DICENTRIC CHROMATID BRIDGES AND POLLEN STERILITY IN *CLEOME GYNANDRA*

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The present investigation revealed that the haploid chromosome number of *Cleome gynandra* (Linn) is n=9 and 4n=36. It is essentially a tetraploid. Meiotic chromosome behaviour in pollen mother cells (PMCs) in this tetraploid at Anaphase I and II were highly irregular. The percentage of chromatid bridges observed during the course of investigation in this tetraploid species was found to be 40.35. In the present investigation the tetraploid species investigated showed 47.60% morphologically fertile pollens, and 52.40% morphologically sterile pollens. It appears that the high frequency of chromatid bridges formed (40.35%) and 52.40% pollen deformity seems to indicate that chromatid bridges were not beneficial to the ecological aptitude of the taxon with regards to its sexual reproduction as deformed pollens often are nonviable. From the results of the present investigation, it was clear that structural alterations in the chromosomes due to largely frequent bridge formation appeared to be part of a common factor contributing to the sterility of pollen grains in *Cleome gynandra*.

Keywords: Cleome gynandra, Pollen grains, Chromatid bridges.

Introduction

Cleome gynandra (Linn) popularly known as cat's whiskers is an erect, annual herb which sometimes becomes woody with age and an abundantly available weed found growing all over world and throughout Nigeria particularly in Adamawa State, in barren land, paddy fields, road sides, open grass lands and in crop fields. In most countries it is never cultivated but grows spontaneously everywhere. *Cleome gynandra* is a native to; Australasia, North, West, Central and East Africa is used all over the world as an important leafy vegetable, eaten as a pot herb, flavoring in sauces and consumed fermented (Kokwaro, 1994). Indigenous knowledge indicates that *C. gynandra* has several nutritional uses (Isa *et al*. 2008b). The weed is used as a medicinal plant to cure diseases such as; dysentery, gonorrhea, malaria, diabetes, rheumatoid arthritis, epilepsy, scurvy and Marasmus, improve eyesight and it provides energy for pregnant and lactating women thereby easing childbirth and helping them regain normal health. In all over the world in different countries it is used for its remarkable nutritional, medicinal and antioxidant properties (Isa *et al*. 2008a). High protein and minerals content of this plant has made it a highly economically important weed.

Investigations carried out by earlier researchers in different species of plants revealed a number of chromosome bridges and fragments in mitotic and meiotic anaphase cells of various species following their exposure to irradiation and to certain chemicals; and such bridges and fragments have appeared spontaneously in mitotic anaphases of various forms. In these cases the bridges and fragments have been shown to result from breakage of the chromosomes with reunion of some of the broken ends so as to produce dicentric chromatids (Malgwi *et al*. 2009).

The presence of bridges at second anaphase, the formation of univalent bridges at first anaphase, and numerous fragments have led to the conclusion that chromosome breakage and reunion occur in meiosis in most species and also in mitosis of few species of plants Gayatri *et al.* (2003). Bridges are formed when chromosome breakage is followed by reunion of chromatids of centric segments in such a way that dicentric chromatids are produced ((Isa *et al*. 2007).

Malgwi & Khan (2003) opined that, the chromosome breakage in meiosis is caused by environmental factors while in mitosis it has a genetic basis with a variation in frequency of aberrations and suggested that the variation was due to inherent differences in susceptibility to spontaneous chromosome breakage.

Whichever way a bridge is produced researchers have attributed its presence in a taxon to high incidences of pollen sterility. Upcott (1937), demonstrated in Tulipa that more than 10% inversions were responsible for considerable sterility of pollen in the taxon. Swanson (1957) concluded that in addition to aberrations usually recorded during meiotic divisions which include presence of chromatid bridges, laggards, fragments and other abnormalities; physiological or environmental factors are also capable of causing deficiencies and duplications during segregation as a result of which unviable pollen grains would be formed in greater numbers. With these controversies, the objective of the present investigation is to look into the behavior of meiocytes especially during chromosomes disjunctions at AI and AII and relate our findings to pollen sterility in *Cleome gynandra* (Linn).

