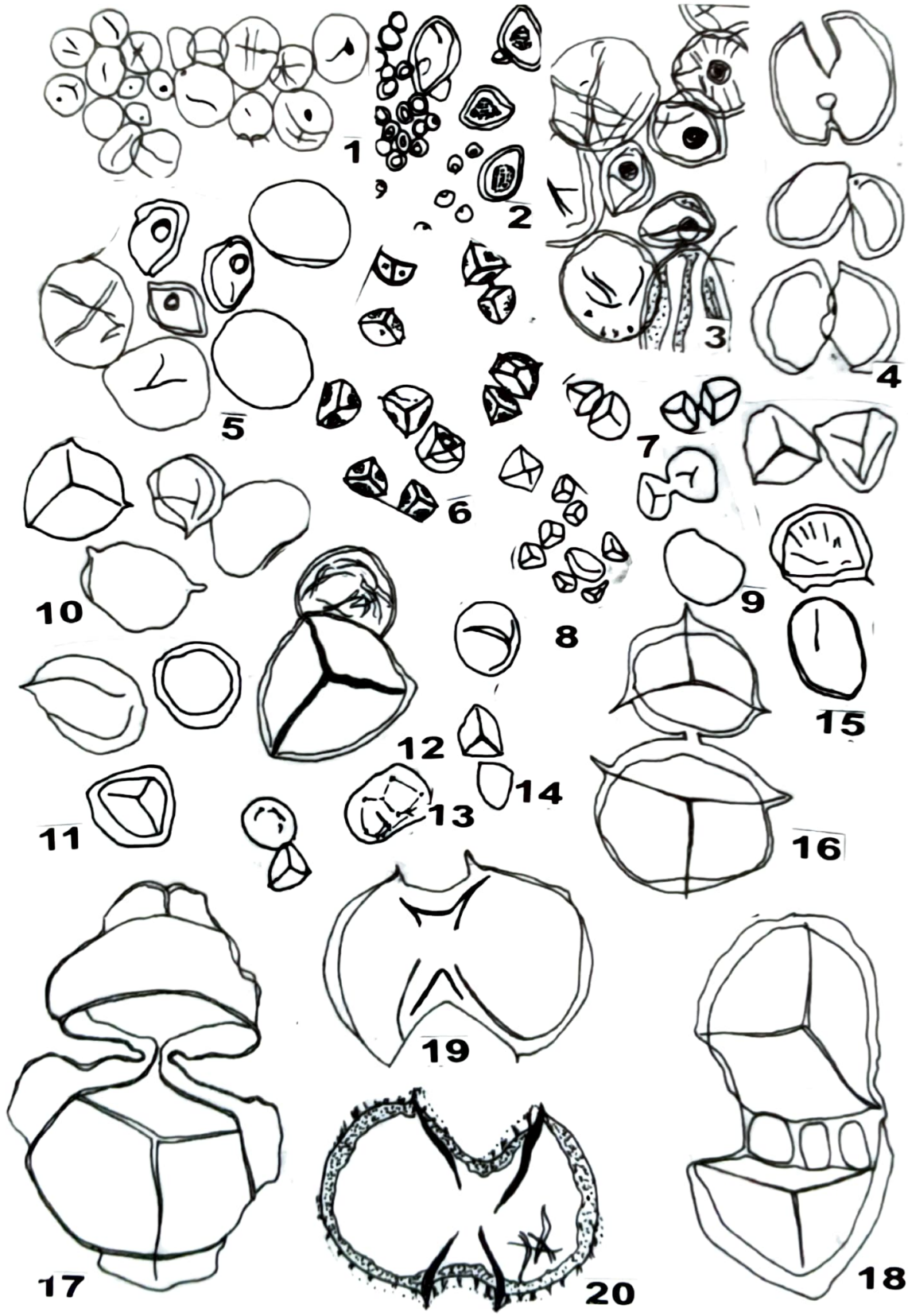


Plate 1



Text-figures 1-20. 1, 4, 13-14, 20. *Isoetes rajasthanensis*. 1. Immature, small sized microspores and large sized, circular, sterile spore, x60. 4. Three jointed microspores, x140. 13-14. Trilete and abnormal microspores, x90. 20. A fused megaspore with spinose exine, x240. 2-3, 5, 7-9, 16-19. *Isoetes coromandelina*. 2. Mixed sporangium with small, monolete spore, x40. 3, 5. Nucleated microspores and anucleated megaspores, x120. 7-9. Trilete microspores and large abnormal spores visible in Text-figures 8 and 9, x60 (text-figure 8), x90 (text-figures 7, 9). 16-18. Jointed megaspores with one or more joints, x240. 19. A fused megaspore, x240. 6, 10-12, 15. *Isoetes tuberculata*. 6. Trilete mega and sterile spores also visible, x8. 10-11, 15. Trilete megaspores and sterile cell visible in three spore tetrads, x40. 12. A trilete megaspore and a circular sterile spore in tetrad, x60 (All camera lucida drawings).

