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Research Article

EFFECTS OF 1:1 MIXTURE OF 8-HYDROXYQUINOLENE AND ALPHA-BROMONAPTHALENE CHEMICALS ON MEIOSIS IN *GYANDROPSIS GYANDRA LINN. (CAPPRIDACEAE)*

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ABSTRACT

Investigations of the effects of 1:1 mixture of 8-hydroxyquinolene and saturated solution of Alpha-bromonapthalene on the meiotic process in *Gyandropsis gyandra* Linn. was carried out with an aim of exploring the nature of any mutagenic impart of the chemicals on normal meiosis in this plant. The combined mixture of 1:1 8-hydroxyquinolene and alpha-bromonapthalene affected first and second meiotic divisions significantly, causing chromosome stickiness (32.74%) at pachytene and anaphase-1. The mixture also disorganized the Metaphase-1 chromosomes causing bivalents to agglutinate into small groups leading to formation of ring bivalents (6.06%). Generally, chromosomes staining, configuration and separations were found to be greatly affected by the mixture. Heavily clumped bivalents characterized anaphase-1 separations in most pollen mother cells. This appeared like highly condensed chromosome masses with stretches of chromatid segments forming bridges which eventually culminated in a reasonably high level of restitution (18.32%). Most pollen mother cells obviously could not go through to second division.

KEY WORDS: Meiosis, restitution, chiasmata, 8-hydroxyquinolene, alpha-bromonapthalene, *Gyandropsis gyandra.*

INTRODUCTION

The possibility of inducing structural changes by exogenous agents such as radiation, viruses and chemicals has been a subject of great interest in the Biological Sciences. After the pioneering works by Brumfield (1943), a wide variety of chemical compounds have shown to be very effective mutagenic agents in both plants and animals. Chemical mutagens induce diverse gene mutations, chromosomal mutations, as well as biophysiological changes. Degradation of DNA and inhibition of DNA synthesis is reported to occur as a result of treatment with the antibiotics mitomicin-C (Reich *et al.*, 1961) and streptonigrin (Radding, 1963). Induction of mutation in plant species is often associated with cytological abnormalities (Burghate *et al.*; 2013). The meiotic process, an event of very high evolutionary stability (Pagliarini 2000), which also provides important clues to the novelties of the karyotype, culminates in the reduction of the diploid chromosome number to haploid. Cytogenetic information and the degree of cytological aberrations, either in mitosis or meiosis, are regarded as dependable criteria used by plant mutation breeders for estimating the effect of mutagens on the crop plants.

The chemical compounds 8-hydroxyquinolene and alpha-bromonapthalene are individually known to interfere with spindle formation during cell divisions, thereby reducing yield in plants [Brumfield, 1943]. Chemical compounds

such as these two which could facilitate a study of the meiotic process (chromosome configurations, behavior and structure) would indeed be important cytological tools. In view of this, a 1:1 mixture of 0.02% 8-hydroxquinolene and saturated aqueous solution of alpha-bromonapthalene was tried on the meiotic nuclear process in *Gyandropsis gyandra* L. with the view to studying their combined effects on the plants meiotic nuclear processes which may suggest their possible usage in the cytological investigation of chromosome configurations and behavior during meiotic division of plants.

MATERIALS AND METHODS

The method of pretreatment of pollen mother cells by Darlington (1965) as adapted by malgwi *et al* (2000) was adapted with slight modifications in the present investigation. The pretreatment mixture for the present investigation was 50mls of 0.02% 8-hydroxyquinolene mixed with 50mls of saturated aqueous solution of alpha-bromonapthalene. The seeds of *Gyandropsis gyandra* L. were planted in the botanical garden of the Department of Plant Science and Biotechnology, Imo State University, Owerri. Young unopened flower buds were harvested between 8.30 – 9.00 am. The flower buds were dropped into some quantity of the 1:1 mixture of 0.02% 8-hydroxyquinolene and saturated aqueous solution of alpha-bromonapthalene in a specimen bottle, and maintained at 10^{0c} in the refrigerator for twenty four hours. After the pre-teatment period, the flower buds were removed and washed thoroughly in running tap water for twenty minutes, rinsed in distilled water for 15 minutes and fixed directly in carnoys fluid (6:3:1) for 24 hours. At the end of fixation period, the flower buds were washed in 30% ethanol and stored in 70% ethanol in the refrigerator at 4^{0c} until required for cytological investigation.

For cytological analysis, flower buds were removed from the 70% alcohol, washed thoroughly in several changes of distilled water for fifteen minutes, then hydrolyzed in normal hydrochloric acid (INHCL) in a water bath for eight minutes at 60^{0C}. They were then squashed directly in 1% aceto-ocein solution. Temporary squashes showing the desired stages were sealed with a nail polish.

