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Cytological Studies in *Eichhornia crassipes* (Mart.) Solms

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ABSTRACT

Chromosomal studies were carried out on *Eichhornia crassipes* and the taxon was found to have 32 individual chromosomes with a haploid chromosome number of 16 (n = 16), indicating a clear tetraploid genome of 2n = 4x = 32. Chiasmata frequencies per nucleus/bivalent were analyzed at diplotene and diakinesis revealing a marked reduction of chiasmata at diakinesis as compared to diplotene. The very low terminalization value of 0.43 showed that most of the chiasmata were terminalized at diakinesis and metaphase I. Multivalent associations were frequent suggesting the presence of a translocation heterozygote. Univalents were also of a frequent occurrence. Chromatid bridges, fragments, laggards and eliminated chromosomes were also frequent at both MI and MII. Secondary association of chromosomes occurred with a preponderance of four and eight over other groups. The occurrence of secondary associations and other aberrations were suggestive that *Eichhornia crassipes* is of hybrid origin. Cytological studies of *Eichhornia crassipes* revealed that chromosome behaviour which includes high frequency of chromatid bridges formed (42.52%), high percentage of univalents (15.24%) and the presence of phragmoplast during microsporogenesis does not influence the prolific behaviour of the plant.

Key words: Chromosome behaviour, chromosome studies, meiotic studies, meiotic irregularities, prophase chromosome pairing

INTRODUCTION

Water hyacinth (*Eichhornia crassipes*) is the world's most noxious aquatic weed (Holm *et al*., 1977) has attracted worldwide attention due to its fast spread and congested growth (Li *et al*., 2006). It is a free-floating, monocotyledonous perennial herb, of the order Lilliales and family Pontederiaceae (Davis *et al*., 2004), with a luxuriant growth clogging major water ways and water bodies (Rambuda and Johnson, 2004) as it inhabits rivers, lakes, ponds, canals, drains, dams, ditches, channels, creeks, lagoons and reservoirs (Levine *et al*., 2003). Water hyacinth, an attractive waterweed with a fibrous root system and dark green rounded leaves produces flowers which are light purple in colour carried in dense spikes projecting above the plant. It reproduces both sexually through the production of vast quantities of seeds which are long surviving and capable of remaining viable for as long as thirty years (FAO, 2008) and asexually through complex vegetative procedure. *Eichhornia crassipes* grows fast forming mats which double every 4-5 days under favorable conditions (Thuiller *et al*., 2005). The plant which produces 33,000 tons fresh weight per hectare annually (Morgan *et al*., 2005) is described as one of the most productive on earth as a single mat may contain 2 million others per hectare that weigh 270 to 400 T (Wilson *et al*., 2005). A single plant has a potential of producing at least 140 million others, hence

the marked and incessant interest in its control (Elam *et al*., 2007). The rate of cell proliferation within any population of cells in plants depends on the rate of cell division which represents the time it takes to complete a cell division cycle and the fraction of cells within the population undergoing cell division. The cell division cycle can be divided into two functional phases, S and M phases and two preparatory phases, G1 and G2. S phase is defined as the phase in which the DNA is replicated. Levetin *et al*. (2001) opined, in *Vicia faba,* the mitotic G-cycle takes about 4.9 h for G1, 7.5 h for S, 4.9 h for G2 and 2.0 h for M phases. This is also suggestive that for a typical taxon it takes close to 20 h for a full mitotic cycle to complete. Water hyacinth however, indicating at least two peaks of active division within the same duration suggests that the taxon is mitotically vigorous and that more than two separate cell cycles (peaks) of active division are possible within the period of 24 h. This probably may suggest why the plant is extremely gregarious in the field and proliferates faster than any other known taxon except bacteria.

The plant has adapted favorably to Lake Geriyo environments by successfully establishing itself vegetatively and stabilizing its genetic system enabling the water hyacinth to rapidly replicate new and successful genotypes capable of exploiting to the maximum the lake habitat they have colonized, proliferating in mass perpetuating their genotype through asexual reproduction. Water is an important resource and its infestation with aquatic weed is a major global problem. Since intensive efforts involving huge expenditure and different control measures including cytological investigations brought no success in abating the menace of water hyacinth, attention needs to be focused on ways and means of utilizing the plant.

Fresh water is a precious resource and its management is of particular importance, so control of excessive growths of water hyacinth has been of wide spread interest for decades (Kateregga and Sterner, 2007). The cost of water hyacinth to communities far outweighs the benefits that might occur through its utilization (Julien *et al*., 1996; Kliber and Eckert, 2005). All efforts to control its growth and spread, including physical, chemical and biological methods have failed miserably or are too expensive to carry out on a regular basis (Nagendra, 2001; Calvert, 2002). Water-hyacinth infestation keeps getting worse despite different types of concerted control efforts; hence the concept of eradication through possible genetic means became imperative. The present investigation desires to dig deeper into the possible reasons for the weeds rapid proliferation in the aquatic environment. Consequently, because of this rapid proliferation and attendant problems of this plant, a great deal of literature concerned with various forms of its control has accumulated in recent years (Elam *et al*., 2007). However despite this attention, little is known about the cytogenetics of the plant in Nigeria.