***Isoetes tuberculata* Gena and Bhardwaja 1984**

Plate 1, figures 1, 4, Text-figures 6, 10-12, 15

Description: Plants aquatic, collected from Atru (Plate 1, figure 1) and Jhalawar. Plants large sized resembling *I. coromandelina*. Leaves less than half exposed and remaining portion submerged. The submerged portion of leaves shows diurnal acid metabolism (Sharma & Harsh 1995) while the aerial portion follows C_3 path of photosynthesis. Microsporophylls are not yet seen. Megaspores are large with triradiate markings and tuberculate ornamentation. Abnormalities in megaspores are visible (Plate 1, figure 4, Text-figures 6, 12) like smaller spores, bigger spores, jointed spores, fused spores, etc. Velum is absent and the upper labium protects the ligule as in *I. coromandelina* (Sharma et al. 1985b, Sharma and Harsh 1993, Sharma & Purohit 2001). In the first week of August, hundreds of plants are seen growing in nature (Plate 1, figure 1). However, no success was achieved in the laboratory for the germination of megaspores of this species.

***Isoetes rajasthanensis* Gena & Bhardwaja 1984**

Plate 1, figures 2-3, Text-figures 1, 4, 13-14, 20

Description: Plants amphibious to terrestrial, medium sized found at Mt. Abu (Plate 1, figure 2), Menal and Chittorgarh during the rainy season. Corm 2-3 lobed, leaves ligulate, green, 4-12 on a plant and 8-16 cm in length. Velum present. Microsporophylls one to three on a plant in the inner whorls of leaves but not always. Microspores monolete (Text-figures 1, 4) and show usual abnormalities, i.e. jointed spores (Text-figure 4), fused spores, sterile spores, etc. Megaspores trilete with spinose ornamentation. Sporal abnormalities present, i.e. smaller spores (Plate 1, figure 3, Text-figures 13-14), jointed spores, fused spores (Text-figure 20), etc. (Bohra et al. 1980, Sharma et al. 1985a, b). During rainy season, a large number of plants are seen growing either on wet soil or along small water streams at Anadara point, Mt. Abu. Plants could not be raised in the laboratory from spores.

***Isoetes reticulata* Gena & Bhardwaja 1984**

Description: It is a small sized plant found growing on wet soil along the margins of ditches and ponds at Atru. Rhizomorph 2-3 lobed with 3-8 leaves on a plant which are 4 to 8 cm in length. Microsporophylls not yet seen. Megasporangium circular to oblong on basal adaxial portion of leaf. Megaspores small or large, trilete. Spore abnormalities common, i.e. presence of sterile spores in tetrad from 1 to 3. Small young plants are seen growing on wet soil during the rainy season. The roots possess an association of VAM fungi (Sharma 1998a, b). Plants could not be raised in the laboratory from spores.

DISCUSSION

Of the four species of *Isoetes* found in Rajasthan, two possess microsporophylls (*I. coromandelina* and *I. rajasthanensis*) while the other two do not have male leaves (*I. tuberculata* and *I. reticulata*). Microsporophylls rarely occur in comparison to megasporophylls. It has been observed that on a plant only 1-3 leaves may have microsporangia whereas all other leaves bear megasporangia. A megasporangium has 200-400 or more megaspores whereas there are thousands of microspores in a microsporangium. In some of the microsporangia, megaspores are also present (Plate 1, figure 6) and make the sporangium of mixed type (Singh et al. 1983). Occurrence of mixed sporangia is a common feature in *I. pantii* (Goswami 2004). Megaspores are trilete while microspores may be monolete or trilete (Srivastava et al. 1996b). During investigation on sporogenesis of *I. coromandelina*, Ekambaram and Venkatanathan (1933) noted the occurrence of 50% sterile spores in a tetrad. This was also supported by Verma (1960, 1961) and Pant and Srivastava (1965). Sharma et al. (1985a) noticed fluctuations in the percentage of sterile and fertile spores in a tetrad. It may be 0 to 100% in *Isoetes* species collected from Rajasthan. There are many types of abnormalities in megaspores, e.g. smaller spores, rounded spores, fused spores, jointed spores, etc. (Pant & Srivastava 1962, Bohra et al. 1980,

Sharma et al. 1985a, b). Similarly, microspores also show abnormalities, i.e. all are not functional (Text-figures 4, 8-9) in tetrad. In suitable conditions, a fertile microspore produces only 4 multiflagellate antherozoids which are functional only in aquatic conditions and not in terrestrial species, i.e. chances of fertilization of archegonia reduces in non-aquatic plants. In aquatic species *I. tuberculata*, male sporophylls are not yet seen and therefore chances of fertilization reduce to zero. Thus all young growing plants of this species, during the month of August, are apogamous and haploid. Similar is the situation in *I. reticulata*, a terrestrial plant without microsporophylls.

Isoetes coromandelina and *I. rajasthanensis* are amphibious to terrestrial and multiflagellate antherozoids cannot reach easily to the archegonia of female gametophytes. Secondly, the number of functional megaspores is not more than 25% (50% sterile, non-nucleated spores and approximately 25% abnormal megaspores in a tetrad). Further, all functional megaspores do not germinate properly and form female gametophytes due to some kind of adverse conditions. Fungal and insect attack is also noticed on megaspores during non-rainy seasons, i.e. during hot summer and chilling cold winter. These reduce the chances of fertilization of egg in the archegonium and formation of functional embryos. It means that out of hundreds of young plants seen at Daosa (*I. coromandelina*) and Mt. Abu (*I. rajasthanensis*), majority are mainly haploid and apogamous. Diploid plants are only a few in number. Cytological study is required for the verification of the present opinion. Very little cytological work has been done on *Isoetes* species of Rajasthan (Bhardwaja & Gena 1992).

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