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PHENOTYPIC CHANGES AND MEIOTIC ABNORMALITIES INDUCED BY PLANT GROWTH REGULATORS IN ZEA MAYS L

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ABSTRACT

The Maize is ranked the third cereal crop in the world after rice and wheat. Plant growth regulators (PGRs) are synthetic hormones that are effective or more than natural plant hormones. This study aimed to assess phenotypic changes and meiotic abnormalities induced in maize influenced by PGRs. Two single hybrid genotypes of maize, as well as, two PGRs including gibberellic acid (GA_3) and ethephon were used in this study. Maize grains were soaked in two different concentrations of each PGR for 12 hours before sowing in the field. The experimental design was a randomized block design arranged in split-split plots with three replications per treatment. GA_3 displayed a wide spectrum of meiotic anomalies in the H_1 genotype, the most being stickiness, varied asynchronization, stray bivalent, multivalent and late movement of chromosomes toward the opposite poles. Meanwhile, meiotic anomalies induced by GA_3 in the H_2 genotype include stickiness, chromosome scattering and varied asynchronization. Ethephon induced meiotic abnormalities in the H_1 genotype were related to laggards, stickiness, disorientation of bivalents, multivalent with non-oriented on the equatorial plate, disturbed polarity, chromosome scattering, cytoplasmic attachment and stray bivalent. Other aberrations induced by ethephon in the H_2 genotype include non-oriented bivalents at metaphase I. The spectrum of meiotic aberrations was found higher in H_1 than in H_2 genotype, as well as, at metaphase than anaphase and telophase. These abnormalities can cause male sterility. This finding can open novel approaches in plant breeding programs of the species that are devoted to hybridization where manual cross-pollination is difficult and time-consuming.

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INTRODUCTION

Maize, *Zea mays L*., 2n = 2x = 20 chromosomes, is one of the most important cereal crops grown widely over the world. It was used for human and animal nutrition because it has rich nutrients. It was used directly in human nutrition. Most maize products in the world were used for animal feeding (Kün 1997). Maize is the grain used in the industry of starch, glucose, oil and dry food as raw material. It has the greatest yield among all warm and cool climates in the world. China is the second largest producer of maize in the world (Ci *et al*. 2012). The total area cultivated with maize in China was more than 33 million ha in 2011, which produced a total of 192 million tons (National Bureau of Statistics in China 2012). Maize productivity in China accounted for about 13.9% of the national total. The total area cultivated with maize in China was mechanized management produced high yields (Liu *et al*. 2012). However, high plant densities were needed to obtain high yields per cultivated area. The high densities resulted in thinner maize stems which increased the risk of lodging and had a detrimental influence on yield (Tokatlidis*et al*. 2011).

The risk of lodging leads to 5-25% yield losses annually in the United States of America (Norberg *et al*. 1988). By using conventional breeding tools and biotechnology techniques, maize crops are improved toward producing shorter plants that havestronger stems (Teng *et al*. 2013). The first molecular marker in corn is the maize map which was published in 1986 (Helentjaris*et al*. 1986). The genetic map in maize contains 116 loci. The genetic linkage maps of corn consist of thousands of classical mutation loci, quantitative trait loci (QTL), expressed sequence tags markers (EST), restriction fragment length polymorphism (RFLP) andsimple sequence repeat (SSR). Pollen grain development in the anther takes place in two main stages, referred to as microsporogenesis and microgametogenesis (Borg *et al*. 2009). Through microsporogenesis, microsporocytes successively undergo meiosis I and meiosis II to produce tetrads of haploid microspores. Microspores sequentially enter two rounds of mitosis after entering microgametogenesis and gradually develop trinucleate pollen grains (Borg *et al*. 2009). Pollen genesis involves the reduction in chromosomal number segregated during microsporogenesis. Recombination (RECB) is a component of the cohesin complex that holds sister chromatids together. The shugoshin (SGO) protects RECB from phosphorylation and ensures stepwise cohesin release in meiosis (Shao *et al*. 2011).

