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MEIOTIC CHROMOSOMAL ANOMALIES RESULTING FROM FUNGAL INFECTION OF MAIZE COMPARED WITH THOSE RESULTING FROM GAMMA IRRADIATION[1]

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ABSTRACT

Meiotic chromosomal anomalies resulting from fungal infection with *Fusarium verticillioides* on some maize hybrids in Egypt were studied compared with those resulting from gamma irradiation. Fungal infection and gamma irradiation treatments elicited similar types of chromosomal anomalies. The percentage of these chromosomal anomalies types differed between treatments. The results illustrated that the differences among all doses of radiation as well as between infection and uninfection treatments were more clearly in case of stickiness % and unorientation % at metaphasic stages, respectively. The results also indicated that the percentage of total abnormal meiotic cells (T. Ab.%) was increased as the doses of gamma-irradiation increased. Similarly, most of the mean values of T. Ab.% in case of infection were highest than their corresponding of uninfection. On the other hand, the results indicated diminishing the effect of infection with the exposure to radiation. In the same times, the results indicated that the infection treatments induced highly increases in multipolarity and bridges in case of non-irradiated plants compared with those resulting in case of irradiated plants. Generally, the results indicated that the fungal infection caused a significant increase in meiotic chromosomal anomalies percentage and significant decreased in pollen viability percentage. This effect of infection treatment were lowest than that the effect of irradiation treatment.

INTRODUCTION

Chromosomal anomalies are one of the most frequently produced classes of mutation that resulted by the action of both physical and chemical mutagens (Gecheff 1996).

Maize, *Zea mays* L bioassay to be described is a particularly favorable experimental assay for the study of chromosomal aberrations that may be scored in both mitotic and meiotic cells and hence pollen grains (Verma *et al.,* 1977). In early studies, maize was used to induce mutants and to make comparisons between radiation and chemically induced chromosomal mutations (Graf 1957).

Gamma irradiation is one of the main physical mutagens for mutation studies in plants. This mutagen has been used widely for monitoring of genetic effects.

On the other hand, maize is naturally contaminated with different fungi including *Fusarium* spp., e.g *Fusarium oxysporum* , *F. verticillioides* and *F. graminearum* (Fadl Allah, 1998). The filamentous ascomycete *Fusarium verticillioides* (synonym, *Fusarium moniliforme* Sheldon; teleomorph, *Gibberella moniliformis* [synonym, *Gibberella fujikuroi* mating population A]) is the major pathogen of maize worldwide and most commonly

reported fungal species infecting maize causing seedling disease, root rot, stalk rot, and ear or kernel rot (Munkvold *et al.*,1997).

The fungus develops inside young plant, moving from the roots to the stalk and finally to the cob and kernels. *Fusarium verticillioides* are of the major concerns of maize is the production of mycotoxins which are genotoxic agents (Sekhon *et al.*, 2006).

In many studies, chromosome stickiness, univalents, multivalents, laggard, chromatin bridges, micronuclei and other abnormalities were observed as the effects of physical and chemical mutagens (Kumar and Singh 2004).

In the present investigation, meiotic chromosomal anomalies resulted from fungal infection by *Fusarium verticillioides* on some economical maize hybrids in Egypt were studied compared with those resulted from gamma irradiation.

MATERIALS AND METHODS

Genetic Materials:

Six maize hybrids *Zea mays* L. were used. The seeds of hybrids were obtained from Maize Research Program, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. These Hybrids are: Giza10 (SC10), Pioneer 30k8 (SC30k8), Giza 324 (TWC324), Gimaza 327 (TWC327), Giza 352 (TWC352) and Pioneer 3084 (SC3084).

Isolation and Identification of causal pathogen:

Fusarium verticillioides was isolated from naturally infected ear collected from the infected maize plant. The infected seeds were separated and tested using the deep freezing method (ISTA, 1996).

The isolated fungus was identified in consultation with the Commonwealth Mycological Institute description sheets, Danish Government Institute of seed Pathology publications, Booth (1985), Burrges *et al.* (1988) and Moubasher (1993).

Preparation of the pathogenic fungus Inoculums:

Inoculums of *Fusarium verticillioides* pathogenic fungus were prepared in autoclaved cornmeal sand water substrates (1:1:2, w/w/v) in flasks 500 ml. Each flask (containing 200 ml. medium and autoclaved for 20 minutes) was inoculated with three disks 7.0mm diam. of pathogenic fungi which taken from 7 days old culture of PDA (plated in a 11-cm. diameter Petri dish) , then incubated at 24 ± 2°C for 3 weeks (Embaby 2006).

Pathogenicity:

The used soil in the pathogenicity test and control was made up soil + Farmyard Manure, this soil sterilized use Soil Solarization Method one month before being infested with fungal isolates according to El-Shanawany *et al.*, (2004). Soil solarization was accomplished by covering moist soil with tarpaulin and exposed to direct sun light. The inoculum of fungus was mixed with the soil after solarization at the rate of 1 g. inoculum / 1000 g. soil in pots (40 cm. high and 30 cm. diameter) three days before planting.

Gamma-rays irradiation:

The seeds (dry and soaked in water at 10 h.) of six hybrids were exposed to gamma rays emitted for Co^{60} at the National Center for Radiation Research and Technology (NCRRT). Seeds (dry and soaked) were exposed to zero, 15 and 30 kr. Irradiated and non-irradiated seeds (dry and soaked) of six hybrids were planted directly after irradiation treatment in infected and uninfected soil in pots.

Determination of meiotic chromosomal anomalies and Pollen grains viability:

At flowering time, about 50 days after planting, floral buds were collected and fixed in farmer solution (3:1 absolute ethanol-acetic acid) for 24 h. they were transferred to 70% alcohol and stored at 4 °C. For each treatment three floral buds were chosen and about five slides were prepared for each bud. to determine the chromosomal anomalies in meiosis, slides were prepared using the chromosomal squash technique with 2% acetocarmine stain (Kumar and Rai 2007).

