

Meiotic Analysis and Pollen Viability in *Asparagus racemosus* var. *javanica* (Kunth) Baker

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Abstract: *Asparagus racemosus* var. *javanica* (Kunth) Baker is a diploid ($2n=20$) plant belonging to the family Liliaceae. Meiotic chromosome behavior and pollen viability accounts for fertility and seed setting. Hence, the present study was undertaken to analyse meiotic chromosome behavior. Meiosis showed abnormalities like chromosome stickiness, micronuclei, dicentric bridges with laggards, snapping of bridge, unequal distribution of chromatin material, precocious movement of chromosome, change in orientation and unreduced gametes etc without any treatment. Pollen viability was found to be maximum in acetocarmine: glycerine compared to TTC (2,3,5- triphenyl tetrazolium chloride).

Keywords: *Asparagus racemosus* var. *javanica* (Kunth) Baker, Aceto-orcein, chromosome behavior, pollen fertility.

Introduction

Liliaceae, one of the largest plant families with about 240 genera and 4,000 species distributed throughout the world but the genus *Asparagus* which has been recently moved from the subfamily Asparagaceae to a newly created family Asparagaceae in Liliaceae. Its habitat is common at low altitudes in shade and tropical climates throughout India, Asia, Australia and Africa. (Simon, 1997) Out of several species of '*Asparagus*' grown in India, *Asparagus racemosus* is a woody climber growing to 1-2 m in height. The leaves are like pine needles, small and uniform. Flowering is during July to September. Flowers are white in color. *Asparagus racemosus* is most commonly used in indigenous medicine. Locally it is called 'Shatavari'.

Like many other Liliaceous genera, the cytological studies carried out in *Asparagus* confined that *Asparagus* has ploidy from diploid to hexaploid with basic number of $x=10$ as reported by Darlington and Wylie (1955). However, to the best of our knowledge, there are few published records on meiotic behavior in *Asparagus* which revealed a high incidence of inversion heterozygosis with a remarkable variety of meiotic chromosome behavior and allied irregularities. Increasing information on the meiotic behavior of *Asparagus racemosus* chromosome may give important insights on the numerical and structural chromosome changes involved in the evolution of the genus.

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To analyze plant fertility by meiotic chromosome behavior, seed production and pollen viability in *Asparagus racemosus* without any treatment was the main aim of this study which was not reported earlier is mentioned here and an attempt is made to interpret them and their significance in the light of modern concepts. Basic data on chromosome number, meiosis and pollen fertility are important in the study of biodiversity for germplasm characterization and its applications in plant breeding. Pollen based plant research may be a far cry, but pollen has come to be acknowledged as a single-celled experimental system, offering a fantastic array of information varying from genetics to physiology covering also haploid culture and breeding programmes.

Materials and Methods

Plant Material:

Asparagus racemosus was collected from Melghat forest of Amravati district and grown under suitable condition in pots containing garden soil and maintained in the departmental garden. Voucher specimens are deposited in the Herbarium of Botanical Survey of India, Western Circle, Pune. (*Asparagus racemosus* voucher no. ASPRVD-1) and in the Herbarium of Department of Botany, S.G.B. Amravati University, Amravati.

Cytological Preparation and Meiotic Analysis

Freshly collected young inflorescence were fixed in a Cornoy's solution I for 24hrs

then washed in distilled water and preserved in 70% ethanol at 4°C. Anthers were dissected from the flowers for chromosome count from PMC and the smears were made in 2% Aceto-orcin as well as 2% Acetocarmine. All meiotic phases were analyzed. Occurrence of chiasma was also observed at diplotene and diakinesis. Abnormalities that might impair the meiotic product were taken into account. All observations were made both from temporary and permanent preparations. Photomicrographs were taken mostly from freshly prepared slides using Trinocular Fluorescence Microscope (AXIOSTAR PLUS, M/S Carl Zeiss, Germany).