Mutation of either Rec8 or Sgo1induced premature segregation of sister chromatids and caused severe chromosome mis-separation. The nuclear division cycle is required for faithful chromosome segregation (Cheeseman *et al*. 2006). Kinetochore protein directly interacts with the mini-chromosome instability complex to form a visible chromosomal bridge between sister kinetochores to ensure co-orientation of sister kinetochores toward the opposite poles during meiosis I (Li and Dawe 2009). Aurora B kinase plays a significant role in kinetochore orientation and connection with the microtubules by correcting incorrect chromosome-microtubule interactions before the cell cycle proceeds (Meyer *et al*. 2013). MIS12 is a central kinetochore component required for equal chromosome segregation during meiosis I in maize (Afreen and Varma 2015). The loss function of either Ndc80 or Mis12 causes severe chromosome mis-segregation and aneuploidy. Impairment of chromosome regulator condensation (RCC1), a guanine nucleotide exchange factor, phosphorylation or methylation leads to defective spindle assembly, as well as, abnormal chromosome segregation (Peters 2006). Cell division cycle regulates chromosome separation at anaphase by binding to and activating anaphase-promoting complex/ cyclosome complex for the destruction of securing (Peters 2006). Pollen genesis and germination in maize are two important steps towards successful pollination. Pollen genesis and functioning are extremely vulnerable to environmental stressors. Abiotic stressis greatly reduced pollen viability (Tang *et al*. 2011). Water insufficiency at the meiotic stage leads to pollen sterility in maize, wheat and rice (Zhuang *et al*. 2007). Cold stress increases the rate of male sterility in sorghum by disrupting the formation of pollen mother cells and cycle progression at the leptotene stage in meiosis I (Brooking 1976).

Ethephon (2-chloroethyl phosphonic acid) is a growth regulator in plants that inhibits stem elongation and increases stem thickness. Thereby it is stimulating plant resistance to lodging (Dahnous*et al*. 1982). Ethephon decline lodging by 85-93% but also slightly reduced yield by 2 - 6 % (Khosravi and Anderson 1991). Plant growth regulators (PGRs) have been used recently to control lodging in maize (Schluttenhofer*et al*. 2011). In some species gibberellins (GAS) promote flowering whereas in others inhibit flowering and promote the expression of vegetative parameters, depending on the genotype, day length, and the time of treatment (King *et al*. 1987). In corn GA-deficient dwarf 1 (d1) mutant induced more leaves than normal, reflecting that GA stimulates flowering in maize (Poethig 1985). GA application restores dwarf mutants in maize to a normal height (Fujioka *et al*. 1988). In combination with GA3, auxin stimulates cell division. GA3 levels decrease in plants under drought deficit (Khatami *et al*. 2015). Besides, indole butyric acid (IBA) stimulates cell division in meristematic zones and the ability of the plant to absorb nutritive material that leads to increased grain productivity (Arteca 1996). GA3 also increases the length of plants and the growth rate of cells through stimulating mitosis, as well as, increasing chlorophyll pigment and the effective age of leaves which leads to increased grain yield per area. Additionally, Cao and Shannon (1997) found that the application of GA3 increased starch accumulation and protein secretion in endosperm suspension cells of maize. Cytokinins (CKS) play a significant role in plant growth and development by regulating mitotic activity and promoting cytokinesis (Carle *et al*. 1998). GA3 stimulates cell elongation, cell division and mitotic activity (MacDonald and Little 2006). Besides, Tabur and Demir (2009) found that exogenous application of 24-epibrassinolide decreased about 50% the mitotic index and showed a high number of chromosomal abnormalities. Furthermore, Kartal *et al*. (2009) stated that homobrassinolide application increases mitotic activity, as well as, mitotic abnormalities if compared with the control. Meanwhile, Tabur and Demir (2009) found that the mitotic index remarkably decreased with triacontanol, as a new plant growth regulator discovered in the last two or three decreases, pretreatment in the root meristem of barley increased chromosomal aberrations. To meet the increasing requirements of maize foods, it is important to increase production and productivity. Increasing maize productivity was carried out with the plant genotypes that are tolerant to biotic and abiotic stresses (Li *et al*. 2019). The application of plant growth regulators (PGRs) was related to the adaptation to drought stress via spicing the mRNA protein doubling mechanism (Neill *et al*. 2019). PGRs were classified into two major groups namedgrowth inhibitors andbiostimulants. Both groups fall into five types namely; auxins, cytokinins, gibberellins, abscisic acid and ethylene (Rademacher 2015). So, Zhang *et al*. (2017) stated that the application of PGRs could increase the yields of maize by reducing the percentage of plant lodging and increasing plant density. The application of PGR induced optimal maize plant height which decreases by 40 - 90 cm (Spitzer *et al*. 2015). Therefore, lowering plant height is a critical tool to prevent the maize plants from lodging (Zhang *et al*. 2017). Ethephon as a common PGR dramatically decline lodging rate by reduced plant height but also decreased leaf area index and crop growth rate (Ahmad *et al*. 2020). Growth regulators are synthetic chemicals used to decrease the undesirable longitudinal growth of plant shoots without reducing productivity (Rademacher 2000). Furthermore, Evans and Poethig (1995) found gibberellin is the only one of several factors regulating phase change in maize. Thus, the aim of this study was to determine the cytogenetic and developmental effects associated with the application of gibberellic acid and ethephon on maize and their properties of effects on maize productivity.

