Bionature, 31 (1), 2011 : 13-22

POPULATION CYTOGENETIC STUDIES CONFIRM X-Y MECHANISM IN *ISOETES PANTII* (ISOETACEAE : PTERIDOPHYTA)

HIT KISHORE GOSWAMI

24,Kaushalnagar, P.O. Msrod, Bhopal, (MP) 462047 India

ABSTRACT

Chromosomal surveys of *Isoetes* species in the ponds in and around Narsinghgarh area in Central India have revealed the evolution of X-Y chromosome mechanism. While *Isoetes pantii* Goswami and Arya is still characterized by variable chromosme number on account of its being a natural hybrid, the plants with 2n=36,39 and 2n=48 always possess heterosporous microsporangia containing highly abnormal megaspores of enormous evolutionary significance. Origin of sex chromosomal mechanism appears to coincide with the evolution of heterospory within the microsporangia, a feature not yet known in any other living plant. On the basis of earlier and present experimental approaches by DNA finger printing, the emphasis is laid on resemblances of some genomic DNA sequences of *I pantii* to a few human Y chromosomal sequences. Brief comments on recent discoveries of human Y chromosome MSY region DNA sequences by different authors in a bryophyte, *Marchantia polymopha* and in several other plants, have also been discussed particularly with the intention that our hypothesis advanced during 1990s that the plants have some sequences from human genome or vice versa, appears to be valid. All these observations also support our earlier hypothesis advanced on the basis of genomic studies as well as computer search (DNA blasting) of a part of *Isoetes genomic* DNA with the human genomic data, that the DNA sequences, must have ceaselessly replicated and randomly distributed among evolving cells before the bisecting of evolutionary lineage to plant and animal cells during Pre Cambrian.

Key words: Sex Chromosomes in Pteridophytes/ X-Y mechanism in *Isoetes pantii*, Chromosomal variables in natural hybrid; Genomic reshuffle

Introduction

A fundamental discovery that megaspores regularly develop inside the microsporangium of *Isoetes pantii* Goswami and Arya and that too, some of the abnormal megaspores resemble fossil lycopods(Goswami, 1975a) paved the way to search for cause and consequences of probable genomic reshuffle within the *I. pantii* genome. This was believed that regular production of some megaspores resembling fossil lycopods must be due to rare combination of genes so as to revive those genes which were once active during Upper Triassic (150-160 million yeas ago). These spores have been compared with Rhaetic genus, *Nathorstisporites* Jung; Goswami, 1975 a, Goswami and Goswami, 1986). Additionally, population cytogenetic studies revealed yet more scintillating observation that *I pantii*

* e-mail : hkgbhopal@hotmail.com; Paper accepted November 2010.

has evolved sex chromosomal mechanism (no other species of the genus has a sex chromosome). The evolution of sex chromosomal mechanisms in the genome of *Isoetes pantii* (Goswami, 1975b; Bhu and Goswami,1990) became instrumental for the search of chromosomal variations in the genus from different localities in Central India. Chromosomal surveys over these years have demonstrated variable counts along with the origin of a new line of chromosome evolution based on n=12 chromosomes, unlike the conventional path of n=11 so well established for the genus *Isoetes,* except an aneuploid count for *Isoetes hystrix* n=10 by Manton (Manton, 1950; Hickey, 1984; Takamiya, 2001; Takamiya, Watanabe and Ono,1994, 1996; Liu, Gituru and Wang, 2004). So the presence of "ancestral megaspores" (megaspores resembling fossil lycopods) inside the microsporangia and such plants being characterized by X and variable B chromosomes with mitotic counts 2n=48; 2n=36 ±1 to 2B (or 1 may be a Y chromosme) guided the path to probe the genomic DNA of *I pantii* with some DNA sequences from human Y chromosome. Probing *Isoetes* genomic DNA with some DNA sequences from the human Y chromosome (Goswami and Chandorkar, 1994) was also conceived due to peculiar amalgamation of human Y chromosome DNA sequences from various organisms mainly apes, demonstrating all basic mechanisms of gene transfers and rearrangements of chromosomal segments with primitive as well as highly specialized (MSY) regions. This is already known that the evolution of a new chromosome (Stebbins,1971; Goswami,1993; Watson *et al*, 1991; Willard, 2003) is based on two fundamental phases, viz.; the new chromosomes (be auto or sex chromosomes) arise *de novo* from within the genome by series of breaks, translocations and other aberrations. Secondly, these phenomenon are assisted by molecular mechanisms like heterochromatinization with occasional out crossing. We have observed these events to be operative for evolution of B, X and Y chromosome in the natural populations of *Isoetes* studied cytologically for several years (Bhu and Goswami, 1990; Bir and Verma, 2010). Our search for some resembling sequences in *Isoetes pantii* with human Y chromosome DNA stretches was practically based on above assumptions which have been truly validated by excellent studies of Okada *et al* (2000) and Ishizaki *et al* (2002) who have demonstrated the presence of some MSY region sequences of human Y chromosome in *Marchantia polymorpha*. These and additional studies (Armstrong and Filatov, 2007) have finally proved that the sex chromosome in plants may also possess primitive as well as highly specialized sequences.