Pollen fertility

Pollen viability was evaluated using two different stains acetocarmine glycerine (1:1) and 0.5 % TTC. At least 200 to 300 pollen grains per plant were evaluated (Alexander, 1969).

$$\text{Pollen fertility \%} = \frac{\text{No. of stained pollen}}{\text{Total no. of pollen}}$$

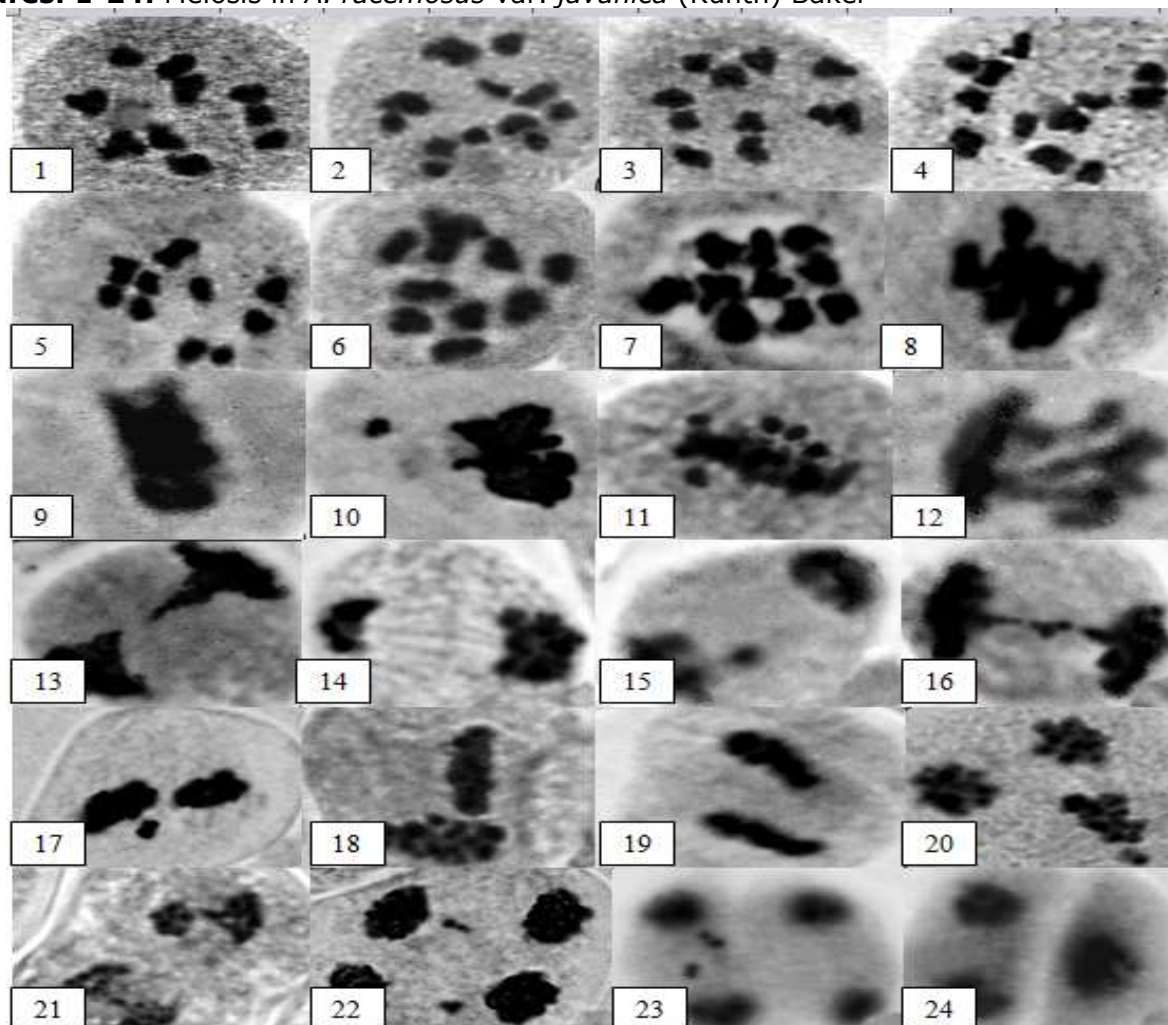
Results

Table.1: PMCs showing meiotic irregularities in *A. racemosus* var. *javanica* (Kunth) Baker