Control was made up of few flower buds which passed through similar process as above but without the pretreatment chemicals. Photomicrographs of desired stages were taken using motic images plus 2.0 digital camera mounted on a trinocular microscope connected to Laptop computer. Each set of experiments was repeated three times with a period of 2 days apart, so as to ascertain the actual effect of the chemical mixture.

RESULTS: Prophase stages of meiosis in all the treated buds in the three separate repetitions appeared irregular and highly affected by the chemical mixture. Chromosome agglutination caused by an extreme segmental clumping occurred (Plate 1A). Several PMCs also showed groups of heavily stained masses of chromosomes. First prophase stages were indistinguishable. At Metaphase 1 (M-I) plates, non-specific sticky adhesions of bivalents appearing as if the cells were still in prophase stage were seen. The percentage frequencies of these phenomena in the three separate replicate treatments are presented in Table 1. Two hundred and fifty (250) PMCs were analyzed for each first and repeated treatment.

Table 1:. Frequency of non-specific sticky adhesions of bivalents in PMCs of treated and non treated flower buds of *G. gyandra* L.

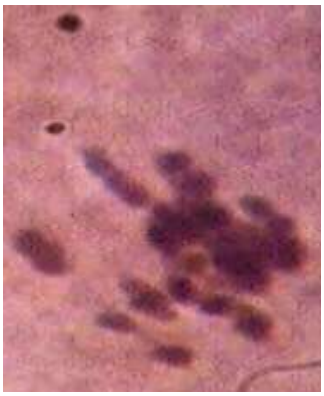
Treatment	Plant part	Frequencies(%)			Mean frequency(%)
		REP i	REP ii	REPiii	
Treated PMCs	Flower buds	33.73	32.93	31.57	32.7
Untreated [Control]	Flower buds	2.75	2.63	2.97	2.7

From the analysis in table 1, the 1:1 8-hydroxquinolene and alpha-bromonapthalene mixture produced very high percentages of non-specific sticky adhesions of bivalents while the abnormality was negligible in the control buds. Only 13.27% of diakinesis-metaphase-1 cells showed the chiasmata well defined with good chromosome staining. Staining was also well improved in these PMCs. Another observation made on the treated PMCs which occurred less in the control is the frequent formation of ring bivalents (Plate 1B). Analysis of this phenomenon is presented in Table 2.

Table 2: Frequencies of occurrence of Ring Bivalents

Treatment	Plant part	Frequencies(%)			Mean frequency(%)
		REP i	REP ii	REPiii	
Treated buds	Flower buds	5.33	6.75	6.10	6.06
Control (Untreated)	Flower buds	0.53	0.47	0.83	0.61

Analysis of Table 2 shows that ring bivalents occurred more in the treated flower buds [6.06%], a phenomenon that is quite negligible in the control buds (0.61%).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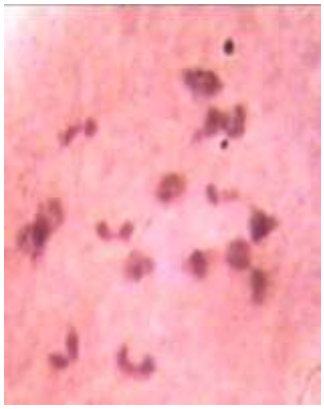
B


C

Plate 1:

A. Non-specific sticky adhesion of chromosomes
B. High frequency of Ring bivalents
C. Chromatid bridge and non-disjuncted chromosomes

M-I plates were also irregular in a large number of PMCs. Analysis of M-I plates with regular chromosome distributions of 18-18 was only 25.60% while M-I plates with irregular restituted nuclei with 36 chromosomes was 19.87%.

Table 3: Frequency of unreduced nuclei

Treatment	Plant part	Frequencies(%)			Mean frequency(%)
		REP i	REP ii	REPiii	
Chemical mixture pretreatment	Flower buds	24.6	15.3	19.7	19.87
No chemical pretreatment	Flower buds	1.02	0.84	0.89	0.92

In the untreated PMCs this abnormality was very minimal (0.92%)(Table 3). Anaphase I and telophase I [T-I] in treated buds showed stretches of segments connecting the two lumps of chromosomes mass at the poles appearing like chromatid bridges (Plate 1C). Second division was not easy to follow and at the end of meiosis 57.63% of the pollens were deformed.