Number of meiotic chromosomal anomalies types in metaphisic stages and anaphisic stages was determined.

Pollen grains viability was estimated using Lugol's iodine staining technique (Molina *et al.*, 2006). Pollen grains were stained a dark-blue being considered viable, while those non stained pollen grains were considered non viable.

Experimental designs:

A factorial experiment in a completely randomized design according to Compton (1994) and Subedi and Ma (2005) was used. In this design, factor A was the infection treatment (infected with fungal and uninfected); factor B was the irradiation treatment (irradiated seeds with 0.0, 15 and 30 Kr. of gamma rays); factor C was soaking treatment (So. dry and soaked seeds) and factor D was obtained six hybrids. The experimental data were subjected to analysis of variances using the general linear model (SAS 9, 2004).

RESULTS AND DISCUSSION

Types of resulting meiotic chromosomal anomalies:

Fungal infection and gamma irradiation treatments elicited similar types of chromosomal anomalies. The percentage of these chromosomal anomalies types differed between treatments. Normal meiosis cells at metaphasic and anaphasic stages as well as wide spectrum of chromosomal anomalies are shown in Figures 1 up to 4. In metaphasic stages, different types of chromosomal anomalies were noticed.

These types named, stickiness (Figure 2-A), precocious movements (Figure 2-B), fragmentation (Figure 2-C), multivalent formation (Figure 2-D), univalents (Figure 2-E), secondary association (Figure 2-F), unorientation (Figure 2-G) and other abnormalities (Figure 2-H) such as scattering (Figure 2-H1) and cytoplasmic connections (Figure 2-H2).

In the same time, in anaphasic stages, different types of chromosomal anomalies showed types named, stickiness (Figure 3-A),

laggard chromatin (Figure 3-B), bridges (Figure 3-C), unequal separation (Figure3-D), multipolarity (Figure 3-E) and other abnormalities (Figure3-F) such as forward movement (Figure3-F1), micronuclei (Figure 3-F2), and non-synchronous division (Figure 3-F3).

Chromosome stickiness has been documented to be due to genetic or environmental factors. Similar results were reported by Gaulden (1987). He postulated that sticky chromosomes may result from the defective functioning of 1 or 2 types of specific non-histone proteins involved in chromosome organization, which are needed for chromatid separation and segregation (Kumar and Rai 2007). The presence of univalents and multivalents at metaphase has been reported in different plants as barley (Kumar and Singh 2003) and broad bean (Bhat *et al.,* 2007a). Mutagens induced structural changes in chromosomes and mutations might be responsible for the failure of pairing among homologous chromosomes. Univalents may originate from the absence of crossing over at pachytene or from synaptic mutants. Chiasma are responsible for the maintenance of bivalents, which permit normal chromosomes segregation. Multivalent formation could be attributed to irregular pairing and breakage followed by translocations and inversions. Stray bivalents at metaphase I and II seemed to be caused by spindle disfunction (Bhat *et al.,* 2007b). The observed precocious chromosomes migration to the poles may be resulted from univalent chromosomes at the end of prophase I or precocious chiasma terminalization at diakinesis or metaphasel. Precocious migration of univalents to the poles was a very common abnormality among plants (Consolaro *et al.,* 1996). Secondary associations were resulted from modified chromosomes arrangement due to the duplication, interchanges or stickiness (Kumar and Singh 2003).

Unorientation and scattering of chromosomes may either be due to the inhibition of spindle formation or the destruction of spindle fibers formed. The behavior of these and of the laggard chromosome was characteristic in that they generally lead to micronucleus formation (Kumar G, Rai 2006). Multipolarity or disturbed polarity, forward movement, cells with unequal chromosomes separation and non-synchronous division might have appeared due to improper spindle function or spindle disturbances (Bhat *et al.,* 2007a; Kumar and Rai 2009). Laggards might be due to the delayed terminalization, stickiness of chromosomal ends or due to failure of the chromosomal movement (Jayabalan and Rao, 1987). Bridges seemed to be a result of non-separation of chiasma due to stickiness. At anaphase, about 50% of the cells displayed chromosomal bridges, often with fragments. These bridges may be resulted as the consequence of crossing over and associated with inversions or chromosome rings. In some cases, the irregular outline of bridge formation through delayed separation of the chiasmata and also due to later replication of heterochromatin or chromatin stickiness was detected (Viccini and Carvalho 2001). Micronuclei may be resulted from lagging chromosomes and fragments, which usually failed to be included in the daughter nuclei (Kumar G, Rai 2009). Cytoplasmic connections refer to chromatin movement as a consequence of mechanical or procedural defect is ruled out (Baquar and Husain 1969).

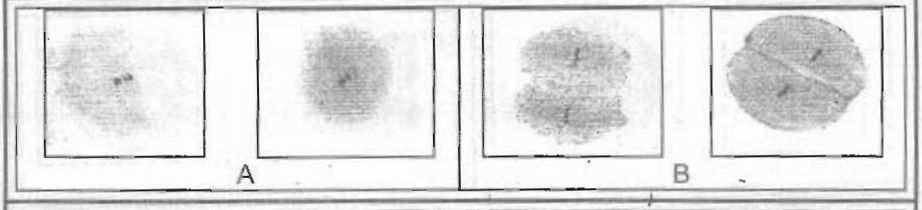

Figure 1: Normal meiotic cells at metaphasic stages, A- metaphase I and B metaphase II using digital microscope magnified 40 X 10x.