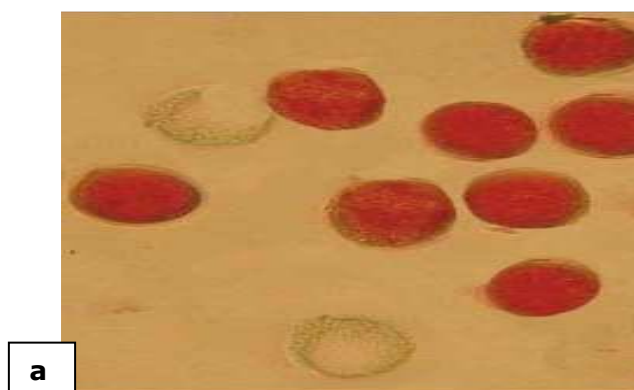
Sr. No.	Phases	No. of PMCs analyzed	Percentage of abnormal PMCs	Abnormalities
1	Diakinesis	191/269	71.00	Multivalent chromosome configurations
2	Metaphase-I	198/258	76.74	stickiness/micronuclei/ equatorial arrangement of chromosome
3	Anaphase-I	135/272	49.63	Dicentric bridge formation with laggards/Snapping of bridge/ Unequal distribution of chromatin material
4	Telophase-I	158/267	59.17	Laggard formation/precocious chromosome movement
5	Metaphase-II	95/162	58.64	Change in orientations/ unoriented univalents
6	Anaphase-II	78/175	44.57	Undistributed chromatin material/change in orientation/ formation of laggards
7	Telophase-II	69/135	51.11	Laggard formation
8	Tetrads	89/231	38.52	Unreduced gametes

Table.2: Pollen viability in *A. racemosus* var. *javanica* (Kunth) Baker by using Acetocarmine: glycerine (1:1)

Sr. No.	Total no. of pollen	No. of viable pollen	No. of non-viable pollen	Percentage of viable pollen grain	Mean of pollen viability
1	40	34	06	85.00	81.36 %
2	30	28	02	93.00	
3	45	39	06	86.00	
4	56	52	04	92.85	
5	43	40	03	93.02	
6	34	29	04	85.29	
7	37	23	14	62.16	
8	51	42	09	82.35	
9	49	37	12	75.51	
10	59	35	24	59.32	
By using 0.5 % TTC (2,3,5- triphenyl tetrazolium chloride)					
1	37	20	17	54.05	36.66 %
2	34	30	04	88.23	
3	20	09	11	40.90	
4	34	12	22	35.29	
5	31	09	22	29.03	
6	38	12	36	31.57	
7	36	03	33	08.33	
8	06	02	04	33.33	
9	28	06	22	21.42	
10	29	07	22	24.13	

Figures: 1-24: Meiosis in *A. racemosus* var. *javanica* (Kunth) Baker

Figures:1) Diakinesis showing 9II+2I, **2)** Diakinesis showing 2III+4II+6I, **3)** Diakinesis showing 9II+2I, **4)** Diakinesis showing 8II+4I (4I are in group), **5)** Metaphase I showing 3III+4II+3I **6)** Metaphase I showing 1IV+8II **7)** Metaphase I showing 10II with 6 II overlapping **8)** Metaphase I showing sticky chromosomes **9)** Metaphase I arranged on equatorial plate **10)** Metaphase I showing micronuclei **11)** Metaphase I showing micronuclei **12)** Anaphase I showing dicentric bridges with laggards **13)** Anaphase I showing snapping of bridge **14)** Anaphase I showing unequal distribution of chromatin material **15)** Telophase I showing laggards **16)** Telophase I showing precocious movement **17)** Metaphase II showing unoriented univalents **18)** Metaphase II showing change in orientation **19)** Metaphase II showing change in orientation **20)** Anaphase II showing undistributed chromatin material **21)** Anaphase II showing change in orientation **22)** Anaphase II showing laggards **23)** Telophase II showing laggards **24)** Tetrad showing unreduced gametes.