This paper also reports, based on repeated experiments, the prevalence of X-Y chromosome mechanism in about 30-40% plants of *Isoetes pantii* in the ponds in and around Narsinghgarh area (M.P, India) from where the original population was reported in 1968 and thereafter.

Methods and Comments

Collection of Plants

There have been about one dozen places regularly visited in Central India from where a few *Isoetes* plants were randomly collected two or three times every three years. Table 1 presents only those localities from where chromosome studies have been repeatedly carried out listing places of collections and variable chromosome numbers for each species. Two voucher specimens of plants are maintained as HKG/Hetero-XY/2004 in Bionature herbarium / collection at Bhopal.

Chromosome preparations

Basic approaches and staining schedules have already been published (Goswami, 1975b, 1996 ; Bhu and Goswami, 1990 and thereafter).Mitotic chromosomes have been mostly photographed after root tip processing (Figs 1-3) but rarely, leaf tips of young leaves had also yielded good results. Meiotic studies (Fig.4-5) have been carried out on young sporangia. Staining procedures have included conventional acetic-orcein (1.5%), Schieff's reagent/ reaction and by C- banding. In order to band for constitutive heterochromatin, the roots were pretreated in 0.002M 8-hydrooxyquinline for 6 hrs at 16-18C and fixed in acetic acid-ethanol (1:3) for 4 hrs. Root tips were squashed after hydrolysis in 1N HCL at 60c on extra clean slides in 45% glacial acetic acid under cover slip. Slides were frozen by keeping in freezer or inverting on dry ice for one day. After gently removing the cover glass 10% acetic acid was dropped on the squashed tissue by a dropper gradually for 2-3 minutes. The slide was dried again. Then saturated Ba(OH)2 drops were put on the slide so as to keep the tissue almost dipped for 5 minutes at room temperature. Results were also obtained by immersing slides in 8% (w/v) aqueous solution of barium hydroxide for about 8 minutes at room temperature. Then slides were washed in distilled water by keeping slides tilted so that water ran without damaging the tissue. After 20 minutes wash, slides were incubated in 2 X SSC (0.3M sodium citrate+0.3M sodium chloride;1:1) at 60-61C for 30mnts. Finally, slides were stained in 5% Giemsa solution with M/15 Sorensen buffer (pH 6.8), for 10 mnts, rinsed in distilled water, air dried and mounted in euparol (Fig.3). Photographs were taken under Nikkon inverted microscope using automated camera attachments with light green filters.

Some of the slides were also stained with 1.5% acetic orcein instead of Giemsa, and rarely through, differential staining was also seen on *I pantii* but not on root tissues of *I.sampathkumarani* Rao and *I. dixitie* Shende probably suggesting that heterochromatin in *I pantii* becomes easily exposed when compared to other species. This is worth recording here that roots after pretreatment and fixation were also processed for wax (56C) blocks for microtomy and sections were cut at 10 mμ (micron) and processed for staining. Intact cell walls sometimes revealed reliable chromosome differentiation.