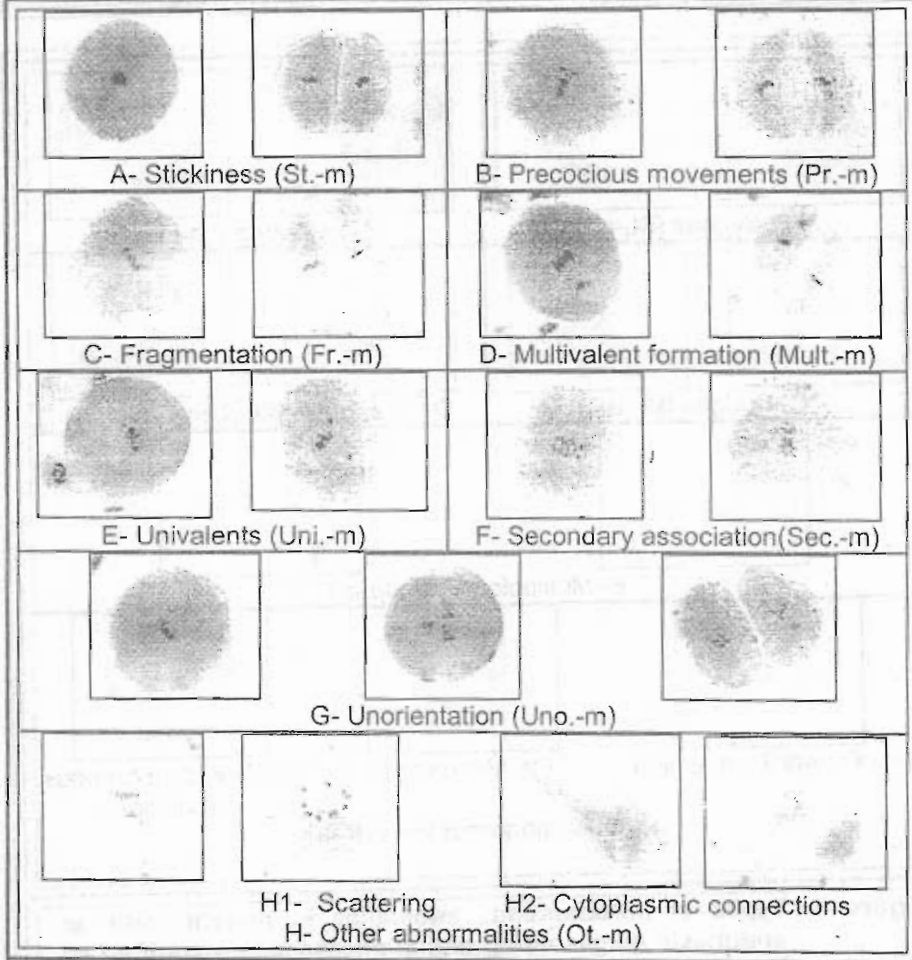

Figure 2: Types of chromosomal anomalies in meiotic cells at metaphasic stages using digital microscope magnified 40 X 10x.

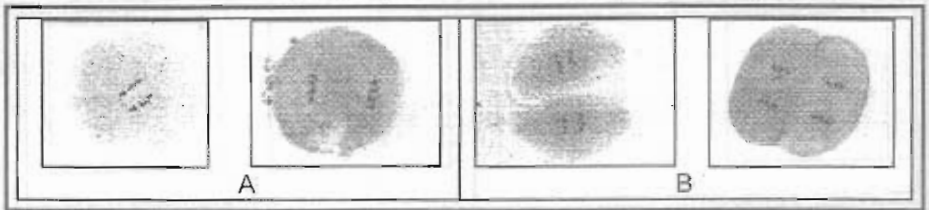

Figure 3: Normal meiotic cells at anaphasic stages, A- anaphase I and B anaphase II using digital microscope magnified 40 X 10x.

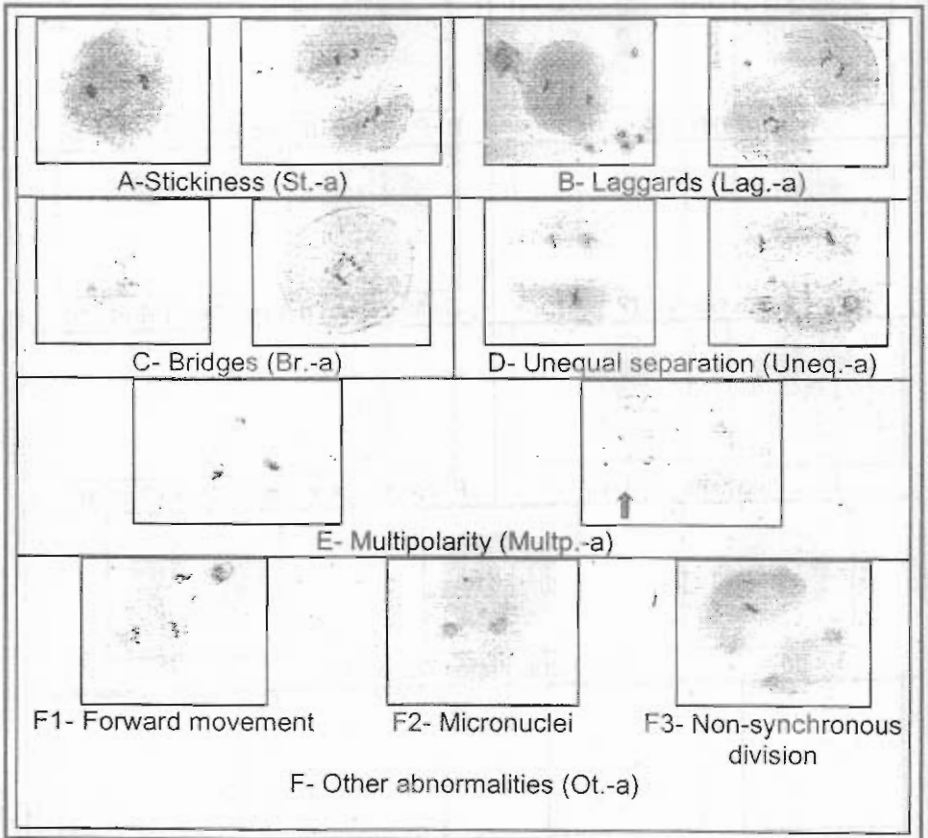

Figure 4: Types of chromosomal anomalies in meiotic cells at anaphasic stages using digital microscope magnified 40 X 10x.