MATERIALS AND METHODS

Genetic stocks: *Zea mays* L. was used as a genetic material in this study. The seeds were obtained from a maize research program belonging to Field Crops Research Institute, Agriculture Research Center, Egypt. The seeds include two single hybrids namely single hybrid 3062 (H_1) and single hybrid 3198 (H_2).

Experimental site: The experiments were performed in a special production farm in Sherenkash Village belonging to El-Mansoura Center, about 2500 m above sea level during the summer growing seasons of 2017 and 2018. The soil texture was clay loam. The foliar application of two growth regulators was applied in two physiologically sensitive growth phases i.e. 4 to 6 leaf, as well as, at flowering stages. Genotypes constituted the main plots and the plant growth regulators formed the subplots.

Plant growth regulators: Two plant growth regulators were used in this study. These including gibberellic acid were used at two concentrations 100 ppm and 500 ppm. In addition, ethephon was used at two concentrations 1000 and 3000 ppm. Ethephon is converted in plant cells into ethylene which is a potent regulator of plant growth and ripeness (Zhang *et al*. 2002). Gibberellic acid (GA_3) is also called gibberellin. Its chemical formal is C_{19} H_{22} O_6. The plants in their normal state produce large values of GA_3.It stimulates plant growth and elongation of cells. It helps plant growth if used in small amounts. GA_3 stimulates the cells of germinating seeds to produce mRNA molecules that encode hydrolytic enzymes. GA_3 can stimulate rapid stem and root growth. It can induce mitotic division in the leaves of some plants, as well as, increase the rate of seed germination (Edwards 1976). All solutions were prepared fresh on the morning of application day.

Field planting: The field experiments were performed in clay loam soil in Sherenkash Village near the city of Mansoura. The experiment was conducted in a split-split plot design arranged with a completely randomized block design with three replicates. The maize genotypes constituted the main plots and the concentrations of plant growth regulators formed the subplots. Each experimental plot had a totalizing useful area of 24 m^2 including eight planting rows of 3 meter length, 0.7 m width and 0.3 m distance between rows. The experimental areas were surrounded by two guard rows considered as border. Each two holes were 20 cm apart. Four grains of maize were hand-dropped per hole at 7 cm depth. The grains were coated with the soil to prevent from drying, birds, pests and fungi. The treatments were randomized within the main plots, as well as, in the sub-plots. The M_1 plants were self-crossed in the first growing season to produce populations segregating for homozygous genotypes. The M_1 grains were grown in the second season to generate M_2 families which may contain double mutants that are assumed to be extremely