Sex Chromosomes

Presence of sex and B chromosomes within the genome have been adjudged by series of conventional tracking of mitotic and meiotic divisions over the years. Since the earliest observation (Goswami,1975b) we have had always found a lagging large sub-metacentric chromosome at meiosis metaphase I. B chromosome also lagged behind at meta and anaphases of both the mitotic and meiotic divisions. No pairing of X or B or Y chromosomes among themselves or with any other chromosome has been observed during early phases of meiotic divisions; these always keep distance in movements (Figs. 4 & 5). Other chromosomes pair regularly and chromosomal segregations, otherwise, have been normal.

Distribution of heterochromatin on each chromosome was well documented (Figs.1 & 2) by Feulgen's stain (euchromatin stained darker than the heterochromatin) and C-banding made the reverse (Fig.3) picture; heterochromatic parts on chromosomes stained darker). On the basis of these staining schedules and also by counter staining with acetic-orcein X and B chromosomes were identified with certainity. This became clear that a bisexual plant of *I.pantii* (bearing both micrsporangia and megasporangia) always possesses a heterosporous microsporangium which

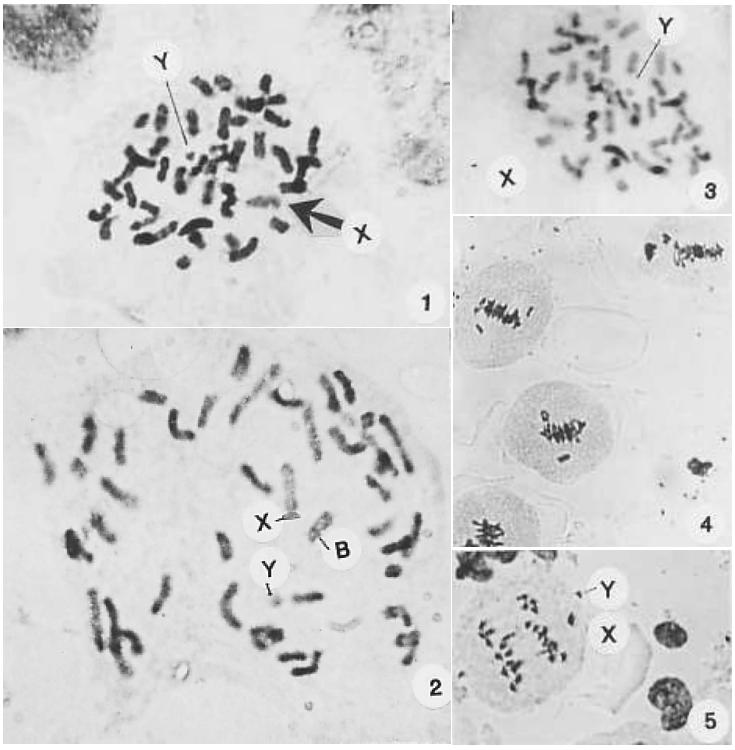

Fig.1. Feulgen stained root tip mitosis in ***Isoetes pantii*** showing 2n= 48 chromosomes; X chromosome is arrowed while Y and B chromosomes are indicated with lines. The squash was mounted in 0.5% luke warm acetic- orcein so as to provide depth in staining (ca., X 2450).

Fig.2. Another plant with 2n=48 chromosomes in root tip mitosis. Feulgen staining has clearly shown submetacentric X chromosome and the B chromosome while Y chromosome is smaller more or less telocentric (ca. X 2450). This metaphase plate also shows a few more chromosomes with less amount of euchromatin which becomes more clear in C-banding (Fig.3)

Fig 3. C-banded root tip metaphase showing distribution of the constitutive heterochromatin among 36+3 chromosomes. The darkly stained upper half and faintly stained lower half is the typical feature of the submetacentric X chromosome. B chromosome is larger than the Y chromosome (ca, X 2000)

Fig.4. Metaphas I in microspore mother cells (stained with 1.5% acetic-orcein) shows regular laggards; X chromosome ; while B or Y may also be seen in some microspore mother cells (ca. X 2000)

Fig.5. Acetic- orcein (1.5%) stained microspore mother cell showing two chromosomes on one pole (X and Y or B) and one chromosome (B or Y) on the other pole in Anaphase I in the microsporogenesis in *I.pantii* . We have already reported variables , viz. X and 2 B and X , Y and B chromosomes in *I pantii* (ca.,X 2000)