Means and relative increasing for chromosomal anomalies:

The means of meiotic chromosomal anomalies % in metaphasic (metaphase I and II) and anaphasic (anaphase I and II) stages are shown in Tables 1 and 2, respectively. These results showed that radiation effect led to unemergence of soaked seeds from all hybrids in infected and uninfected soil specially the dose of 30 Kr. in the same time, SC10 hybrid failed to emergence in 15 and 30 Kr. (except in 15kr./DS) as well as SC3084 hybrid which failed to emergence in 15kr./SS and TWC324 hybrid failed to emergence in 30kr./DS in uninfected soil .

The mean performances of non-irradiated plants were lowest those of their corresponding of the mean performances of irradiated plants with 15 and 30 Kr. as well as the mean performances of irradiated plants with 15 Kr. were lowest than those the mean performances of irradiated plants with 30 Kr. It could be also regarded that in most types of chromosomal anomalies, the mean performances of uninfected plants were lowest than those the mean performances of infected plants. The frequencies of stickiness and unorientation at metaphasic stages, respectively were highest than the frequencies of the other chromosomal anomalies types. On the other hand, the frequencies of bridges and multipolarity at anaphasic stages were lowest than the frequencies of the other chromosomal anomalies types. The results illustrated that the differences among all doses of radiation as well as between infection and uninfection treatments were more clearly in case of stickiness % and unorientation % at metaphasic stages, respectively. This finding indicated that more effect of radiation and infection treatments were appeared in a defect in the function of specific non-histone proteins involved in chromosome organization, as well as the inhibition of spindle formation. From the results of Table 2, it could be regarded that the maximum frequency (52.6% in hybrid TWC327) of total meiotic abnormal cells percent (T. Ab.%) was observed at 30 Kr. followed by 15 Kr. (31.8 in hybrid SC3084) in uninfected plants.These results indicated that the percentage of total abnormal meiotic cells was increased with the increasing of the doses of gamma-irradiation. Similarly, most of the mean values in case of infection were highest than their corresponding in case of uninfection in T. Ab.%.

It could be regarded from the recorded results in Table 3, that the increases ratio for most meiotic chromosomal anomalies means of all infected hybrids at D.S. and S.S. treatments were more than those the meiotic chromosomal anomalies means of all uninfected hybrids. This finding indicated that the fungal infection induced chromosomal anomalies formation. This effect of fungal infection which may be resulted from the effects of mycotoxin has somewhat harmony with that previously obtained by Agar and Alpsoy (2005). They stated that the effect of aflatoxin G1 (AFG1) toxicity induced chromosomal aberrations in *Vicia faba* and *Zea mays*. Their results showed that 0.1, 0.2, 0.4 ppm concentrations of aflatoxin G1 increased chromosomal aberration. Also, indicated the decrease in the increasing ratio for infection treatment with the increases of irradiation doses for most meiotic chromosomal anomalies types. This finding indicated diminishing the effect of infection with the exposure to radiation.