The objectives of this research therefore were to characterize water hyacinth chromosome behaviour during microsporogenesis and to report on any anomalies that may occur during the course of meiotic division.

MATERIALS AND METHODS

Source of materials and study site: The flower buds of water hyacinth (*Eichhornia crassipes*) used for the present investigation was obtained from Lake Geriyo, situated at the outskirts of Jimeta-Yola metropolis, Adamawa State, Nigeria (Fig. 1). It extends between longitudes 12°25'E and 12°11'E and latitudes 9°81'N and 9°17'N. The natural lake started as a small gulley but was later filled with water from rains and some influx from the Benue river. The lake came into recognizable existence in 1950.

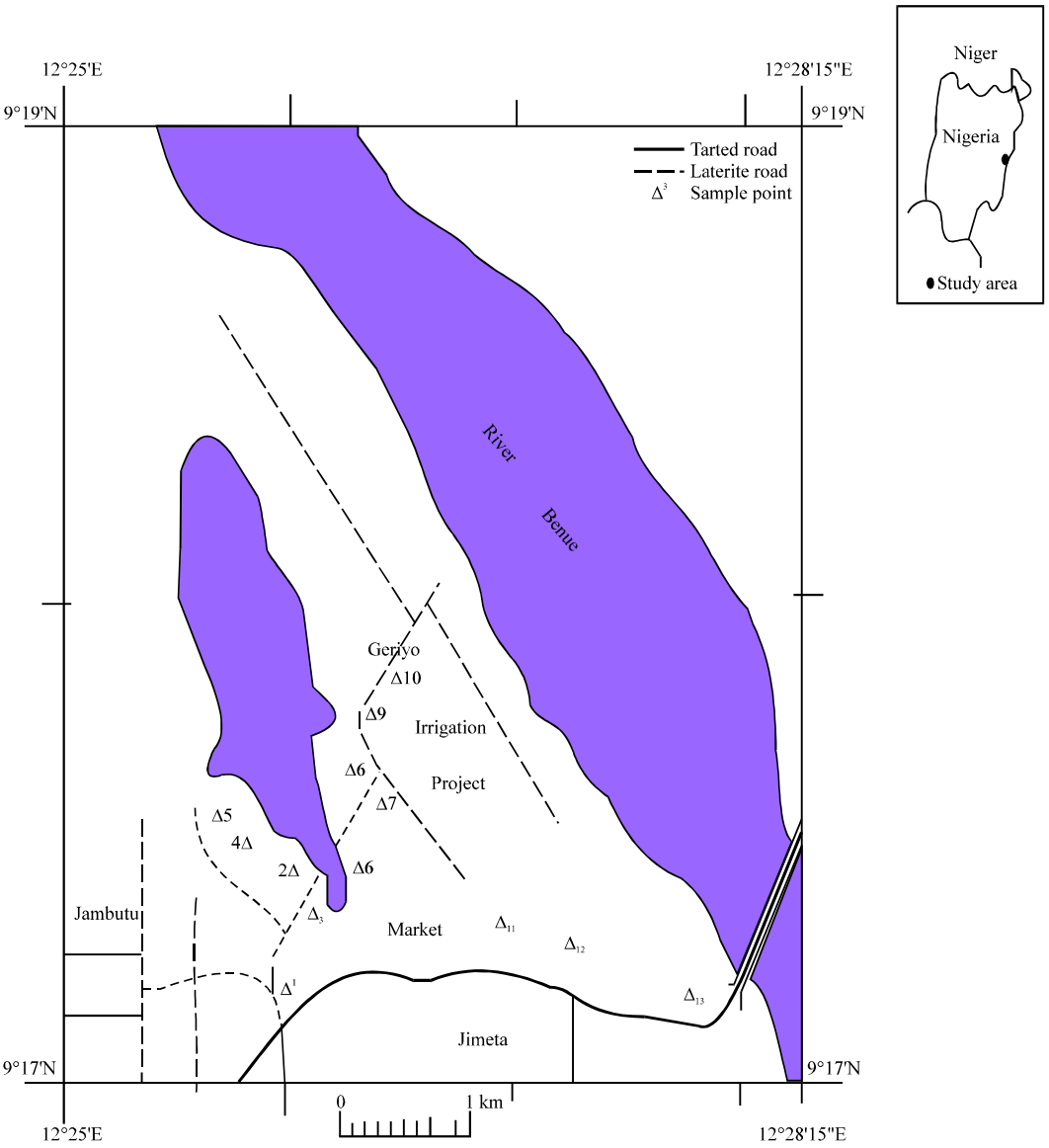

Fig. 1: Map of the study area showing sample locations (Upper Benue Basin 1990 and Fieldwork 2006)