a
Viable and non-viable pollen in
acetacarmine and glycerine (1:1)



b
Viable and non-viable pollen in
0.5 % TTC

Result and Discussion

Different meiotic irregularities have been observed (Table.1) of which Metaphase I and Diakinesis shows highest percentage of abnormal PMCs which is above 70% and remaining are in range of 30-60 %. Highest number of bivalents and univalents could be seen in diakinesis and metaphases (Plate I; fig 1,2,3,4,5,6 and7). 20-30 % trivalents were observed in PMC's. 70 % of PMC's showed 10 bivalents, 9 bivalents and 2 univalents whereas 30 % PMC's with other multivalent configuration and very rarely quadrivalents (fig 6). In *A.officinalis* occurrence of high percentage of univalents, laggard chromosomes clumping etc., and reduction in pollen fertility may indicate lack of complete homology between its parental genomes. This species is under cultivation for a long period which may be the reason for heterozygosity and heterogeneity of its genome (Sheidai and Inamdar, 1992). This may be due to genotype variation as shown in *Secale cereal* (Hazoreka and Rees, 1967; Roseweir and Rees, 1962). Cell containing non congression metaphases can be seen where the whole chromosomes set had the tendency to build up a equatorial plate (fig 9), apart from one or two chromosomes were noticed to lie freely in cytoplasmic area (fig 10 and 11). This may lead to the formation of micronuclei in mitotic cycle.

Chromosome stickiness either involves a few bivalents or the whole chromosome complement in MI (fig 8). Sheidai (2001) reported that during anaphase I and II cells shows stickiness, preventing segregation while in some they did not move towards pole normally and results in $n=60$ as observed in *A. gonocladus*.

Anaphase-I with dicentric bridges (fig 12), snapping of bridge (fig 13) and unequal distribution of chromatin mass, (fig 14) was observed. Laggards (fig 15) and their precocious movement (fig 16) were found at telophase-I. M-II also showed sticky mass with micronuclei, disturbed metaphase-II (fig 11,18 and 19) and anaphase-II might be due to the disturbance of spindle apparatus which results change in orientations (fig 20 and 21), change in orientation with chromatin bridge and laggards (fig 22). Laggards and fragments were observed at telophase-II (fig 23). Tetrads were observed with unreduced gametes (fig 24). The unreduced meiocyte formation and doubling of the chromosome

number through both mechanisms of meiotic anaphase failure and syncyte formation do not seem to disturb such a balance as judged from the high pollen fertility and fruit set observed in *Asparagus* species. The existence of polyploidy in the genus *Asparagus* has been noticed by several workers (Darlington and Wylie, 1955; Kar and Sen, 1985 and Sheidai and Inamdar, 1997).

The most common type of meiotic abnormalities was laggards. This abnormality was observed in both first and second meiotic division in *Asparagus*. The induction of laggards at metaphase I in diploids may be extending in all subsequent meiotic stages. Lagging chromosomes did not reach the poles as a result of the absence of spindle fibers or irregular formation. This has a role in the micronuclei formation. In another case, PMCs were mitotically divided without reducing the chromosome number, chromosomes move towards the poles by separating longitudinally and form diads in which chromosome number was not reduced.

In *Chlorophytum comosum* Chromosome Bridge was one the anomaly encountered in most of meiocytes which includes linking chromosomes together in the metaphase and causing them to form one or more than one bridge in the anaphases, a process which could continue up to telophases. The thickness of bridges observed and the number of chromosomes involved in their formation varied among different meiocytes. This leads to loss of genetic material and give rise to polyploids. In *Chlorophytum comosum* sticky chromosomes were observed from early stage of prophase and continued to the final stage of meiosis (Gudadhe et al., (2012). Detail meiosis of *A. officinalis* (Flory, 1932) and autotetraploid *A. racemosus* has been worked out (Venkateshwarlu and Raju, 1958).