Table 1: Means of meiotic chromosomal anomalies % in metaphasic stages

Types of chromosomal anomalies			St-m%		Pr-m%		Fr-m%		Mult-m%		Uni-m%		Sec-m%		Uno-m%		Ot-m%		Ta-m %		Tn-m %	
Treatments			Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.
Doses	So.	Hybrids																				
0.0Kr.	DS	SC 10	1.6	2.0	0.2	0.7	0.4	0.9	0.1	0.1	0.3	0.4	0.7	0.7	2.0	0.9	0.8	1.2	6.0	6.9	57.5	47.4
		SC30k8	1.0	0.7	1.3	0.5	0.6	3.1	0.1	0.4	0.9	0.7	1.2	0.9	0.8	2.9	0.8	0.5	6.8	9.6	67.9	40.3
		TWC324	1.8	1.9	0.1	0.8	0.6	1.8	0.0	1.0	0.4	0.4	1.3	0.7	1.5	2.2	0.4	1.8	6.1	10.6	60.1	55.3
		TWC327	1.4	0.9	0.4	1.0	1.2	1.3	0.1	0.5	0.5	0.6	1.0	0.6	0.4	1.9	1.0	1.1	5.9	7.7	57.2	51.9
		TWC352	1.9	1.1	0.1	0.8	0.6	1.8	0.5	1.0	0.3	0.5	0.5	0.7	2.3	2.6	0.6	0.9	6.7	9.3	62.2	48.9
		SC3084	1.8	1.9	2.1	1.5	1.8	1.5	0.3	0.9	0.5	0.8	2.9	1.4	2.1	1.2	0.7	1.0	12.2	10.0	63.8	55.8
	SS	SC 10	2.6	3.7	1.6	0.4	0.7	1.8	0.0	0.4	0.8	0.6	0.5	0.3	1.1	2.0	0.9	1.1	8.2	10.4	57.2	56.5
		SC30k8	0.5	2.5	1.5	5.2	1.7	1.3	0.1	0.4	0.6	0.8	1.0	2.0	0.7	2.3	0.5	1.0	6.5	15.5	66.3	62.3
		TWC324	1.6	6.0	0.3	3.1	0.9	2.1	0.1	2.3	0.4	0.8	1.0	2.6	1.5	3.3	0.5	1.4	6.4	21.5	60.9	59.9
		TWC327	2.0	4.1	0.5	2.2	1.6	1.3	0.6	1.9	0.1	0.7	0.3	1.9	3.0	2.8	0.5	0.8	8.6	15.6	60.5	59.1
		TWC352	0.7	2.3	0.7	1.6	1.7	1.0	1.0	0.9	0.2	1.3	1.1	2.1	3.6	2.7	0.6	0.7	9.5	12.7	53.0	58.7
		SC3084	2.3	1.4	1.8	0.6	1.0	1.0	0.6	1.5	0.2	0.2	2.3	3.1	3.2	4.6	0.9	0.5	12.3	12.8	60.6	54.3
15 Kr.	DS	SC 10	2.8	9.3	1.6	1.6	2.7	3.3	1.2	0.4	1.3	3.2	2.7	2.4	1.8	2.5	1.7	0.9	15.9	23.7	44.1	47.4
		SC30k8	4.5	3.4	3.9	3.3	3.9	4.1	0.5	0.5	3.5	1.2	3.5	2.6	1.1	4.1	2.3	1.8	23.2	21.0	52.7	42.6
		TWC324	2.6	6.0	0.6	2.4	3.6	3.3	1.8	2.5	0.4	1.8	1.9	1.5	2.9	3.9	1.3	1.7	15.2	23.0	48.3	60.9
		TWC327	3.5	6.7	2.1	3.1	3.9	3.7	2.8	1.9	1.2	1.5	1.0	2.9	3.4	3.6	0.6	1.0	18.4	24.4	44.6	49.5
		TWC352	2.9	4.4	2.0	2.5	3.1	3.3	2.4	2.4	0.2	0.9	1.7	2.4	4.1	5.1	2.0	2.0	18.4	22.9	46.7	43.1
		SC3084	5.1	4.0	3.4	1.3	3.0	1.5	2.0	1.1	1.0	0.4	5.2	4.1	3.2	4.8	1.1	0.6	23.9	17.9	44.0	56.4
	SS	SC 10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		SC30k8	8.3	4.9	1.8	1.0	2.3	1.6	0.5	1.6	1.0	1.3	3.3	1.9	2.1	4.0	1.2	0.6	20.7	16.8	54.6	39.7
		TWC324	2.0	3.9	0.5	2.0	3.7	3.0	1.0	2.7	0.5	1.0	1.9	1.8	1.7	3.8	1.6	1.0	12.8	19.2	52.3	59.6
		TWC327	4.0	5.6	0.4	1.5	2.6	1.7	1.9	2.0	0.6	0.5	1.6	2.1	2.0	2.3	0.9	1.3	13.9	16.9	51.1	54.8
		TWC352	6.4	4.1	0.8	1.7	2.0	1.7	1.0	1.5	0.3	0.4	1.9	5.1	3.7	4.1	0.7	1.5	16.7	19.9	46.5	53.2
		SC3084	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30 Kr.	DS	SC 10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		SC30k8	5.9	5.6	2.4	1.3	2.3	4.0	2.3	1.6	1.1	1.0	3.2	3.3	5.2	2.7	2.3	1.7	24.7	21.2	42.7	39.7
		TWC324	-	4.3	-	3.3	-	3.9	-	3.2	-	1.5	-	2.8	-	5.8	-	1.6	-	26.2	-	44.7
		TWC327	10.1	12.3	4.1	2.6	6.1	6.1	4.8	4.4	2.3	2.0	7.1	1.1	3.7	4.8	5.1	3.6	43.4	36.9	31.8	42.3
		TWC352	4.9	11.7	3.2	2.7	2.6	5.2	1.7	3.7	0.9	2.3	5.7	1.0	3.4	3.5	1.9	2.2	24.4	32.4	48.3	33.5
		SC3084	3.7	5.0	5.3	2.4	4.6	3.4	2.3	1.8	2.6	3.6	5.9	1.8	3.6	3.0	3.1	2.9	31.0	22.6	46.2	46.6
	SS	SC 10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		SC30k8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		TWC324	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		TWC327	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		TWC352	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		SC3084	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Inf. = infection Uninf.= uninfection So.= soaking treatment DS = Dray seeds SS = Soaked seeds Kr.= measurement unit of radiate