Cytological techniques: For meiotic division, pollen mother cells are analyzed. Small unopened flower buds of anthers collected from the Lake, were dissected and fixed directly in Carnoy's fluid (6:3:1) for 24 h. Processed anthers were preserved in 70% alcohol and stored in a refrigerator at 4°C. Anthers were removed from storage and stained and squashed directly in 1% acetic Orcein after hydrolysis in 10% hydrochloric acid (HCl) for 6 min in a water bath. Excess stains were removed using a blotting paper applied to the edges of the cover slips. Temporary squashes showing good meiotic stages were selected and cover slips sealed using nail varnish applied to the edges.

Photomicrography: Photomicrographs of good meiotic stages were taken using Motic Images plus 2.0 mL digital camera mounted on Kyowa Research microscope.

RESULTS

The earlier stages of meiosis in the present investigation were not suitable for critical analysis, however, at zygotene, homologous chromosomes came to lie side by side in pairs. The chromosomes at this stage showed a granular structure and the stained nucleus revealed in addition to the chromatin reticulum a clear pulsating spherical body i.e., nucleolus (Plate 1a) attached to a specific region on one of the chromatin segments. The point of attachment of the organizer was highly condensed and deeply stained.

The arrangement of chromosomes in the zygotene nucleus is not always at random in most pollen mother cells PMCs. In a few PMCs, the chromosomes were observed to be densely clamped to one side leaving the remainder of the nucleus clear (synizesis arrangements). At this stage pairing or synapsis of chromosomes in intimate associations were observed. Synapsis was observed

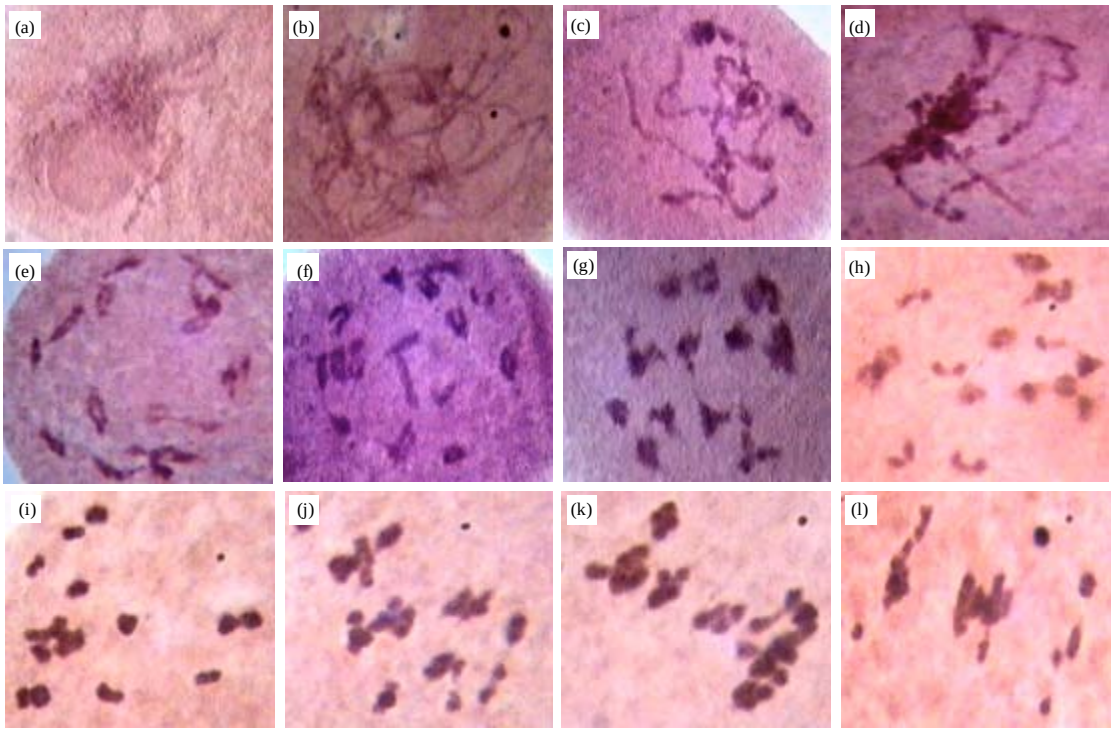

Plate 1(a-l): Different stages of meiosis in *Eichhornia crassipes* (Mart.) Solms, (a) Zygotene nucleus showing clear spherical nucleolus, (b) Pachytene attachment of bivalents to the nuclear wall, (c) Showing pachytene nucleus, (d) Pachytene-Showing non-specific sticky groups of bivalents, (e) Diplotene-Showing attachment of bivalents to the nuclear wall, (f) Showing Late diplotene nucleus (g) Diakinesis-Prometaphase, (h) Late Diakinesis, (i) Prometaphase showing 16 clear bivalents 8 of which are ring bivalents, (j) Late Diakinesis-with 16 bivalents, (k) MI-Showing a disturbed spindle indicating 2 large groups of associating bivalents and (l) MI-Showing orientation of bivalents indicating two linear associating quadrivalents, PMC: Pollen mother cells, AI: Anaphase I, AII: Anaphase II, MI: Metaphase I, MII: Metaphase II, PMCs: Pollen mother cells

to begin in most PMCs at several places along the whole length of the chromosomes. In most segments it was proterminal, beginning at the ends and proceeding towards the centromere.