All living organisms, irrespective of their complex organization, meiotically reduce their chromosome number to generate haploid gametes at the start of sexual reproduction, which compensate for fertilization and maintain the diploid chromosome number over the generations (Golubovaskya, 1979 and Pagliarini, 2000). Correct chromosome segregation is required for regular cell division and to generate balanced gametes.

Chromosome distribution is generally irregular between these diads. Size of the nuclei is also different in diads with unequal chromosome number (Silva-Stort, 1984). The laggards attributed to irregular orientation of chromosomes (Dimitrave and Gadeva, 1997). Other most prominent meiotic anomalies noticed were disorientation of spindles during the second meiotic divisions owing to spindle irregularities. Disturbances in chromosomal movements occur due to suppression of spindle movement which in turn may be due to changes in cytoplasmic viscosity (Kostoff, 1930; Sax, 1937; Sheidai and Inamdar, 1991).

Sticky metaphases with clumped chromatin material and the precocious chromosome accessions were observed commonly in most of the stages. Chromosome stickiness is caused due to genetic and environmental factors and several agents have been reported to cause chromosome stickiness Pagliarini (2000) and Gaulden (1987) postulated that stickiness may result from the defective functioning of one or two types of specific non histone proteins involved in chromosome organization which are needed for chromosome separation and segregation.

Randomly scattered chromosomal fragments or micronuclei are often associated with irregular shape spindles. The normal functioning of spindle apparatus is crucial for chromosome alignment during metaphase and correct segregation of chromosomes to poles (Shabrangi et al., 2010). Sometimes disturbances during the meiotic course cause abnormalities in the process which can lead to sterility of gametes as well as variation in their genetic constitution. Meiosis is basically normal with few abnormalities involving univalents, chromosome bridges, laggards, micronuclei and polyploid. Rothfels and Nambiar (1975) demonstrated that the bridge may originate from chiasma formation in heterozygous inversions.

A. racemosus var. *subecerosa* shows regular quadrivalent and hexavalent formation which is related with superior survival potential of this species as it grow wild (Grant, 1975).

Pollen fertility:

The meiotic abnormalities obstruct the normal cell division and partially affect pollen

fertility. High meiotic stability ensures high pollen fertility. The amount and quality of pollen produced by a flower is an important component of fitness. Pollen quality is often equated to pollen viability, i.e., the proportion of pollen grains that are viable while viability can be measured in number of ways (Stanley and Linkens, 1974).

The pollen viability in acetocarmine glycerine was found to be 81.36 % (Table No. 2) while in 0.5 % TTC it was 36.66 % (fig a,b). In *A. racemosus* var. *javanica* (Kunth) Baker the longest pollen viability and the smallest pollen volume were found. Pollen viability is always greater than germinability. Both the variables were differently affected by environmental and genetic factors. In the present study fresh pollen grains immediately after anthesis were taken. Germination of pollen is generally most successful immediately after anthesis and viability deteriorates rapidly in most plant species (Kearns and Inonye, 1993).

Pollen viability decreases significantly within one hour of exposure to air after dehiscence. Several staining test has an advantage as an indicator to check pollen viability because these are faster and easier than pollen germination. Pollen viability was greater in acetocarmine glycerine medium as compared to TTC. As reviewed by Aizen and Harder (2007) many genetic studies demonstrate that poor quality pollen can also reduce seed production which is interpreted as a quality limitation. Pollen limitation and low fruit and seed set are affected by pollen viability (Stone et al., 1995)

Conclusion

Meiosis depicted multivalency, laggards, bridges, micronuclei without treatment. High crop yield generally depends on viable pollen grains. The meiotic cycle is highly irregular but the seed set and germination is 100 %, suggesting that the irregularity is not at geneic level but some other factors like the cell environment or external factors must have been responsible. Pollen fertility and viability have a paramount importance in hybridization programme. Pollen viability was extremely high in *Asparagus* in acetocarmine than TTC.

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