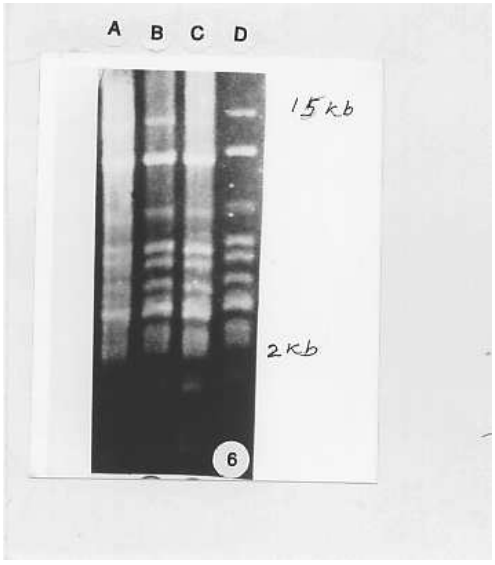

Fig.6. Electrophoresis of amplified genomic DNA samples from mature sporophylls Only from one bisexual plant of *I pantii* using a mixtutre of primers from human DNA sequences CT52Y and PY6). Lowest band in the Lane C is less than 2kb which has not been seen in any other sample (?).
A. Megasporophyll sample

B. Microsporophyll sample : sporangium with large megaspores and microspores

C. Microsporophyll : sporangium contained many large megaspores, abnormal megaspores and thousands of microspores (plant ascertained for sex chromosomes)

D. Mixture of primers run for control.

contains thousands of three types of microspores (alete, monolete and trilete) and dozens of abnormal meagaspores resembling fossil lycopods (Goswami and Goswami, 1986; Bhu and Goswami, 1990). Such plants were sorted out for genomic DNA studies.

Present RAPD study

Isoetes plants have offered unique experimental opportunity. This lycopod has a single large adaxially placed sporangium at the base of each sporophyll. Since 30-40 sporophylls form a rosette and the basal part is embedded in the corm this is always possible to turn down the sporophylls on side and locate whether the sporangium is a female sporangium (megasporangium) or a male one (microsporangium). Practically in all bisexual plants outermost whorl of sporangia are megasporangia but the microsporangia are often in the third whorl of sporophylls (all *Isoetes* plants do not bear both megasporangia and microsporangia). The most significant part of this trial of experiment is that the genomic DNA was extracted from the microsporophylls and megasporophylls only after ascertaining the chromosome number from the roots of the plants and ascertaining that the plant did possess 2n=48 chromosomes and or the squashes revealed the presence of X and Y chromosomes.

Genomic DNA extractions were done by standard protocol as earlier (Bajpai and Goswami, 2002). Briefly; 2gms of leaves were

homogenized with lysis buffer, followed by phenol-Chloroform, Isoamyl extraction; washing with 70% ethanol and finally eluted in 80 µl of double distilled water. The DNA was then amplified by polymerase chain reaction (PCR). Each reaction (25µl) contained 2mM MgCl2, 10mM tris-KCl, 4mM of a mixture of two primers PY-6 and CT52-Y, 2m MdNTPs and lU of Taq DNA polymerase. The PCR amplifications consisted of 40 cycles of 94 C /15 s to denature the DNA , 35 C / 30 s to anneal the primers and 72 C /60 s for elongation. A final extension step of 72 C for 7 min was performed after the 40th cycle. The experiment was repeated once. The RAPD products were separated by electrophoresis in 1.2% agarose gels stained with ethidium bromide and visualized and photographed under ultraviolet light.