In a few segments it was procentric, beginning at the centromere and proceeding towards the end. Where pairing was observed to have been initiated, the points of contact between two homologues appeared as dark spots (Plate 1b). At pachytene, most nuclei appeared to be stable; all the chromosomes or parts of chromosomes which were still unpaired, continue to remain unpaired (Plate 1b, c). The chromosomes which are now visibly thicker appeared to be present in the haploid number but each thread can be recognized as two segments closely oppressed forming a bivalent. The points where pairing was initiated were more deeply stained than the remaining parts of the chromosomal segments. These were probably the centromeric regions (Plate 1d).

Paired chromosomes at diplotene were completely separated except at their points of chiasmata. The frequency of chiasmata per bivalent at diplotene has been analyzed and the result of analysis presented in Table 1. The frequency of chiasmata varied between 1.38 and 2.31 per bivalent. However, the total number of chiasmata per individual nucleus varied considerably.

At diplotene, the chromosomes are in a less highly contracted state (Plate 1e, f) than at diakinesis (Plate 1g, h). At diakinesis, the compact bivalents were well spaced out in the nucleus and members of these pairs no longer attract but repel one another. As at diplotene 16 bivalents were usually seen (Plate 1i and j). There is an even distribution of the bivalents in most PMCs at diakinesis. The bivalents at this stage consequently assumed a more rounded shape (Plate 1k, l) with the homologues joined to each other largely at their terminal ends. Most PMCs indicated primary association of bivalents and the most commonly occurring associations are 4 groups of 3 bivalents and four singles (Plate 2m). However, multivalent associations were frequent. The frequency of multivalents observed at diakinesis is presented in Table 2. The haploid number of

Table 1: Frequency of chiasmata at diplotene in 120 PMCs in *Eichhornia crassipes*

Average No. of chiasmata per nucleus	No. of PMCs analyzed	Average frequency of chiasmata per bivalent
37	19	2.31
36	17	2.25
34	16	2.13
31	16	1.94
28	15	1.75
25	13	1.56
23	13	1.44
22	11	1.38
Total		14.76
Average frequency per bivalent		1.85

Table 2: Frequencies of multivalents observed at diakinesis in 328 PMCs

No. of PMCs analyzed	Chromosome compliments	Percentage
253	16 bivalents	77.13
35	15 bivalents, 2 univalents	10.67
10	14 bivalents, 1 trivalent, 1 univalent	3.05
7	14 bivalents, 1 quadrivalent	2.13
15	14 bivalents, 4 univalents	4.57
5	13 bivalents, 1 quadri, 2 univalents	1.52
3	13 bivalents, 1 trivalent, 3 univalents	0.92
328	Total	100.00

chromosomes were found to be sixteen with a regular formation of 16 bivalents per pollen mother cell. The highest and lowest percentage frequencies of PMCs analyzed was recorded as 10.67 and 0.92, respectively. At the diakinesis stage, chromosomes of bivalents were held by one or two chiasmata. The frequency of chiasmata at this stage was found to have reduced, probably due to the now highly contracted nature of the chromosome segments. It was observed to vary from 1.00 to 1.88 per bivalent. The result of analysis is presented in Table 3.

Metaphase I nucleus usually showed 16 bivalents in the spindle equator. Multivalents are usually not visible at this stage because of the extreme compact nature of the chromosomes. However, a polar view of metaphase I plates showed few multivalent configurations. Lateral view of the spindle equator at this stage showed some non-orientate univalents, bivalents and sometimes multivalents (Plate 2n showed some of this phenomenon). Occasionally metaphase I plates with 32 unreduced chromosomes were also seen (Plate 2o).