short dwarf plants. Fertilizer application as recommended by the Egyptian Ministry of Agriculture followed high yield practice was applied according to Tedesco *et al*. (2004) with a base fertilizer gift of 75 kg urea (N) ha^{-1}, 75 kg triple super phosphate ($P_2 O_5$) ha^{-1}, 90 kg potassium chloride (K_2O) ha^{-1}, as well as, a top dressing with 150 kg ha^{-1} of urea. This application was done at seven expanded leaves stages and 75 kg ha^{-1} (46.5% N) at the tassel stage to reach a maximum grain yield per area. The experiment was rain-fed. During the growing seasons, no herbicides or pesticides were used (Zhang *et al*. 2014). The grains were sown with four seeds per hole. Twenty-three days after sowing, the number of plants was reduced to one plant per hole to adjust the plant density to the required value in each treatment. Tillers had been removed immediately after the appearance. Axillary branches were not produced. Planting time in late April is the main key to proper corn cultivation. For better comparisons of grain yield per plant tillers were removed immediately after appearance and additional cobs on the main culm were not removed. The experiment was irrigated whenever soil moisture was reduced by furrow irrigation at weekly intervals. The plots were regularly hand-weeded Sulistiono *et al*. (2021).

Phenotypic analysis: For each maize genotype and treatment, ten selected plants per sub-plot were used for measuring vegetative traits and yield components. The plants were harvested by hand from each sub-plot at maturity during the late days of September. Ear weight after sum drying using a fixed water content of 14.0% was determined on ten selected ears per sub-plot in each treatment according to Noein and Soleymani (2022). Lodging at the milk stage was minimal (< 3%) in both growing seasons. The lodging faced the experiment caused by field management or a typhoon (maximum wind speeds of 18 ms^{-1}) and they did not recover (Zhang *et al*. 2014). Plant height was measured by cm using meter rulers when the plants became to bloom from the ground surface to the plant terminal (Hütsch and Schubert 2017). All measurements were taken from the central rows. The two outer lines were considered as a border. At physiological maturity, ears were manually harvested, shelled, sun-dried first and then transferred to oven dried at 60 ^{o}C until they reached a constant mass. Then, the ear weight was determined and expressed by grams at a standard moisture of 130 g kg^{-1} according to Leolato*et al*. (2017). All variables were evaluated using ten phenologically uniform plants that were previously tagged on each split-split plot according to Leolato*et al*. (2017).

Measurements

Leaf area: Leaf area is often unutilized for measuring plant growth, being directly related to photosynthesis and transpiration rate, among other physiological processes. In this trait, Blanco and Folegatti (2005) demonstrated that the leaf area is a key variable in studies of plant growth and photosynthetic efficiency. In addition, Favarin *et al*. (2002) stated that leaf area is used as a yield indicator and it can be useful for crop technical evaluations. It was expressed as m^2 when the plants were 50 days old using the fresh weight method. Ten disks were taken from the fresh leaves using cork piercing 1.5 cm diameter and then weighted. All the plant leaves were weighted to be applied in the following formula.

$$\text{Leaf area } (m^2) = \frac{\text{Fresh weight of total leaves per plant} \times 10 \times \text{area of disk}}{\text{Fresh weight of 10 disks}}$$

Biochemical analysis

Chlorophyll content: Chlorophyll pigment concentration was measured according to Oron *et al*. (1988). About 0.5 grams of the plants fresh weight were taken to be extracted pigments concentration in 4.5 ml methanol in a test tube container. The container was left in a cool temperature, dark place for 24 hours. Chlorophyll was measured at 20 days of plant old using a Spectrophotometer at wavelengths 663 and 645 nm. The absorption spectrum of different pigments of chlorophyll a and b was determined and transferred to their concentrations according to Lichtenthaler and Wellburn (1983) as follows;

$$\text{Chlorophyll a } (\mu g \,/\, mg\ FW) = \frac{[12.25\ (A663) - 2.79\ (A645)]\ \text{volume (ml)}}{\text{Weight of leaf tissue (mg)}}$$

$$\text{Chlorophyll b } (\mu g \,/\, mg\ FW) = \frac{[21.5\ (A645) - 5.1\ (A663)]\ \text{volume (ml)}}{\text{Weight of leaf tissue (mg)}}$$

In addition, total chlorophyll was determined according to Ahmed *et al*. (2020) as follows;