Table 2: Means of meiotic chromosomal anomalies % in anaphasic stages

Types of chromosomal anomalies			St-a%		Lag-a%		Br-a%		Uneq-a%		Multp-a%		Ot-a%		Ta-a %		Tn-n %		T.Ab%		Total PMCs. observed	
Treatments			Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.
Doses	So.	Hybrids																				
0.0Kr.	DS	SC 10	0.3	0.7	0.5	0.3	0.0	0.1	2.2	4.4	0.0	0.1	0.5	0.8	3.5	6.3	33.0	39.3	9.5	13.3	1315	1374
		SC30k8	0.2	0.8	0.6	2.6	0.0	0.6	1.5	4.8	0.1	0.1	0.5	1.7	2.9	10.7	22.5	39.5	9.6	20.2	1144	1204
		TWC324	0.1	0.4	0.4	1.5	0.0	0.4	2.5	3.7	0.0	0.5	0.3	0.5	3.4	7.0	30.4	27.1	9.5	17.6	1387	1064
		TWC327	0.4	0.7	0.4	1.2	0.0	0.3	1.7	2.8	0.0	0.4	0.6	1.1	3.1	6.4	33.7	34.0	9.1	14.1	1289	1321
		TWC352	0.3	0.6	0.9	1.8	0.2	0.1	2.0	3.7	0.0	0.2	0.6	1.5	4.0	7.9	27.1	33.8	10.6	17.2	1320	866
		SC3084	0.1	0.8	0.2	1.2	0.1	0.1	1.5	2.4	0.0	0.2	0.2	1.3	2.0	6.0	22.0	28.2	14.3	16.0	975	1607
	SS	SC 10	0.4	0.3	0.5	0.8	0.3	0.5	2.1	2.0	0.2	0.0	0.5	0.3	3.8	3.9	30.8	29.2	12.0	14.3	2046	1708
		SC30k8	0.4	1.5	0.9	0.6	0.0	0.1	2.3	2.4	0.0	0.4	0.3	1.0	3.9	5.9	23.3	16.3	10.4	21.4	999	1390
		TWC324	0.2	0.4	0.4	0.8	0.0	0.2	2.8	1.3	0.0	0.0	0.6	0.2	4.1	3.0	28.6	15.6	10.5	24.5	1639	1008
		TWC327	0.4	0.8	0.2	0.4	0.0	0.3	1.8	1.8	0.0	0.1	0.5	0.9	3.0	4.2	27.9	21.1	11.5	19.8	951	973
		TWC352	0.0	0.8	0.2	0.9	0.0	0.1	2.3	2.2	0.0	0.1	3.4	0.9	5.8	4.9	31.7	23.7	15.4	17.6	880	773
		SC3084	0.1	1.2	0.6	1.3	0.0	0.0	0.7	1.1	0.1	0.7	0.6	1.5	2.2	5.8	25.1	27.1	14.5	18.6	811	834
15 Kr.	DS	SC 10	1.7	0.6	1.2	1.6	0.3	0.7	3.1	2.1	0.4	0.2	1.8	0.9	8.7	6.1	31.4	22.9	24.5	29.7	1153	898
		SC30k8	0.8	0.7	0.8	3.8	0.0	0.5	1.7	1.1	0.2	0.1	1.1	1.8	4.6	7.9	19.5	28.6	27.7	28.9	722	1138
		TWC324	1.6	0.9	1.1	0.5	0.3	0.2	3.6	2.7	0.6	0.3	1.0	0.3	8.2	4.8	28.3	11.3	23.4	27.8	727	1349
		TWC327	0.9	0.6	2.1	0.7	0.2	0.3	4.1	2.4	0.7	0.0	1.3	0.3	9.2	4.3	27.8	21.4	27.7	28.7	906	983
		TWC352	1.4	1.8	1.0	1.3	0.0	0.1	2.3	0.9	1.0	2.0	1.7	3.5	7.5	9.5	27.4	24.6	25.9	32.3	973	1076
		SC3084	1.4	0.6	2.2	1.6	0.6	0.8	1.8	3.3	0.3	0.4	1.6	1.7	7.8	8.3	24.3	17.4	31.8	26.2	643	1208
	SS	SC 10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
		SC30k8	1.0	2.5	2.0	2.8	0.8	0.9	2.4	3.9	0.1	0.7	0.4	2.9	6.7	13.8	17.9	29.7	27.4	30.5	1397	911
		TWC324	1.7	0.7	0.9	1.4	0.3	0.7	3.9	1.7	0.9	0.2	2.4	0.5	9.9	5.4	25.0	15.8	22.8	24.5	927	917
		TWC327	0.9	0.9	0.9	0.8	0.7	0.5	3.6	2.1	0.0	1.0	0.5	1.5	6.7	6.7	28.3	21.6	20.6	23.6	1819	1204
		TWC352	0.8	0.5	1.0	0.9	0.3	0.7	2.5	3.4	0.8	0.5	0.9	1.0	6.2	7.0	30.7	19.9	22.9	27.0	779	622
		SC3084	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
30 Kr.	DS	SC 10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
		SC30k8	2.0	0.9	2.0	1.9	0.4	0.8	2.6	9.8	1.0	0.4	2.3	0.5	10.3	14.2	22.3	25.0	34.9	35.3	1007	1511
		TWC324	-	0.7	-	0.8	-	0.1	-	3.3	-	0.5	-	0.4	-	5.7	-	23.4	-	31.9	0	727
		TWC327	1.6	1.0	1.7	1.0	1.0	0.5	2.4	2.8	0.6	0.8	1.9	1.1	9.3	7.2	15.6	13.6	52.6	44.1	690	805
		TWC352	1.9	1.9	1.6	1.5	1.1	0.5	2.9	2.8	0.8	1.2	1.8	2.8	10.0	10.6	17.2	23.5	34.5	43.0	1323	660
		SC3084	1.1	1.6	1.3	2.2	0.4	1.0	2.2	3.4	0.3	0.7	1.1	2.1	6.3	11.1	16.6	19.7	37.3	33.7	774	956
	SS	SC 10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
		SC30k8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
		TWC324	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
		TWC327	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
		TWC352	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
		SC3084	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0

Inf. = infection Uninf.= uninfection So.= soaking treatment DS = Dray seeds SS = Soaked seeds Kr.= measurement unit of radiate PMCs. = Pollen mother cells

The maximum increasing ratio (7.0) in case of Multp.-a% was shown for non-irradiated treatment followed by (4.67) in case of Br.-a%. These types of meiotic chromosomal anomalies were previously mentioned as the lowest in frequencies. This indicated that the infection treatment induced highly increase ratio in multivalent formation which may be resulted by spindle disfunction. This finding has somewhat harmony with that illustrated previously by Styer and Horace (1984). They treated maize roots with solutions of moniliformin (a metabolite of *Fusarium moniliforme* Sheldon). They mentioned that higher concentration caused a disruption of the spindle apparatus.

On the other hand, the present data in Table 4 showed that increase ratio for all meiotic chromosomal anomalies means of all irradiated hybrids (with 15 and 30 Kr, respectively) were more than meiotic chromosomal anomalies means of all non-irradiated hybrids at D.S. and S.S. treatments. This result indicated that the increasing of irradiation doses induced high increase ratio in all induced meiotic chromosomal anomalies types for non-irradiation treatment. This finding was in agreement with those obtained by Kumar and Rai (2009). They found that gamma rays induced the highest percentage of chromosomal abnormalities in gametic cells of *Zea mays* L. They also illustrated that the maximum frequency of chromosomal anomalies in gametic cells was induced by irradiation with 800 Gy followed by 600 Gy, 400 Gy and 200 Gy respectively. They also indicated the decrease in the increasing ratio of irradiation treatments with the infection for most meiotic chromosomal anomalies types. The maximum increasing ratios (14.0 and 10.0 at 30 and 15Kr., respectively) in case of Mult.-a% were shown for uninfected treatment. These findings indicated diminishing the effect of irradiation treatment with infection.