At Anaphase I, chromosomes which characteristically failed to orientate on the M-1 plate were observed either to lag, divide or were eliminated from the normal chromosome fronts. This state was characterized by chromatid bridges (Plate 2p-r), belated separation of chromosomes (Plate 2s), lagging, eliminated chromosomes and fragments. The frequency of bridges, fragments, laggards and eliminated chromosomes on Anaphase I plate has been analyzed and presented in Table 4 with the highest and lowest percentage frequencies of chromatid bridges, fragments, laggards and eliminated chromosomes was recorded as 5.91 and 3.54, respectively. Complete cytokinesis occurred at the end of Metaphase II before the onset of Anaphase I and completion of Telophase I. The two daughter nuclei at the end of Anaphase I were found to have been surrounded by their own

Table 3: Frequencies of chiasmata observed at diakinesis in 80 PMCs

Total No. of chiasmata per nucleus	No. of PMCs analyzed	Average frequency of chiasmata per bivalent
30	20	1.88
28	15	1.75
28	12	1.50
26	10	1.44
22	8	1.25
18	6	1.13
28	9	1.00
Total		9.95
Average frequency per bivalent		1.42

Table 4: Frequency of chromatid bridges, fragments, laggards and eliminated chromosomes on A1 plate in 508 PMC

No. of PMC showing clean separation	No. of bridges	No. of fragments	No. of laggards	No. of eliminated chromosomes	Frequency of PMC showing clean separation	Percentage
323	-	-	-	-	323	63.58
30	1	1	-	-	28	5.91
28	-	1	1	-	26	5.51
25	1	-	1	2	21	4.92
22	1	1	2	2	16	4.33
22	-	-	1	3	18	4.33
20	1	-	-	3	16	3.94
20	1	-	-	4	15	3.94
18	-	1	-	3	14	3.54
508					477	100.00

individual cytoplasm and showed complete division (Plate 2t). Metaphase II plates were characterized by both regular and irregular chromosomes distributions which probably might have been due to elimination of chromosomes from normal chromosome fronts during first anaphasic chromosome separations. Some Metaphase II plates showed 16-16 non synchronized division (Plate 2u).

At Metaphase II stage, initiation of cell plate formation was observed. Plate 2v showed 16-16 bipolar chromosome separation and the initiation of cell plate formation. Plate 2w showed the completion of cell plate formation indicating bridges and belated separations and Plate 2x showed the telophase II belated separation.

The frequency of distribution of chromosomes on metaphase II plate was analyzed and presented in Table 5 while the character and frequency of secondary association were analyzed and presented in Table 6.

With orientation which completed at metaphase II the chromatids of each dyad was found to have separated and moved to the poles at Anaphase II. This stage was also characterized by completion of cell plate formation indicating bridges, fragments, laggards and eliminated chromosomes (Plates 2w, x). The frequency of chromatid bridges and lagging chromosomes have been analyzed and presented in Table 7.

Other forms of abnormality observed at Anaphase II-Telophase II were that Anaphase II-Telophase II plates were also characterized by restitution nuclei. Some poles were observed to have 32 unreduced single chromosomes in some cases both poles were unreduced. At the end of

Table 5: Frequency of different chromosome distribution on MII plate in 433 PMCs

No. of chromosomes on Metaphase II plate	No. of eliminated chromosomes	No. of PMC analyzed	Percentage
16-16	-	226	52.19
16-15	1	55	12.70
16-14	2	41	9.47
15-15	2	39	9.01
15-14	3	37	8.55
14-14	4	35	8.08
Total		433	100.00

Blank spaces signifies nil

Table 6: Frequency of secondary association on MII plate

	No. of chromosomes							
No. of single chromosomes	2	3	4	5	6	7	8	Frequency
16	-	-	-	-	-	-	-	40
14	1	-	-	-	-	-	-	20
12	2	-	-	-	-	-	-	20
10	-	2	-	-	-	-	-	18
11	1	1	-	-	-	-	-	15
11	-	-	-	1	-	-	-	15
10	3	-	-	-	-	-	-	13
10	-	2	-	-	-	-	-	12
10	-	-	-	-	1	-	-	13
9	-	-	-	-	-	1	-	14
8	2	-	1	-	-	-	-	14