Total chlorophyll ($\mu g \,/\, mg$ FW)= chlorophyll a + chlorophyll b

Cytological analysis: At the time of flowering after 45 days from sowing the treated grains in the field, young flower buds from 15 plants selected randomly from each treatment dose and control were separated to be fixed in Farmer's solution. For meiotic studies young male flower buds containing pollen mother cells (PMC_S) at five cm long before appearing from the meristems top of the plant were excised and then fixed in Farmer's fixative freshly prepared solution consisting of 3 absolute alcohol : 1 acetic acid for 24 hours and rinsed in 70% ethyl alcohol. Anther squash technique was used to prepare slides. One flower was taken to be stained with acetocarmine (2%) and cytological preparations were done using acetocarmine methodology according to Turker *et al*. (2008). Microscopic observations were made with a Nikon light microscope using 10 x 40 magnification. Well spread the meiotic cells in the anthers containing flower and scored the various types of cytological effects induced by each plant growth regulator. After the staining procedure, the meiotic cells were seen to have been stained red violette. The results of this study are expected to appear chromosomal abnormalities associated with the application of plant growth regulators based on abnormal chromosomal behavior in meiosis. These observations were made during the growth and flowering stage of plants. More than 20 dividing PMC_S from each treated plant and the control populations were examined and analyzed. Photographs were recorded from freshly prepared slides using a Nikon research photomicroscope according to Rai *et al*. (2010).

STATISTICAL ANALYSIS

The data were subjected to analysis of variance (ANOVA) and the significance of the experimental treatments was detected using the F-test at 0.05 significant level. In addition, the least significant difference (LSD) test was used to compare the differences between means at 0.05 and 0.01 probability levels. The data were also subjected to analysis of variance using split-split plot analysis according to Steel and Torie (1960).

RESULTS AND DISCUSSION

Plant height: The results described in Table 1 showed that all concentrations of GA_3 significantly decreased the plant height of the H_1 genotype among M_1 and M_2 generations. In contrast, both concentrations of GA_3 significantly increased plant height of the H_2 genotype among M_1 and M_2 generations, except GA_3 - 100 ppm significantly decline plant height if compared with the control. Both concentrations of ethephon significantly decreased the plant height of both hybrid genotypes among M_1 and M_2 generations. The higher concentration of ethephon recorded the lowest rate of plant height among both hybrid genotypes in the M_1 and M_2 generations. The results are supported by the findings of Kaya *et al*. (2006), who pointed out that gibberellic acid sprayed on maize has effectively contributed to increasing the length and elongation of the stem. Besides, Azizi (2012) reported that GA_3promoted plant cells todivision and elongation, as well as, helps to stimulate leaf area and grain weight which have positively affected on yield components.These findings are in line with Ghodrat *et al*. (2012), who reported that GA_3 increases the ability of plants to absorb nutrients from the soil and the activity of cell division in meristematic zones of plants which finally leads to increased grain yield, as well as, increases the sink strength via promoting the length and growth rate of cells.The reduction of plant height under the influence of ethephon

is due to the inhibition of cellular division and elongation (Davies 2010). These results agreed with Zhang *et al*. (2014), who found that the application of ethephon decline plant height and ear position and improves the resistance of maize to lodging. Confirming the results tabularized in Table 2, hybrid genotypes, PGRs, concentrations of PGRs, the interaction between PGRs by their concentrations, as well as, the interaction between hybrids by PGRs by their concentrations achieved significant effect on plant height among M_1 and M_2 generations. In addition, the interaction between hybrids by PGRs, as well as, the interaction between hybrids by concentrations of PGRs appeared the same trend on plant height in M_2 generation. The results reflected that plant height was significantly impacted by hybrid genotype, PGRs, the interaction between hybrids by PGRs, concentrations of PGRs, the interaction between hybrids by concentrations, PGRs by their concentrations, as well as, hybrids by PGRs by their concentrations. Thus, plant growth regulators are artificial chemical substances used to decrease plant height in order to control maize lodging (Schluttenhofer*et al*. 2011). The effect of PGRs on vegetative and perhaps generative plant growth is greater dependent on the dosage and application time of PGRs and probably varies with the maize genotype as seen in this study (Hütsch and Schubert 2018).