Results and Discussion

Chromosome variations

Interspecific and intraspecific chromosome variations along with the presence of one B chromosme has been known for Indian species of *Isoetes* (Ninan, 1958; Pant and Srivastava, 1965; Goswami, 1996) Table 1 indicates that *I .coromandelina* L and *I.sampathkumarani* Rao, posses same chromosome numbers reported in earlier studies then their suspected natural hybrid *I pantii*, (Goswami and Bhu, 2000) which clearly demonstrated variable chromosomes' count adhering to multiples of n=12 (Bhu and Goswami 1990; Goswami 1996, 2004; see also, Bir and Verma, 2010).This is expected that hybrid segregates in a population may naturally show many variables in both chromosomal (Taylor and Hickey, 1992; Musselman *et al.* 1995) structure and functions on account of imposed genomic clashes (Goswami and Goswami ,1986; Landry, Hartl and Ranj, 2007). Instable counts of chromosomes in hybrid segregates of *Isoetes* may be due to genomic clash at the chromosomal level also besides, many morphological rarities encountered over several years. This aspect needs to be studied in detail.

DNA studies

Pioneering experiments of Walter Nagl (1991) were the first to demonstrate the presence of two human gene sequences in a plant, *Phaseolus.* The genomic DNA of several cultivars of the beans, *Phaseolus coccineus* L and *P. vulgaris* L was Southern hybridized with a cDNA probe of human aromatase cytochrome P450, the enzyme converting androgens in to estrogens. Evidences for the existence of an aromatase homologous DNA sequence in these plants was obtained. Our earlier studies on genomic DNA of *Isoetes pantii, Isoetes coromandelina* and *I. sampathkumarani* were carried out by RAPD cross matching with the human chromosome primers (Goswami and Chandorkar, 1994)) which indicated positive presence of DNA stretches akin to human Y chromosome primer sequence PY-6 . Since the initial experiment was carried out at Institute of Human genetics, Heidelberg (courtesy : Peter Vogt Goswami and Chandorkar, 1994) the same was repeated after a few years. Prof Walter Nagl (Kaiserslautern) informed that *Isoetes pantii* DNA did possess some DNA stretches akin to DNA from the human Y chromosome sequence but Peter Vogt also suspected some contamination. This was indeed a matter of non reliable faith during 1990s because one would not expect the same DNA sequences in a plant similar to human Y chromosome locus. Our follow up studies by Southern hybridization techniques with a very specific human Y chromosomal probe DYS1 (Goswami and Bhu, 2000: courtsey: Late Dr Lee) had clearly indicated that the specific Y chromosome sequences were positively

signaled only in the genomic DNA of microsporophylls (sporophylls bearing heterosporous microsporangia), and not in any other sample of sporophylls from other species). But now this has been shown by more sophisticated experiments by Okada and his associates (Okada *et al.* 2000; Ishizaki *et al*, 2002) that the liverwort *Marchantia polymorpha* not only has X and Y chromosome mechanism but has human Y chromosome specific DNA MSY -sequences (see also reviews, Tanurdzic and Banks, 2004; Armstrong and Filatov, 2008).

Our major hypothesis, in mind, as already mentioned, was that recently evolved sex chromosomes in *I pantii* might have followed the same two principles; ie, new chromosomes arise *de novo* from within the genome by heterochromatinization, chromosome breaks and gene transfers by tranlocations, and secondly, some basic DNA sequences as found in evolutionarily recent (*Homo sapiens*) human Y chromosome might be also be primarily present in these primitive land plants which existed hundreds of millions of years before the genus *Homo* evolved. There are several gene sequences in bacteria and many microorganisms which are exactly copied in the human genome. This tenders a support to our hypothesis that the DNA sequences replicated ceaselessly and got dispersed in early phases of evolution in trillions of cells during Pre Cambrian (?) before bisecting in to plant and animal cells (Bajpai and Goswami,2002; Goswami, 2009). Genomic reshuffle can always revert back to genic expressions as also demonstrated by series of computer based genomic studies which show several small and larger DNA stretches to be common in *Isoetes* and *Ginkgo* at many loci of human chromosomes (Goswami *et al*, 2006; Goswami, 2009). Morphologically, this has been best displayed by the regular production of megaspores resembling fossil lycopods inside the microsporangia of *I. pantii.* Furthermore, sex chromosomes in *I. pantii* have strictly adhered to cytological characteristics of movements during mitotic and meiotic divisions. While all chromosomes pair and regularly segregate in meiosis, no pairing of X or B or Y chromosomes among themselves or with any other chromosome has been observed (Figs. 4 & 5).