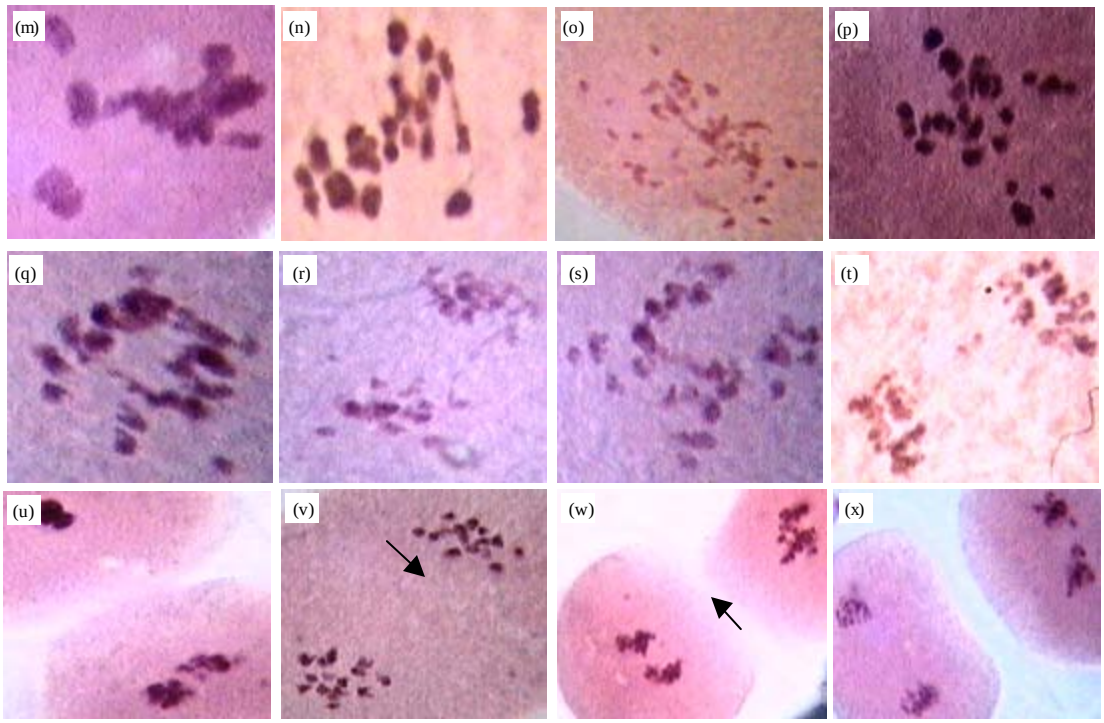

Plate 2(m-x): Different stages of meiosis in *Eichhornia crassipes* (Mart.) Solms, (m) MI-Showing primary association of bivalents-4 groups of 3 and 4 single ones, (n) MI-Orientation of bivalents and 2 non-orientate bivalents, (o) Restituted nuclei showing 32 single chromosomes, (p) AI-Indicating one broken bridge and a fragment, (q) AI with a broken bridge and an acentric fragment, (r) AI-Showing broken chromatid bridges (s): Early AI showing belated separations, (t) Anaphase I, (u) Metaphase II-showing non synchronized division, (v) Metaphase II-showing 16-16 bipolar chromosome separation and the initiation of cell plate formation (phragmoplast arrowed), (w) A-II showing completion of cell plate formation indicating bridges and belated separations and (x) Telophase II showing belated separation, Plate 2v: The phragmoplast is a plant cell specific structure that forms during late cytokinesis (cell plate formation)

Table 7: Frequency of chromatid bridges, fragments, laggards and eliminated chromosomes on AII plate in 450 PMCs

No. of clean separations	No. of chromatid bridges	No. of fragments	No. of laggards	No. of eliminated chromosomes	Frequency of PMC showing clean separation	Percentage
297	-	-	-	-	297	66.00
40	-	1	1	-	40	8.89
29	1	-	1	2	29	6.44
23	1	-	-	2	23	5.11
17	1	2	-	1	17	3.78
15	-	1	-	3	15	3.33
15	-	-	2	3	15	3.33
14	-	-	-1	4	14	3.11
Total					450	

Anaphase II, four distinct quartet groups of chromosomes on each pole were seen. This will later re-organize into a quartet of nuclei at the end of Telophase II stage. The quartet of nuclei will later develop into four distinct pollen grains.

DISCUSSION

The taxon, *Eichhornia crassipes* used in the present investigation was found to be essentially a tetraploid where each chromosome is present four times as pairing is usually possible only between two and four sets of homologous chromosomes (Malgwi *et al.*, 2008). This means that any larger primary association of chromosomes other than these numbers is not expected. In the present investigation, enough time has been given to the study of prophase pairing of chromosomes particularly at zygotene and pachytene where it was possible to follow segmental pairing and other forms of chromosome behaviours. The high percentage of unpaired chromosomes and a high terminalization value could mean a special adaptation to ensure sexual sterility (Darlington, 1965). In this taxon, pairing was observed between two, three, four or more homologous segments and as the prophase stage progresses, chiasmata were formed in the paired regions of the segments which later was found to result in the formation of multiple associations of three, four, five, six or even seven chromosomes at diplotene. Pairing was observed to have come to an end at late pachytene and totally lost by diplotene when an open bivalent structure becomes visible. It is, in general, a process governed by chromosome homologues and the associations during meiosis in most PMCs analyzed characterize water hyacinth chromosome behaviour during microsporogenesis.