DISCUSSION

Sensitivity of the chromosomes to the chemical mixture was well indicated in both the first and the second meiotic divisions among the three set of chemical pretreatments. The 1:1 0.02% 8-hydroxyquinolene and aqeous alpha-bromonapthalene applied for the period of Twenty four hours was found not only to hampere the clarity of bivalent chromosomes at prophase-I and M-I, but also inhibit spindle formation of both M-I and M-II and also to induce chromosomes stickiness. Chromosome stickiness has been the subject of cytological studies in several plant species. Among well-known maize mutants are those displaying chromosome stickiness (Defani-Scoarize *et al.* (1995, 1996), Caetano-Pereira *et al.* 1995a,b, 1997, 1998b). Chromosome stickiness may occur spontaneously when no external agents are involved as in the so-called hereditary stickiness (Gaulden, 1987) where a spontaneous mutation in the structural genes coding for the proteins involved in chromosome separation and segregation results to failure of these two processes, thereby fostering chromosome clustering. Chromosome stickiness is also induced by exposure to external agents such as chemical mutagens and radiation, which will act as mutagens. Gaulden 1987 postulated that these mutagens exert direct action on one or more types of specific non-histone proteins involved in chromosome organization leading to defective functioning of these proteins. Pessim *et al;* 2015 adds that in severe cases, the action of these agents provokes formation of single or multiple pycnotic nuclei which culminates in chromatin degeneration. In the present investigation, the percentage difference in frequency between the treated buds and the untreated ones with regard to this abnormality (32.74% and 2.7% respectively) indicates that the chemical mixture of 1:1 0.02% 8-hydroxyquinolene and aqeous alpha- bromonapthalene may have been responsible for the high level of stickiness in the treated buds.

Where diptotene plates were clear in the treated buds, the distal chiasmata of the bivalents remained unseparated resulting in circular or ring shapes. Ring bivalents as observed in the three different treatments were fewer in the untreated buds. This is in an indication that in this species, normal chiasma movement begins interstitially and moves towards the terminals. The negligible frequency of the ring type bivalents in the untreated buds (0.61%) suggests an accelerated terminalization of chiasmata once initiated. The difference in frequency of ring bivalents between the treated and the untreated PMCs (6.06% and 0.61% respectively) is an indication that although 8-hydroxyquinolene and aqeous alpha-bromonapthalene when applied separately are capable of slowing down or stopping spindle formation in somatic cells, however their combined effect in the ratio experimented seems to be also effective in slowing down chiasma movements. This presumably may have been due to an induced increased

reduction of chromatid repulsion forces by the chemical mixture leading to very slow chiasma movement, thus the distal ends of the chromatids remain attached by their terminal chiasmata. Low chiasma frequency as observed in this investigation has been reported in other taxa. Aguiar-perean *et al*. 1984 documented that low chiasma frequency leads to the appearance of univalent chromosomes which get lost during the separation process and their loss imparts negatively on pollen fertility and seed production. Piaglarini *et al*, 2000 reported a positive correlation between chiasma frequency and combining ability in many inbred lines of maize. Koduru and Rao 1981 attributed low chiasma frequency to precocious chiama terminalization or presence of asynaptic/desynaptic genes. Irrespective of their originating source Pessim 2015 concludes that they lead to similar behavior – Univalents exhibiting precocious ascension at metaphase one or remaining as laggards at anaphase one; either of which may precipitate micronuclei formation at telophase down to meiosis two.

The comparatively higher frequency of first division restitution (FDR) in the treated PMCs (19.87%) as contrasted with the low frequency in the untreated PMCs suggests that the chemicals used in the treatment may have induced this phenomenon. This is an indication that mixtures of these chemicals when properly applied could find use in important cytological procedures such as inducement of sexual polyploidization (Otto and Whitton, 2000). Reporting on the three major sources of origin of meiotic restitution in plants, Storme and Mason (2014) stated that alteration in meiotic spindle dynamics; defects in cell plate formation and omission of meiosis I or II are some of the main sources of origin of this anomaly. In the present investigation, the 1:1 mixture of 8-hydroxyquinolene and alpha bromonaptalene used in treating the PMCs could have initiated any one or more of these processes resulting in high frequency of unreduced nuclei (19.87%) in the treated flower buds.

CONCLUSION

The investigation into the cytological effects of mixture of 1:1 8-hydroxyquinolene and alpha-bromonapthalene on meiocytes of *Gyandropsis gyandra* (L.) showed that the chemical mixture induced anti-spindle action, the slowing down of chiasma terminalization and sticky chromosomes appearances at prophase ending in first division restitution (FDR). First meiotic division was found to be the principal phase affected by the mixture. Result indicated that second meiotic division was virtually blocked by the chemical mixture as chromosome staining and bivalent structure were greatly affected. Chiasmata became dormant as chromosomes went through non-specific clumping. While the biochemical mechanism of action of the chemical mixture was not investigated in this study, it is likely that the depolymerization of the cell's microtubules and/or the blockage of the tubulin trransport (Artivinli, 1881) by the chemical mixture may have been part of the pathways for action.

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