Sex chromosomes in other plants

Among other plants, Smith (1956) mentioned about a marine alga, *Fucus* and a bryophyte, *Sphaerocarpus donelli.* to possess a sex chromosome. Heteromorphic sex chromosomes were also discovered in *Ginkgo* and *Cycas* (Lee, 1954 and Abraham and Mathew, 1962, respectively; see also, Armstrong and Filatov, 2008), Recent discovery of sex chromosomes in *Marchantia polymorpha* and that too, that *Marchantia* has DNA sequences exactly similar to some of the human MSY region of Y chromosome (Tanurdzic and Banks, 2004) have convinced that some human DNA sequences may also be present in plants. Referring to flowering plants Tanurdzic and Banks (2004) mentioned that sex determination is a process that leads to the physical separation of male and female gamete producing structures. This is getting revealed that sex chromosomal mechanism is often associated with dioecism and many plants are being discovered wherein X and Y mechanism controls the male and female sex of the plants. Among lower plants a bryophyte *Marchantia polymorpha* has been studied extensively within the past decade but sex chromosomes among angiosperms have been known for quite some time in many plants viz. *Melandrium, Silene latifolia, Rumex acetosa, Cannabis sativa,* and several others, (Ye *et al*, 1991; Charlesworth, 2002; Tanurdzic and Banks, 2004; Armstrong and

Filatov, 2008). Infact plants have diversified modes of sexual expression ranging from hormonal to independent genes' expression, further leading to sex controlling genes being localized on specific chromosomes (Charlesworth, 2002). All reviews have so far indicated that there are no set rules for sexual differentiation and evolution of sex chromosomes (Armstrong and Filatov, 2008). As hypothesized on the basis of computer blasting of some genomic DNA sequences with the human genome (Bajpai and Goswami, 2002) there can not be any set rule because, in the early phases of evolution in Pre Cambrian-Cambrian, the DNA sequences must have been ceaselessly replicating and getting incorporated randomly among evolving cells in trillions before bisecting in to plant and animal cells. There would have been millions of individual cells with same and different DNA sequences and on genomic reshuffle during evolution, very many combinations would have arisen and survived. Dioecism can not be strictly correlated with X-Y mechanism and also, not all sex chromosomes of one species can have resembling sequences with all related species.

In Silene latifolia, for example, Y chromosome mostly closely resembles the X- degenerate class of sequences of the human Y chromosome. The sex of each haploid plant of the bryophyte, *Marchantia* is decided by distinct sex chromosomes, with males having one very small Y chromosome and no X chromosome. Female plants of *Marchantia* have one X chromosome and no Y chromosome. The genus has a very small genome size of 280 Mbp distributed among eight autosomes and one sex chromosome. A small portion of the *Marchantia* Y chromosome has been sequenced and Okada associates (Ishizaki *et al*, 2002) have detected similarities with the euchromatic male specific region (MSY) of the human Y chromosome.

These discoveries finally prove, that experimental observations during 1990s and thereafter (Goswami and Chandorkar, 1994; Bajpai and Goswami, 2002) were not wrong and origin of sex chromosome must have followed the same DNA stiching and molecular alignment as has been instance with the origin of sex chromosome in man. This has been a permanent misfortune that our work on *Isoetes* DNA has always been suspected to carry contamination from human DNA (???????). However, I have aptly and repeatedly demonstrated the presence of X and Y chromosomes and mitotic and meiotic movements over generations of this quillwort. No contamination of any sort can simulate such a situation; though this would have been decidedly reliable, had these chromosomes would have been tagged and photographed with sex chromosome probes, as per the modern requirements as has been demonstrated by Japanese group on *Marchantia polymorpha* sex chromosomes.

Acknowledgements

This entire set of experimental work has been carried out in many laboratories during 1993-2006 for which I feel greatly indebted to many colleagues and friends. This paper is dedicated to the memory of my beloved student Late Dr Arun Kumar Bajpai who had carried out extensive technical and financial support and shared all academic challenges, posed out by this kind of unconventional work for almost a decade until his sad demise in 2008. I am also grateful to the late Dr I. H. Lee who had helped me in my molecular genetic studies during my short term stays at Taegu, South Korea. My special thanks are also on record for the genuine help of Professor Dr. Jennifer Geiger for the critical review of the manuscript and very helpful suggestions.