Statistical analysis for the cytological studies results:

For appropriate statistical analyses of the cytological studies the results showed that the total normal meiotic cells percent (N.M.C.%) was determined for all plants depending on the results of total abnormal meiotic cells percent (T.Ab.%) trait. Also, determined the pollen viability percent (P.V.%) trait.The results of the analyses of variance are presented in Table 5. The results indicated highly significant differences for all factors of N.M.C.% and P.V.% traits except the effect of infection factor on N.M.C% which indicated a only significant difference for this factor of this trait. Interactions between all studied factors were highly significant in case of (N.M.C.%) also, most of interactions between all studied factors were highly significant in case of (P.V.%) except (AxB). In the same time (AxBxCxD) were significant as well as (AxC) and (AxD) were non-significant.The results also indicated that the mean squares of infection effect were less than those of radiation effect mean squares. These findings indicated that infection effect was less than those radiation effect on these traits.

Table 3: Relative increasing for chromosomal anomalies means of all infected hybrids at D.S. and S.S. treatments compared to chromosomal anomalies means of all uninfected hybrids at DS and SS treatments

Doses of irradiation	chromosomal anomalies in metaphasic stages									chromosomal anomalies in anaphasic stages							T.Ab%
	St-m%	Pr-m%	Fr-m%	Mult m%	Uni-m%	Sec-m%	Uno-m%	Ot-m%	Ta-m %	St-a%	Lag-a%	Br-a%	Uneq-a%	Multp-a%	Ot-a%	Ta-a %	
At 0.0Kr (inf/uninf)	1.48	1.74	1.48	3.23	1.50	1.23	1.32	1.46	1.50	3.10	2.31	4.67	1.39	7.00	1.36	1.73	1.57
At 15 Kr (inf/uninf)	1.17	1.25	0.86	1.19	1.23	1.11	1.47	0.94	1.15	0.84	1.18	1.50	0.83	1.12	1.17	1.00	1.10
At 30Kr (inf/uninf)	1.27	0.66	1.16	1.06	1.21	0.37	1.00	0.77	0.90	0.74	0.90	0.80	1.75	1.07	0.78	1.09	0.94
Means of increase ratio (inf/uninf)	1.26	1.09	1.07	1.32	1.27	0.81	1.28	0.97	1.12	1.04	1.30	1.38	1.20	1.33	1.10	1.19	1.14

Inf. = infection Uninf.= uninfection So.= soaking treatment DS = Dray seeds SS = Soaked seeds Kr.= measurement unit of radiate

Table 4: Relative increasing for chromosomal anomalies means of all irradiated hybrids (with 15 and 30 Kr, respectively) at DS and SS treatments compared chromosomal anomalies means of all non-irradiated hybrids at D.S. and S.S. treatments

Cases of infection treatment		chromosomal anomalies in metaphasic stages									chromosomal anomalies in anaphasic stages							T.Ab%
		St-m%	Pr-m%	Fr-m%	Mult-m%	Uni-m%	Sec-m%	Uno-m%	Ot-m%	Ta-m %	St-a%	Lag-a%	Br-a%	Uneq-a%	Multp-a%	Ot-a%	Ta-a %	
15Kr	At uninf. (15kr/0.0Kr)	2.75	1.78	2.77	4.83	2.11	2.13	1.41	1.86	2.21	4.80	2.60	3.50	1.51	10.00	1.67	2.16	2.20
	At inf. (15kr/0.0Kr)	2.17	1.29	1.68	1.84	1.77	1.93	1.52	1.20	1.70	1.40	1.41	2.20	0.91	2.20	1.45	1.25	1.54
	Means of increase ratio (15kr/0.0Kr)	2.41	1.47	2.13	2.56	1.91	2.02	1.47	1.47	1.90	2.25	1.78	2.57	1.16	3.50	1.54	1.58	1.80
30Kr	At uninf. (30kr/0.0Kr)	3.88	4.22	3.55	9.33	3.78	4.78	2.16	4.43	3.89	6.80	3.40	7.00	1.28	14.00	2.40	2.57	3.49
	At inf. (30kr/0.0Kr)	3.32	1.61	2.90	3.05	3.23	1.43	1.60	2.40	2.34	1.60	1.36	2.40	1.63	2.80	1.40	1.63	2.10
	Means of increase ratio (30kr/0.0Kr)	3.54	2.57	3.17	4.56	3.45	2.94	1.84	3.24	2.96	2.90	2.00	3.71	1.48	4.67	1.83	1.98	2.64

Inf. = infection Uninf.= uninfection So.= soaking treatment DS = Dray seeds SS = Soaked seeds Kr.= measurement unit of radiate PMCs. = Pollen mother cells

Table 5: Analyses of variances and the mean squares for normal meiosis cells percent (N.M.C.%) in M_1 generations

S. O. V.	D.F.	N.M.C.%	P.V.%
Factor (A)	1	40*	813**
Factor (B)	2	71072**	98081**
Factor (C)	1	29864**	19843**
Factor (D)	5	2751**	1523**
A x B	2	714**	308*
A x C	1	247**	288
B x C	2	8594**	5937**
A x B x C	2	198**	421**
A x D	5	155**	69
B x D	10	1886**	1327**
A x B x D	10	249**	276**
C x D	5	1237**	1459**
A x C x D	5	235**	296**
B x C x D	10	2599**	2043**
A x B x C x D	10	189**	181*
Error	144	9	80

Factor A = Infection (a=2) **Factor B = Radiation doses (b=3)**
Factor C = soaking treatment (c=2) **Factor D = Hybrids (d=6)**
***, ** Significant at 0.05 and 0.01 levels of probability, respectively**

The results of N.M.C.% and P.V.% means which presented in Table 6, indicated that the means of uninfected plants for most treatments were significantly exceeded the infected plants in these traits. The results indicated that means of N.M.C.% and P.V.% traits decreased with the infection.

The results also indicated that the means of N.M.C.% and P.V.% traits for all non-irradiated plants were significantly exceeded than those the irradiated plants with 15 and 30 Kr. as well as irradiated plants with 15 Kr. which were also significantly exceeded than those irradiated plants with 30 Kr. This results indicated that the means of N.M.C.% and P.V.% traits decreased with the increasing of irradiation doses. These results were in accordance with the results obtained by Kash and Al-Gwaiz (1995). They indicated that the treatment of wheat seeds (*T. Aestivum L.*} with the above doses of radiation caused a highly significant increases in the number of abnormal cells, while pollen fertility percent decreased with increasing gamma-ray doses.

