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# INDUCED CYTOMIXIS AND MALE STERILITY IN *HORDEUM VULGARE* L

BY

Soliman A. Haroun and H. M. El Wadie

Biology Department, Faculty of Education, Tanta University,

Kafr El Sheikh 33512 Egypt and collage of Education,

King Saoud University Abha.

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## INTRODUCTION

Radiation induced chromosomal aberrations are widely known in crop plants. The mode of action and origin of these aberrations have been extensively studied in different plants by a number of workers (Haroun & Ali 1993, Das & Roy 1989, Lgnacimuthu & Sakthivel 1989, Reddy & Annadurai 1992 and Gutuam &Richarria 1992), either to study their mutagenic effects or their use in mutation breeding technique to create desirable genetic variation in economic crops.

Gamma rays not only induce mitotic aberrations but also affect meiotic chromosomes and pollen fertility (Haroun & Ali 1993). They were found to affect chiasmata frequency which is an indication of the degree of recombination. A number of workers have reported the effects of gamma rays on many genera showing various effects mostly depending on the dose of treatment and the nature of the plant material.

Transmigration of chromatic material from one cell to another (cytomixis) has been reported by many workers in different plants, and during microsporogensis for a wide range of angiosperms which represent genetically unbalanced types such as mutants (Gosttschalk 1970), plants treated with various chemicals like laurel alcohol,

EMS (ethyl methyl sulphonate) or chemical mutagens (Datta & Biswas 1984), fixative (Linnert 1955), and temperature anomalies (Basavaiah & Murthy 1987).

Up till now and to our knowledge this is the first report on cytomixis induced by gamma irradiation. This phenomenon in PMC's and its role to induce aneuploid and polyploid number of chromosome was investigated in addition to the meiotic disturbance and reduction in chiasmata frequency to create male sterility in cereal crops as barley.

## MATERIALS AND METHODS

Mature and healthy grains of *Hordeum vulgare* L (2n = 14) were treated with gamma irradiation at doses of 2.5, 5, 10, 20, 30, 40, and 50 kr. the treated grains were grown under normal field conditions. For meiotic studies young spikes with flowers in pre-anthesis stage were collected between 8-10 am., fixed in 3:1 (v/v) ethyl alcohol and acetic acid glacial for 24h. at room temperature. The material was stored in 70% ethyl alcohol at 4 $c^{o}$ till use. Anthers were stained and squashed in 2 % acetocarmine. Meiotic chromosomes, chiasmata frequency, cytomixis and stainability of pollen grains as indicator for viability were studied in 20-40 pollen mother cells at diakenesis and metaphase I for each treatment. Photographs were taken from temporary slides.

## RESULTS AND DISCUSSION

Meiotic studies.

Meiotic aberrations were observed at most of the meiotic stages. Lagging chromosomes at anaphase I and II (Fig. 2-f,h) and micronuclei at the tetrad stage (Fig.2-i) were the most common types. No doubt that such aberrated cells induced by gamma rays would subsequently show aneuploid number of chromosome and more or less show a high degree of sterility as reported by Das & Roy (1989), Major & Khanna (1988), Mishra & Roghuvanshi (1989), Venkateswarlu et al (1988), Reddy &

Annadurai (1992) and Haroun & Ali (1993). This was found to affect pollen fertility, which is negatively correlated with doses applied.

Mean chiasmata frequency per cell and per bivalent as well as the depression index were recorded in table 1. It is obvious that cells showing high numbers of chiasmata were more frequent in control and at low doses of treatment, where no cells with 11 and 12 chiasmata were recorded. This is in contrast with the high treatments where cells showing low numbers of chiasmata were being more frequent. Such a reduction in chiasmata frequency by gamma rays was previously reported by Hamadi & Godward (1989), and Venkateswarlu et al (1988). On the other hand Lawrance (1961) found that chiasmata frequency increased at the lower doses. The depression index of chiasmata frequency was found to be increased at higher doses (40 and 50 kr), where the number of bivalents become reduced because of the tendency of chromosomes to associate in groups of more than two pairs (Fig. 2-e), leading to a reduction in chiasmata frequency.

The reduction in chiasmata frequency could be due to chromosomal structural changes or due to the upsetting of genetic systems which control the distribution of chiasmata on the bivalents, as recorded by Jones (1974). In the present study it seems that the first suggestion is more likely to be the cause of meiotic aberrations in pollen mother cells affect chiasmata frequency, and pollen fertility is subsequently reduced. (Fig.1) .

Cytomixis

Cytomixis was observed in pollen mother cells after irradiation either in form of cytoplasmic connections or direct fusions between cells in most treatments applied in the present investigation. Both types of cytomixis were recorded in meiosis I at all doses and in meiosis II at doses of 30, 40 and 50 kr. treatments.

The majority of PMC's observed were seen mostly connected in series of 3-5 cells (Fig. 2 - a,b), either by direct fusion or with cytoplasmic connections, and sometimes with both. Partial migration of chromatin material was observed (Fig. 2-c), where cells show aneuploid chromosome numbers (2n + 2 or 3) as reported by Spare & Deshpande (1987), and Basavaiah & Murthy (1987). Also, the whole migration of chromatin material (Fig. 2-d) was observed, mostly causing the formation of polyploid cells as stated by Bahl & Tyagi (1988).