TABLE 1. CHROMOSOME SURVEY IN *ISOETES* POPULATIONS IN NARSINGHGARH AREA, (MP)

Name of Species.	Chr.count	Locality	Reference
1. *I coromandelina* L	2n=22=1 2n=33+1	Narsinghgarh Narsinghgarh	Bhu & Goswami,1990. Goswami, 1975, 1996; n=11 +1 -- -do--
Goswami & Bhu, 2000			
2. *I. sampathkumarani* Rao	2n=66 +1	--do-------	Bhu & Goswami, 1990
3. *I pantii* Goswami & Arya	n= 33 +0 2n= 24 2n= 36+3	- --do------ Narsinghgarh Narsinghgarh	Goswami & Bhu, 2000 Goswami & Bhu, 2000 Goswami,1975; Goswami & Goswami, 1986
	2n= 36, 39, 48	--do------	Bhu & Goswami,1990
	2n= 36-39; 48	-- do----	Goswami & Bhu, 2000
4. *I. fuchsii* Bhu *et al*	2n=60	Maksudangarh Guna	Bhu, Goswami, Sharma (& Bajpai (2001)
5. *I muricata*	2n=60	Bhopal	(similar to hybrid segreagate, no.6)
(*erroneously* reported as *I. muricata*) (*Isoetes* sp from Bhopal) Bhopal			Bhu & Goswami,1990
6. *I. pantii; var.hybrida*	2n=60	Narsinghgarh	Goswami, 2004 ;

REFERENCES:

Abraham, A. and Mathew, P.M. (1962). X and Y chromosomes in *Cycas.* Ann. Bot., **26:** 261-266.

Armstrong,S.J and Filatov, D.A (2008). A Cytogenetic view of sex cghromosome evolution in plants. Cytogenet Genome Res **120:** 241-246

Balpai, A.K., and Goswami, H.K. (2002). Human Genome may share some genes with plant gene pool. *Bionature*, **22:** 63-65.

Bajpai, A.K., Goswami, R., and Goswami, H.K. (2004). *Isoetes* V. Genomic reshuffle: DNA sequences. *Bionature.* **24:** 25-32.

Bhu I. and Goswami, H. K. (1990). A new line of chromosomal evolution in *Isoetes*. *Bionature* **10:** 45-53.

Bir, S.S. and Verma, S.C. (2010). Chromosome Atlas of Indian Pteridophytes. Bishen Singh Mahendra Pal Singh, Dehradun

Charlesworth, D. (2002). Plant sex determination and Sex Chromosomes. Heredity. **88:** 94-104

Goswami, H K. (1975a). The morphogenetics of mixed sporangia in *Isoetes* J.Ind.bot Soc. 54 (3-4) 210-218

Goswami, H. K. (1975 b). Chromosome studies in natural population of *Isoetes pantii* with heterosporous sporangia. *Cytologia* **40:** 543-551.

Goswami, H.K. (1993). Origin and Evolution of Chromosome system. In J.B.S. Haldane and Evolution. Pp 117-134. Catholic press Ranchi

Goswami, H.K. and Chandorkar, M. (1994).Highly conserved DNA sequences in *Isoetes pantii.* Ind. Fern J. 11. 53- 55

Goswami, H. K. (1996). Three decades with *Isoetes.* Indian Fern Journal **19:** 51-61.

Goswami, H. K. (2004). Some observations on *Isoetes pantii* complex: New segregate in nature. Indian Fern J. **21:**150-157

Goswami, H.K. (2009). Dispersal of Genes in Evolution. II. Conserved DNA sequences are evolutionary signals in the human genome. Bionature. **29:** 39-46

Goswami, Indira and Goswami, H.K. (1986). Genetics of natural variants VI. Some abnormal spores of *Isoetes pantii* Goswami & Arya resemble fossil lycopods. Bionature **6:**23-28

Goswami, H. K. & Bhu, I. (2000). *Isoetes pantii* is a Natural hybrid : *I. sampathkumarini x I. coromandelina.* Bionature **20:** 9-18.