Materials and Methods

Small flower buds were collected from dense strands of *Cleome gynandra* (Linn) growing in the wild farms around Girei. Buds were crushed and fixed directly in Carnoy's fluid (6:3:1) mordanted with 2 drops of 90% Orthophosphoric acid for 3 hours and were stored in 70% alcohol in a refrigerator at 4^{O}C until when it was required for use. For chromosome analysis anthers were squashed directly in 1% acetic Orcein, after hydrolysis in 1NHCL at 60 ^{O}C for 8-10 minutes in a glass slide and then examined for their ability to absorb the stain. Grains full of stained materials with regular shape were counted as fertile while grains that were irregular in outline, imperfectly filled or empty of stain were counted as sterile. In this investigation, pollen sterility refers toe percentage of "sterile" grains. Photomicrographs were taken from the temporary squashes showing good dividing stages with Wild M20 photomicroscope.

Results

Analysis of meiotic activities were restricted to Anaphase I and II only. Anaphase I (AI) in this species showed bivalents and multivalents disjoining and migrating to the poles. Some of the univalents that failed to orientate at metaphase I (M-I) either lag behind or are eliminated from the normal chromosome fronts. Anaphase I continues with the clumps separating but still joined by stretches of sticky chromatids. The moving fronts appeared as lamps of sticky stretches. This stage was also characterized by dicentric chromatid bridges, belated separation of chromosomes, Lagging as well as eliminated chromosomes.

The frequency of dicentric bridges, fragments, laggards and eliminated chromosomes on Anaphase I has been analyzed and presented in Table 1.

Table 1. Frequency of chromatid bridges, laggards and eliminated Chromosomes on A1 plate in *Cleome gynandra*

No. of pmc showing clean separation	No. of bridge	No. of laggards	No. of eliminated chromosomes	Frequny of pmc showing clean separation	Percentage (%)
195	-	-	-	195	55.39
-	1	1	-	75	21.31
-	2	-	2	25	7.10
-	1	1	1	15	4.26
-	-	1	2	15	4.26
-	1	2	3	11	3.13
-	1	-	-	09	2.56
-	2	1	2	07	1.99
			Total	352	100%

As seen in anaphase I, the same sticky stretch that appears chromatid bridges extends into a second separation known as Anaphase II. The presence of this phenomenon at this stage indicates that the sticky bivalents passed over with proper disjunction. The frequency of chromatid bridges and other aberrations presented in table II.

Table 2. Frequency of chromatid bridges, fragments, laggards and eliminated chromosomes on A1I plate in *Cleome gynandra*

No. of pmc showing clean separation	No. of bridge	No. of fragment	No. of laggards	No. of eliminated chromosom es	Frequency of pmc showing clean separation	Percentage (%)
150	-	-	-	-	150	59.52
-	2	-	-	-	50	19.84
-	1	1	1	-	25	9.92
-	2	1	-	-	13	5.16
-	1	-	-	1	07	2.78
-	1	-	1	-	07	2.78
				Total	252	100%

In this species, formation of pollen was observed to be highly irregular, judging from the high incidences of morphologically sterile pollen grains. Out of 488 pollen mother cells (PMCs) scored at this stage the clamped unreduced poles of these unsynchronized stages showed 15.73% frequency. They may probably never separate before pollen formation. This probably may probably may result in two types of pollen grains.

Two types of pollen grains were produced at the end of AII. Out of 935 pollen grains analyzed 445 (47.6%) were found to be morphologically fertile and 490 (52.4%) were morphologically sterile. Analysis of observations during pollen formation showed 40.48% abnormalities.

Discussion

The species under investigation demonstrated of PMCs with chromatid bridged at both 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 occur without a fragment simultaneously, it means that the fragments were produced before the breakage occurred. Unclean anaphasic separation characterized by a single bridge and a free fragment was observed in the species investigated. Bridges observed in anaphase II indicated that the plant was heterozygous for an inversion.