It could be also noticed from Table 6 that the hybrid SC10 showed the lowest ratio for N.M.C.% and P.V.% traits. Oppositely, SC30k8 and TWC327 hybrids cleared the highest ratio in these traits.

In the conclusion, the results of cytological traits indicated that the fungal infection caused significant increases in meiotic chromosomal anomalies percent and significant decreased in pollen viability percent. These effects for infection treatment were lowest than those of irradiation treatment.

Table 6: Means for normal meiotic cells percent (N.M.C. %) and pollen viability percent (P.V. %) traits

Traits			N.M.C.%		P.V.%	
Treatments			Uninfection	Infection	Uninfection	Infection
Doses	So.	Hybrids	means	means	means	means
0.0Kr.	DS	SC 10	90.5	86.7	94.1	83.8
		SC30k8	90.4	79.8	91.3	79.4
		TWC324	90.5	82.4	90.0	81.4
		TWC327	90.9	85.9	85.2	77.1
		TWC352	89.4	82.8	88.9	83.8
		SC3084	85.7	84.0	91.5	90.1
	SS	SC 10	88.0	85.7	96.7	88.6
		SC30k8	89.6	78.6	93.9	82.4
		TWC324	89.5	75.5	89.0	89.7
		TWC327	88.5	80.2	84.3	86.1
		TWC352	84.6	82.4	87.5	82.3
		SC3084	85.5	81.4	89.3	86.1
15 Kr.	DS	SC 10	75.5	70.3	75.7	70.2
		SC30k8	72.3	71.1	73.8	67.2
		TWC324	76.6	72.2	78.7	65.5
		TWC327	72.3	71.3	64.9	61.7
		TWC352	74.1	67.7	48.9	81.2
		SC3084	68.2	73.8	79.3	82.0
	SS	SC 10	0.0	0.0	0.0	0.0
		SC30k8	72.6	69.5	83.2	71.9
		TWC324	77.2	75.5	73.1	28.1
		TWC327	79.4	76.4	28.2	25.5
		TWC352	77.1	73.0	91.1	64.1
		SC3084	0.0	0.0	0.0	0.0
30 Kr.	DS	SC 10	0.0	0.0	0.0	0.0
		SC30k8	65.1	64.7	27.0	38.0
		TWC324	0.0	68.1	0.0	22.3
		TWC327	47.4	55.9	47.8	25.5
		TWC352	65.5	57.0	42.2	43.6
		SC3084	62.7	66.3	40.4	38.7
	SS	SC 10	0.0	0.0	0.0	0.0
		SC30k8	0.0	0.0	0.0	0.0
		TWC324	0.0	0.0	0.0	0.0
		TWC327	0.0	0.0	0.0	0.0
		TWC352	0.0	0.0	0.0	0.0
		SC3084	0.0	0.0	0.0	0.0
$LSD_{A\ 0.05}$			0.82		2.41	
$LSD_{B\ 0.05}$			1.00		2.95	
$LSD_{C\ 0.05}$			0.82		2.41	
$LSD_{D\ 0.05}$			1.41		4.18	

$LSD_{A\ 0.05}$ value for comparison between infection and uninfection $LSD_{B\ 0.05}$ value for comparison between irradiation doses $LSD_{C\ 0.05}$ value for comparison between dry and soaked seeds $LSD_{D\ 0.05}$ value for comparison between all hybrids So.= soaking treatment DS = Dray seeds SS = Soaked seeds Kr.= measurement unit of radiation Sd= Standard deviation

الشذوذات الكروموسومية الميوزية الناتجة عن الإصابة الفطرية للذرة بالمقارنة بتلك الناتجة عن التعرض لأشعة جاما

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تمت دراسة الشذوذات الكروموسومية الميوزية الناتجة عن الإصابة الفطرية بفطر الفيوزاريوم فيرتسيلويدس وذلك على بعض هجن الذرة الشامية فى مصر وذلك بالمقارنة بالشذوذات الكروموسومية الميوزية الناتجة عن تأثير التعرض لأشعة جاما . ووجد أن الإصابة الفطرية والتعرض لأشعة جاما يُحدثان أنواعاً متماثلة من الشذوذات الكروموسومية ووجد أن نسبة هذه الشذوذات متباينة بين كلا المعاملتين. وقد أوضحت النتائج وجود تباين فى نسب الشذوذات بين جرعات الإشعاع وكذلك بين معاملة العدوى وعدم التعرض للعدوى هذا التباين كان أكثر وضوحاً فى نسبة الشذوذات من نوع اللزوجة الكروموسومية والتوجيه الكروموسومى الخاطئ على الترتيب . أوضحت النتائج أيضاً أن نسبة الخلايا الميوزية الشاذة تزداد بزيادة الجرعة من أشعة جاما. وعلى نفس المنوال كانت معظم متوسطات صفة نسبة الخلايا الميوزية الشاذة فى حالة التعرض للعدوى أعلى منها فى حالة عدم التعرض للعدوى. ومن ناحية أخرى أوضحت النتائج تضاؤل تأثير العدوى فى ظل التعرض للإشعاع. فى نفس الوقت أوضحت النتائج أن التعرض للعدوى يستحث زيادة نسبية كبيرة فى الشذوذات من نوع التعدد القطبى والجسور الكروماتيدية وذلك فى حالة النباتات المعدية الغير مشعة مقارنة بتلك التى فى حالة النباتات المعدية المشعة. وعموماً فإن النتائج أوضحت أن الإصابة الفطرية تسبب زيادة معنوية فى نسبة الشذوذات الكروموسومية الميوزية وإنخفاضاً معنوياً فى نسبة حيوية حبوب اللقاح ، تلك التأثيرات الناجمة عن الإصابة الفطرية كانت أقل من مثيلتها الناجمة عن التعرض للإشعاع.