Homologous chromosomes were observed to have separated in pairs at diakinesis or as a result of the multiple associations, three or four chromosomes were held intimately by chiasmata to give rise to multivalents in the forms of trivalents and quadrivalents. The moderate percentage frequency of multivalents (7.62%) along with the high percentage of univalents (15.24%) disagrees with earlier reports by Darlington (1965). The occurrence of relatively high percentage of univalents in *Eichhornia crassipes* (15.4%) indicates the possibility of the presence of certain factors which may include one or more of the following:

- The presence of non homologous segments
- Differences in linearity of the genes in the homologous segments
- Structural changes in the chromosomes during the karyotypic evolution of these species or might have originated due to the early disjunction in some of the partially homologous chromosomes as a consequence of which they were unable to form normal bivalents (Swanson, 1957)

The occurrence of multivalents in the forms of trivalents and quadrivalents and sometimes pentavalents as were observed in the *Eichhornia* species investigated is explainable only on the basis and assumption of the presence of a translocation heterozygote. It has been observed that when such an exchange occurs in any given species, pairing amongst interchange segments would show the configuration of a multivalent (Darlington, 1930). The arrangements, orientation and behavior of the multivalents formed may be of such a kind as to result in irregular distribution of the chromosomes at anaphase to the daughter nuclei. Disjunctions of the multivalents were numerically unequal resulting in irregular distribution of the chromosomes so much so that some of the nuclei came to have more or less than the haploid number of chromosomes. It is the opinion of the authors that chromosome conjugation in the form of multiple associations at the pairing stage in the species investigated may have contributed to a certain amount of meiotic irregularities.

As meiosis progressed, there seemed to be considerable reduction in the number of multivalents right from the pairing stage at pachytene to the final terminalization of chiasmata at diakinesis and metaphase I. The reduction at metaphase I of multivalent frequencies has been suggested by Malgwi *et al.* (2008) to be due to dropping off of chiasmata with extreme shortening of the chromosomes or partly due to the localization of the chiasmata to the ends of the chromosomes mates.

There were other observable structures associated with chromosome behavior during prophase. The stained nucleus in most of the PMCs at zygotene and early pachytene showed in addition to the chromatin material of each nucleus, dark rounded bodies, one per nucleus were observed (C.F. Plate 1b). These were the nucleolar structures containing the nucleolus. The nucleolus diminishes in size during and towards the end of the prophase stage and then reappears in telophase. It has been suggested that in late prophase the nucleolus contributes materially to the formation of the visible matrix, while in telophase the nucleolus is formed from the material substances under the influence of a particular chromosome region, the nucleolar organizer (Malgwi *et al.*, 2008). Other bodies observed associated with prophase chromosomes, both at zygotene and pachytene were specialized portions of the chromatin material of most PMCs. These portions unlike the remainder of the chromatin material stain more deeply and are more conspicuous (C.F. Plate 1b). These are the heterochromatic regions of the chromatin segments and are quite distinct from the euchromatic regions which are lightly stained or not at all. Generally, when only a portion of the chromosomes are differentially condensed, the evolved portion are usually located distally or immediately adjacent to the centromere. In the *Eichhornia* species investigated, differential condensation occurred immediately adjacent to the centromere while the long distal segments remain euchromatic.

At metaphase of meiosis, however, such highly condensed chromosomes or chromosomes parts were generally indistinguishable from euchromatin. This is because the whole chromosomes were highly contracted and therefore stained uniformly.

The tetraploid *Eichhornia* species analyzed in this investigation have small chromosomes which are a characteristic of this genus and this agrees with Krishnappa (1971). Going by the assumption that the shorter the chromosome length the fewer the frequency of chiasmata, it is therefore not surprising that the diplotene stages of the present taxon showed a moderate frequency of chiasmata per bivalent at diplotene and diakinesis. Analysis showed that the longer chromosomes at diplotene stage formed more chiasmata (1.85 average) at least two chiasmata per bivalent than at diakinesis with 1.42 average.

Terminalization value for the tetraploid species investigated has been estimated to beless than 1.00 (0.43). This high terminalization value of 0.43 suggested that at diakinesis and metaphase I almost all the chiasmata were terminalized. Higher terminalization value of less than 1.00 may in some cases as opined by Malgwi *et al.* (2008) be due to slow rate of development in the cells ensuring that the chiasmata have more time to become terminalized. The shortness of the chromosomes complements characteristic of the present taxon is consequential to the high terminalization value observed. It is therefore possible that the terminalization value of 0.43 in this investigation is actually dependent on chromosome lengths of the species and not on the level of species ploidy as earlier suggested by Sangowawa (1985).

In the tetraploid *Eichhornia* species investigated, the chromosomes during meiosis showed a characteristic grouping that is conspicuous at metaphase II (C.F. Plate 1j). Fine threads connecting the secondary associated chromosomes as observed by Mochida *et al.* (2004) in *Zea mays* were not

observed on some of the metaphase II plates. Analysis of metaphase II plates (Plate 2u, v) in *Eichhornia crassipes* indicated that there were preponderances of 2 over other groups. However, the most frequent combination of associating chromosomes at metaphase II for this tetraploid was groups of 2 and 4 free chromosomes.

Secondary association as observed in this species has been used as one of the criteria for determining the polyploidy nature of many plants (Stebbins, 1985). He observed that stronger secondary association acts between chromosomes which although identical have failed to form chiasmata in multivalent pairing associations. From the findings of the present investigation, secondary association in *Eichhornia crassipes* species seemed to be due to actual attraction, presumably caused by the same forces that gave rise to primary or normal pairing. However, a certain degree of clumping due to bad fixation is not altogether ruled out.