The percentage of the direct fusion form of cytomixis is higher than cytoplasmic connections. The latter are sometimes represented by two or three connections as was recorded by Bahl & Tyagi (1988). The percentage of cytomixis in relation to dose treatments shows no definite relation either for types of connections or for stages. The dose of 30 kr records the highest value of cytomictic cells even compared with high doses (40, 50 kr.). This dose also represents the minimum dose causing cytomixis in second meiotic division (Table 2).

Generally, the percentage of cytomixis was found higher in meiosis I and decreases as the advancement of division progresses (Bahl & Taygi 1988), but without a definite relationship to gamma irradiation doses.

Cytomixis resulted from radiation in the present work is directly related to changes in chromatin material or chromosome number in the cytomictic cells, depending on the nature and amount of material travelling between cells. In case of partial migration, aneuploid cells are formed having 2n + x where x =2 or 3 chromosomes as was reported & Deshpande (1987) and Bahl & tyagi 1988). If the migration involves the whole set of chromosomes, it mostly leads to the formation either of giant cells or polyploid cells as stated by Gosttschalk (1970).

No doubt changes due to cytomixis and the formation of aneuploids or polyploids leads to the formation of sterile PMC's, which are not able to function, producing variants in the following generations, especially in gametic cells rather than somatic ones. In the present study, the abnormal microspores produced by cytomixis lead to the formation of abnormal tetrads in the final stages of meiosis (Fig 2-j), and aberrant pollen grains appear, being smaller, empty and unstained (Fig 2-g ). In addition to inducing meiotic aberrations, it affects chiasmata frequency and fertility. These pollen grains are non functional and would not be able to grow in competition with the normal pollen grains even if they are functional as previously suggested by Spare & Deshpande (1987).

The absence of cytomixis in the control plants confirms the mutagenic effects of radiation and its role in the induction of this phenomenon in the present work plants.

## SUMMARY

A study of the cytological effects of gamma rays at doses of 2.5, 5, 10, 20, 30, 40, and 50 kr is reported for *Hordeum vulgare* L., a cereal crop plant . Meiotic abnormalities were observed in PMC's and were found to be related to the formation of sterile pollen grains. Not only was adverse correlations recorded between gamma doses and pollen viability, but also with chiasmata frequency. The last parameter is mostly a consequence of chromosomal strctural changes in meiotic division.

Cytomixis was observed in PMC's of treated plants in the form of direct fusion and cytoplasmic connections. Both types were recorded in meiosis I and meiosis II with different intensities of irradiations showing the reduction of cytomixis as meiotic division advances. PMC's showing cytomixis were observed sometimes in series of 3-5 cells. Some cells were observed showing partial migration of chromatin material forming cells having aneuploid numbers of chromosomes, while others show migration

of the whole set of chromosomes by direct fusion, forming giant cells or polyploids. All cytomictic cells formed are aberrant and genetically unbalanced. This consequently form non-or poorly functional pollen grains leading to male sterility in this plant.

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Table 1: Distribution of chiasmata frequencies in PMC's of *hordeum vulgare* treated with different doses of gamma rays.

| treat. Kr | Scored Cells | cells with different Nos. of chiasmata 11 | 12 | 13 | 14 | 15 | 16 | 17 | Chiasmata per cell ±S.E. | Chiasmata per bivalent | depress. index |
|---|---|---|---|---|---|---|---|---|---|---|---|
| cont. | 32 | 0 | 0 | 0 | 2 | 6 | 10 | 14 | 15.12±0.47 | 2.30 | 0.0 |
| 2.5 | 28 | 0 | 0 | 2 | 2 | 4 | 12 | 8 | 15.78±0.16 | 2.25 | 2.11 |
| 5.0 | 26 | 0 | 0 | 3 | 3 | 4 | 10 | 6 | 15.66±0.23 | 2.23 | 2.85 |
| 10 | 22 | 0 | 4 | 0 | 6 | 2 | 2 | 8 | 15.00±0.29 | 2.14 | 6.56 |
| 20 | 34 | 0 | 6 | 4 | 2 | 10 | 6 | 6 | 14.71±0.31 | 2.10 | 8.71 |
| 30 | 40 | 6 | 4 | 2 | 6 | 12 | 8 | 2 | 14.10±0.52 | 2.01 | 12.53 |
| 40 | 28 | 10 | 8 | 4 | 2 | 4 | 0 | 0 | 12.35±0.72 | 1.76 | 23.38 |
| 50 | 30 | 14 | 6 | 6 | 2 | 2 | 0 | 0 | 12.06±0.41 | 1.72 | 25.19 |

Table 2: Percentage of pollen mother cells showing cytomixis by direct fusion, cytoplasmic connections at meiosis I and meiosis II, abnormal ones at different doses of gamma irradiation treatments and pollen grain viability.