The results obtained herein agreed with Li *et al*. (2019), who reported that ethephon inhibits stem elongation and promotes stem thickness, thereby improving resistance to lodging.Furthermore, Dong *et al*. (2006) found that the combination of ethephon with diethyl aminoethyl hexanoate (DA-6) decreased stem length and increased internode diameter below the ear position improving lodging resistance. Furthermore, paclobutrazol is a plant growth regulator that mainly decreases the length of the second internode, resulting in plant height decline. The results confirmed the findings of Leolato*et al*. (2017), who noticed a reduction in plant height of maize with the application of PGR, trinexapac-ethyl. Decreased plant height affected by some doses of PGR may be due to the regulation of physiological processes by synthesizing specific kinase proteins responsible for cell division, differentiation and morphogenesis (Zamaninejad*et al*. 2013). The same pattern was also obtained by Leolato*et al*. (2017), who found that trinexapac-ethyl applied in maize reduced plant height, as well as, decreased 1000 grains weight. Moreover, Spitzer *et al*. (2015) found that ethephon application on maize reduced plant height of about 46 cm. This reduction in plant height was due to the shortening in length of internodes. In addition, Spitzer *et al*. (2015) noted that the preparations containing ethephon considerably reduced plant height and diminished plant yield.

Table 1. Mean values of plant height (meter) at 120 days plant old influenced by plant growth regulators among two maize genotypes

Treatments (ppm)		Single hybrid 3062 (H_1)		Single hybrid 3198 (H_2)	
		M_1	M_2	M_1	M_2
0		2.82	1.75	2.61	1.62
GA_3 - 100		2.39	1.55	2.49	1.68
GA_3 - 500		2.64	1.50	2.63	1.78
Ethephon - 1000		2.03	0.83	2.02	0.99
Ethephon - 3000		1.89	0.64	1.95	0.71
F-test		**	**	**	**
LSD	0.05	0.01	0.03	0.03	0.04
	0.01	0.02	0.04	0.04	0.06

** : Significance at 0.01 probability level.

Table 2. Analysis of variance for plant height among M_1 and M_2 generations of two hybrid genotypes influenced by plant growth regulators

Source of variance	DF	M_1			M_2		
		SS	MS	Calculated F	SS	MS	Calculated F
Treatment (Hybrids x PGRs)	3	1.9172	0.6391	3130.14 **	4.36	1.45	4869.92 **
Replications	2	0.0007	0.0003	1.65 ^{NS}	0.00	0.00	0.29 ^{NS}
Hybrids	1	0.0070	0.0070	34.31 **	0.15	0.15	503.72 **
PGRs	1	1.9097	1.9097	9353.65 **	4.20	4.20	14065.34 **
Hybrids x PGRs	1	0.0005	0.0005	2.47 ^{NS}	0.01	0.01	40.69 **
Error (1)	6	0.0012	0.0002		0.00	0.00	
Main plot	11	1.9191			4.36		
Concentrations	1	0.0126	0.0126	70.35 **	0.07	0.07	268.59 **
Hybrids x Concentrations	1	0.0005	0.0005	2.81 ^{NS}	0.00	0.00	7.93 *
PGRs x Concentrations	1	0.1365	0.1365	761.88 **	0.11	0.11	419.67 **
Hybrids x PGRs x Concentrations	1	0.0100	0.0100	55.84 **	0.02	0.02	89.77 **
Error (2)	8	0.0014	0.0002		0.00	0.00	
Sub-plot	12	0.1611			0.20		
Total	23	2.0802			4.57		

SS, MS: Sum and mean squares, respectively. DF: Degrees of freedom. PGRs: Plant growth regulators.
NS, *, ** : Insignificant differences, significant at 0.05 and 0.01 probability levels, respectively.