Goswami, H.K., Kang, S. C., Goswami, R. and Bajpai, A.K. (2006). DNA sequence do not abide with phylogenetic distance. Bionature. **26:** 1-12.

Hickey, R. J. (984) Chromosomal numbers of Neotropical *Isoetes*. Amer. Fern. J.**74:** 9-13

Ishizaki. K., Shimizu-ueda, Y., Okada, S., Yamamoto, M., Fujisawa,M., Yamamoto, K.T., Fukuzawa, H., and Ohyama, K. (2002). Multicopy genes uniquely amplified in the Y chromosome specific repeats of the liverwort *Marchantia polymorpha.* Nucleic Acids Res. **30:** 4675-4681

Landry, C.R., Hartl, D. L. and Ranj, J. M. (2007). Genome clashes in Hybrids : insights from gene expression . Heredity. 1-11

Lee, C. L. (1954) Sex Chromosomes in *Ginkgo.* Am. J. Bot. **41:** 545-549

Liu, X., Gituru, W.R. and Wang, Q.F. (2004). Distribution of basic diploid and polyploidy *Isoetes* in East Asia. J. Biogeogr.**31:** 1239-1250

Manton, I. (1950). Problems of cytology and Evolution in the pteridophyta. Cambridge University, Press, Cambridge.

Musselman, L.J., Knepper, D.A. , Bray, R.D. Caplen, C. A. and Ballou, C. (1995). A New *Isoetes* hybrid from Virginia . Castanaea, 60, 245-254

Nagl, W. (991). Two human DNA sequence (Aromatase:Telomere) detected in *Phaseolus* (Fabaceae) by respectively blot and in situ hybridization. Polish Bot. Stud. **2:** 157-164.

Ninan, C.A. (1958). Studies on the cytology and phylogeny of the pteridophytes. V. observations on the Isoetaceae. J. Ind.bot. Soc. 37. 93-102.

Okada, S. *et al*. (2000) Construction of male and female PAC genomic libraries suitable for identification of Y chromosome -specific clones from the liverwort Marchantia polymorpha. Plant J. **24:** 421-428 (not read in original)

Pant,D. D. and Srivastava, G.K. (965). Cytology and reproduction of some species of *Isoetes*. Cytologia **30:** 239-251

Smith, G.M. (1956). Cryptogamic Botany Vol I and Vol II. McGrawHill Publications. NewYork.

Stebbins, G.L. (1971). Chromosomal Evolution in higher plants. Edward Arnold Ltd. London. UK.

Takamiya, M. (2001). *Isoetes sinensis var sinensis* in Korea. (Isoetaceae : Pteridophyta). Fern Gaz. **16:** 169-177.

Takamiya, M., Watnabe, M. and Ono, K. (1994). Biosystematic studies on the genus *Isoetes* in Japan I. Variation of somatic chromosome numbers. Journal of Plant Research. **107:** 289-297.

Takamiya, M., Watnabe, M, and Ono, K. (996). Biosystematic studies on the genus *Isoetes* (Isoetaceae) in Japan. II Meiotic behaviour and reproductive mode of each cytotype. Amer. J. Botany. **83:** 1309-1322.

Tanurdzic, M and Banks, J.N. (2004). Sex Determining Mechanisms in Land Plants. The Plant Cell **16:** S61-S71 (Supplement-2004).

Taylor, W.C. and Hickey, R.J. (1992). Habit , evolution and Speciation in *Isoetes.* Ann.Missouri Bot. Gard. **79:** 613-622.

Watson, J.M., Spencer, J. A., Riggs, A.D., and Graves, J.A. (1991). Sex chromosome evolution: *Platypus* gene mapping suggests that part of the human X chromosome was originally autosomal. Proc. Natl Acad. Sci. USA. **88:** 11256-11260.

Willard, H.F. (2003). Genome Biology. Tales of the Y chromosome. Nature., **423:** 810-813.

Ye ,D., Oliveira, M., Veuskens, J., Wu, Y, Installe, P., Hinnisdales, S., Truong , A.T., Brown, S., Mouras, A. and Negrutiu,I. (1991). Sex determination in the dioecious *Melandrium.* The X/Y chromosme system allows complementary cloning strategies . Plant Science. **80:** 93-106.