| treat. Kr | No.of PMC's cell | % of cytomixis D.F. | C.C. | M I | M II | total | % of abn. PMC's | % Poll. fertility |
|---|---|---|---|---|---|---|---|---|
| control | 156 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 91.45 |
| 2.5 | 145 | 1.30 | 0.76 | 2.06 | 0.0 | 2.06 | 5.10 | 87.58 |
| 5.0 | 164 | 1.20 | 0.19 | 1.39 | 0.0 | 1.39 | 5.80 | 85.36 |
| 10.0 | 183 | 1.15 | 1.04 | 2.19 | 0.0 | 2.19 | 6.20 | 86.78 |
| 20.0 | 177 | 2.12 | 0.63 | 2.75 | 0.0 | 2.75 | 6.70 | 86.60 |
| 30.0 | 185 | 10.81 | 3.78 | 13.51 | 1.08 | 14.59 | 6.95 | 83.70 |
| 40.0 | 193 | 5.21 | 1.16 | 6.97 | 0.80 | 07.77 | 8.10 | 78.73 |
| 50.0 | 127 | 5.15 | 1.68 | 7.02 | 0.21 | 07.23 | 8.91 | 75.38 |

D.F. = direct fusion, C.C = cytoplasmic connection, MI = meiosis I, MII = meiosis II

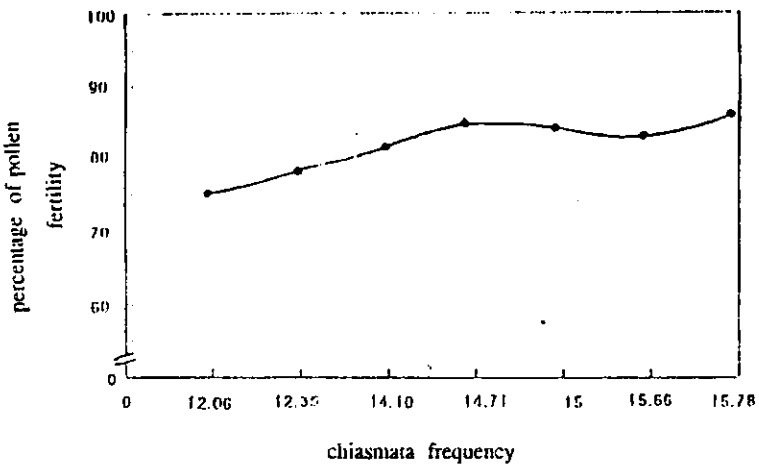


Fig. 1: Relationship between chiasmata frequency and percentage of pollen fertility in PMC's of *Hordeum vulgare* L. treated with gamma rays at seed stage.

Fig. 2-a,b. Series of PMC's showing cytomixis by direct fusion and cytoplasmic connection. 2-c. Cell showing aneuploid chromosome number (9 bivalents). 2-d. Whole set migeration of chromosome by direct fusion. 2-e. Diaknesis showing more than two bivalent associated together. 2-f. Unoriented chromosome at anaphase I. 2-g Small empty and unstained pollen grains. 2-h. Lagging at anaphase II. 2-i. Micronuclei at telophase II. 2-j. abnormal tetrad stage showing five groups.

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# استحداث السيتوماكس والعقم الذكرى فى نبات الشعير

*سليمان عبد الفتاح هارون **حسين مانع الوادعى
*قسم البيولوجى - كلية التربية - جامعة طنطا - كفر الشيخ ٣٣٥١٢ مصر
**كلية التربية - جامعة الملك سعود - أبها

أجريت هذه الدراسة على تأثير جرعات مختلفة من اشعة جاما (٥ر٢-٥-١٠-٢٠-٣٠-٤٠-٥٠) كيلوراد على الانقسام الميوزى لنبات الشعيركأحد المحاصيل الاقتصادية الهامة.

أظهرت الدراسة وجود تشوهات فى أمهات حبوب اللقاح ونسبة عقم تتناسب طرديا مع جرعات الإشعاع المتناولة. كما أظهرت تأثير واضح للجرعات على نسبة الكيازما (أماكن العبور) فى ثنائيات الكروموسومات وربما يكون انخفاض نسبة الكيازما ماسببه التغيرات التركيبية فى الكروموسومات نتيجة للتشوهات الناتجة عن المعاملات الاشعاعية.

شوهدت أيضا ظاهرة السيتومكسس فى أمهات حبوب اللقاح أثناء الانقسام الميوزى عن طريق الانتقال بين الخلايا او عن طريق الخيوط السيتوبلازمية سواء فى الانقسام الأول أو الثانى مع انخفاض النسبة فى الانقسام الثانى عنه فى الأول . مع وجود انتقال كلى لمحتويات بعض الخلايا إلى الأخرى ينتج عنه خلايا عملاقة ذات عدد مضاعف من العدد الكروموسومى كما وجدت بعض الخلايا ذات عدد كروموسومى أكبر من العدد الصبغى لانتقال جزئى لبعض الكروموسومات من خلية إلى أخرى .

لوحظ أثناء الدراسة أن معظم الخلايا التى تحدث بها الظاهرة كانت مشوهه وتبعا لذلك فان عملية التلقيح لا تتم بصورة جيدة وبذلك تزداد نسبة العقم ويؤثر على الإنتاجية .