It is probable from the observations of secondary association in this tetraploid species, that the original basic number in the genus *Eichhornia* is less than 8, probably 4 and that species of this genus are autopolyploids. Observations of secondary association indicated the preponderance of groups of 2 and 4 free chromosomes which seems to support the assumption that the basic number is less than and possibly 4. The presence of 4 single chromosomes seemed to indicate the genome of their putative ancestors and now even after a long period of genome hybridization they are still so alike that they seem to attract each other in one group of 4 free chromosomes.

The species under investigation demonstrated a fairly large number of PMCs with chromatid bridges at Anaphase I and II. Breakage of a chromatid bridge usually produces a fragment but since it has been observed in some PMCs where a bridge occurred without a fragment simultaneously, it meant that the fragments were produced before the breakage occurred. Unclean anaphasic separations characterized by a single bridge and a free fragment were also observed in this species. This is an indication that *Eichhornia crassipes* is heterozygous for an inversion. Bridges were not frequently observed at Anaphase II, although it was unexpected in view of the comparatively low chiasmata frequency of the taxon and the high terminalization value (<1).The percentage of chromatid bridges observed during the course of investigation in the tetraploid species was found to be 42.52%. The chromatid bridges formed may at any point during chromosome migration to the poles at Anaphase stages break up, thus creating an imbalance in the linearity of genes in the chromosomes. Essentially it would either produce chromosomes with additional segments or chromosomes lacking certain segments. The presence of either a deficient or duplicated chromosome in the genotype of any gamete may prove to be lethal or beneficial to the individual. However, these might not be the same in all cases, provided the missing or additional segments were very small (Darlington, 1965). In the present investigation, it appears that the high frequency of chromatid bridges formed (42.52%) seems to indicate that chromatid bridges were not beneficial to the ecological aptitude of the taxon.

The presence of univalents during meiosis appeared to be a common feature in the *Eichhornia* species investigated. They might have arisen in three ways, either from a chromosome which altogether failed to pair at zygotene or one that paired to form a normal bivalent but whose two chromosome segments separated at diplotene because no chiasmata were formed between them. It is also probable that segmental differences in combination with genetic dissimilarity of individual chromosomes, as maintained by Mochida *et al.* (2004) was responsible for the failure of pairing amongst pairable chromosome mates in the *Eichhornia crassipes* investigated. The percentage failure of pairing is high (15.24%) indicating a very weak homology between the four sets of chromosomes of the prophase stage. Thus it is possible that the occurrence of univalents at

diakinesis and metaphase I in this investigation might have been due presumably to the subsequent dissociation of chromosomes following partial synapsis at zygotene or to the taxon's extreme adaptation to asexual reproduction ensuring gene differentiation in homologues. Thus similar genes that usually attract each other have now become essentially dissimilar.

In the present investigation, micronuclei were not too frequent despite the high number of univalents and fragments formed. The reasons for these could come from the fact that cytokinesis occurred twice, one at the end of first division with the formation of phragmoplast and the other at the end of Telophase II. Univalents were able to be dissociated into the cytoplasm.

Relationships between the formation of chromatid bridges and the incidence of unpaired chromosomes have been observed in the *Eichhornia crassipes*. It showed a clear cut relationship between bridge formation and the incidence of unpaired chromosomes percentages. It is also probable that precocious anaphasic separations, genetic factors (e.g., dissimilar in the linearity of the genes) were also responsible for the formation of univalents in water hyacinth in addition to inversion heterozygosity. The fast terminalization of chiasmata along with too many similar segments competing for pairing in the tetraploid were also possible causes of univalents in this investigation which consequently lead to sterility.

CONCLUSION

Phragmoplast which is a system of microfibrils associated with the development of the cell plate in dividing cells particularly in somatic cell divisions is a feature thought to be essentially related to equatorial cell divisions only. Meiosis however is a reductional division that results in the formation of gametes during Microsporogenesis. The present investigation revealed the formation of phragmoplast at anaphase. The presence of phragmoplast during the course of Microsporogenesis in the present taxon seems to essentially confirm that the meiotic first divisions of meiocytes are actually mitotic in nature and that some plant species undergo full equational divisions separating the two poles into two daughter cells mitotically before undergoing the second reductional division which might have contributed to the meiotic anomaly Apart from maize, phragmoplast formation during meiosis has never been reported again especially in hydrophytes.

From the results of cytological studies carried out in this investigation, it is evident that chromosome behaviour does not influence the prolific behaviour of the plant. Water is an important resource and its infestation with aquatic weed is a major global problem. Since cytological investigations brought no success in abating the menace of water hyacinth, the authors' of this paper are of the opinion that further research be carried out on other aspects of the plant such as, karyological studies, reproduction and mitotic index.

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