The high frequency of chromatid bridges in *Cleome gynandra* (40.35%) may at any point during chromosome migration to the poles at Anaphase stages break up, creating an imbalance in the linearity of genes in the chromosomes. It either produces chromosomes with additional segments or chromosomes lacking certain segments, heralding the formation of new ones with either a deficient or duplicated segments (Darlington and Wylie, 1955).

Abnormalities of chromosome aberrations during the cause of meiosis have been the cause of pollen sterility. Such an assumption is in support of Malgwi *et al*. (2009) findings in *Crotolaria species* where they recorded all grades of aberrations and concluded that pollen sterility is closely related to meiotic irregularities. Choudhuri (1975) have correlated pollen sterility with incidence of bridge formation this is in line with Upcott (1937) and Swanson (1957) who demonstrated that chromosome aberrations were responsible for sterility of pollen. The univalents were sometimes lost in the cytoplasm or were eliminated from the normal chromosome fronts. This probably led to the deficiency in chromatin material in some nuclei. Some chromosomes may be present in duplicate at one pole, while the other become deficient for one chromosome. This various anomalies might have led to pollen fertility.

As revealed by earlier researchers, when chromatids are broken at anaphase, each bridge gives rise to two chromatids which may either be deficient in some parts of the segments or may carry in duplicate other portions of the bridge, depending upon the position where the bridge was ruptured. The tetrads with the deficient, chromatids may not develop into viable pollen but will give to a collapsed or deformed pollen grains.

As it is clearly shown from the results of the present investigation which revealed 40.35% chromatid bridges and 52.40% sterile pollen, it is clear that there is a direct relationship between the incidence of chromatid bridges and malformation of pollen. The sum total of the incidence of chromatid bridges correlates directly with the incidence of deformed pollen. Therefore to assume that only the preponderance of chromatid bridges is responsible for pollen fertility or sterility does not seem deep seated. Such fertility or sterility may in all honesty be correlated to general irregularities rather than to chromatid bridges alone. Thus, it is clear that pollen fertility or sterility in the tetraploid species is both genic and chromosomal. Variations in pollen fertility be attributed to the amount of structural changes present in the karyotype of the species. These assumption is in support of Garba & Gorsic (1956) findings in Collinsia. They concluded that in addition to aberrations recorded during meiotic division which was apparent from the presence of bridges, laggards, fragments and other abnormalities, physiological or environmental factors are also capable of causing deficiencies and duplication during segregation as a result of which inviable grains would be formed in greater number (Darlington & Dark, 1932).

Conclusion

Meiotic chromosome behaviour in the taxon *Cleome gynandra* at anaphase I and II were highly irregular leading to formation of chromatid bridges along with chromatin fragments to the order of 40.35%. From the results of our findings, it is our opinion that structural alterations due to bridge and fragment formations were responsible for the sterility of pollen grains. Formation of dicentric chromosomes involving chromatid bridges and fragments was found to be a usual phenomenon in this species indicating that it is of hybrid origin. Hybrids are usually characterized by serious meiotic irregularities, like lagging and eliminated chromosomes along with frequent formation of chromatid bridges and sterility of pollen grains. From the results of the investigation, it is clear that structural aberrations in the chromosomes appeared to be common factor contributing to the sterility of pollen grains. Therefore, there seemed to be a definite relationship between aborted pollen grains and the percentages of aberrations in this tetraploid species indicating a good evidence for regarding them as of hybrid origin. Hybrids are usually characterized by serious meiotic irregularities like lagging and elimination of chromosomes along with frequent formation of chromatid bridges and deformity of pollen grains. All these characteristics of hybrids were observed in this tetraploid species investigated and this may be regarded as characteristic and conclusive evidence of the hybrid nature of this *Cleome* species. The non-synchronization of the meiotic process within anthers and PMCs were clear indication of an unstable genetic system.

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