Table 3. Mean values of leaf area (m^2) developed per plant at 20 days plant old influenced by plant growth regulators among two maize genotypes

Treatments (ppm)		Single hybrid 3062 (H_1)		Single hybrid 3198 (H_2)	
		M_1	M_2	M_1	M_2
0		0.069	0.057	0.059	0.042
GA_3 - 100		0.066	0.052	0.060	0.048
GA_3 - 500		0.063	0.048	0.060	0.055
Ethephon - 1000		0.057	0.030	0.055	0.034
Ethephon - 3000		0.056	0.020	0.054	0.031
F-test		**	**	**	**
LSD	0.05	0.0002	0.0009	0.0003	0.0002
	0.01	0.0003	0.0012	0.0004	0.0003

** : Significance at 0.01 probability level.

Also, Zhang *et al*. (2014) found that diethyl aminoethyl hexanoate as a plant growth regulator reduced plant height in two maize genotypes. Besides, the same authors found that ethephon application reduced plant height and ear position but lowers yield and grain weight especially at the high rates of application. Besides, Cao *et al*. (2015) stated that PGRs significantly reduced ear height and plant height reduced lodging in maize. Ethephon has been reported to have highly significant effects on internode length (Earley and Slife 1969), plant height (Cox and Andrade 1988) and ear height (Kasele*et al*. 1994).

Leaf area: The results mentioned in Table 3 showed a significant decrease in the leaf area of the H_1 genotype in response to GA_3 application among M_1 and M_2 generations. In contrast, the leaf area was significantly increased in the H_2 genotype among M_1 and M_2 generations in response to GA_3. Meanwhile, leaf area was significantly decreased in H_1 and H_2 genotypes treated with both ethephon concentrations among M_1 and M_2 generations. The higher concentration of ethephon has reduced the rate of leaf area. The results indicated that gibberellic acid may stimulate increased cell division and elongation. These results agreed with Kaya *et al*. (2006), who found that gibberellic acid increases the length and growth rate of cells, as well as, increases the effective age of leaves and chlorophyll pigment, which finally leads to increased grain yield. In addition, GA_3 enhances root growth, shoot growth, shoot dry weight and protein accumulation (Lulai *et al*. 2016). The results obtained herein are in harmony with Hütsch and Schubert (2017), who found that the growth retarded plants had higher leaf areas and reduced transpiration rates. The same authors found that the higher shoot growth after GA_3 application was accompanied by a decline in leaf area and an increase in transpiration rate during one week before anthesis. Maize growers needed to use plant growth regulators as an alternative management practices to overcome the negative consequences of high plant densities.The application of ethephon as a plant growth regulator may inhibit the gibberellins biosynthesis that is produced naturally by plant cells, reducing stem length, plant height and leaf area.

Hybrids with erect leaves and short plant height are more responsive to crowding than that with long stems and flagged leaves (Strieder *et al*. 2008). Confirming the results presented in Table 4, the leaf area per plant was significantly influenced by hybrids, PGRs, the interaction between hybrids by PGRs, concentrations of PGRs, the interaction between hybrids by concentrations, as well as, hybrids by PGRs by their concentrations among M_1 and M_2 generations. In addition, the interaction between PGRs by their concentrations appeared the same trend in leaf area developed per plant in M_2 generation. The results indicated that the leaf area developed per plant significantly responded to hybrid genotypes, PGRs, the interaction between hybrids by PGRs, concentrations of PGRs, the interaction between hybrids by concentrations of PGRs, PGRs by theirconcentrations, as well as, hybrids by PGRs by their concentrations. The results obtained herein are in line with the findings obtained by Shekoofa and Emam (2008), who decided that application with ethephon was associated with shortening in plant height, leaf area index, decreasing lodging but also slightly decline yield by 2 - 6%. In addition, Kamran *et al*. (2020) stated that higher photosynthetic rate and longer duration rate of green leaf area were mainly responsible for increasing grain productivity after the application of paclobutrazol as a plant growth regulator. The flowering stage is a crucial phase for the formation of maize yield. When the plants begin to grain filling, leaf area function gradually declines leading to reduced photosynthetic leaf area and turned to leaf senescence. High photosynthetic area during this period is important for increasing dry matter and accumulation yield (Yang *et al*. 2017). These facilitate higher photosynthetic compound production and transportation to the maize ear. Leaf senescence was affected by the levels of photosynthetic pigments and the antioxidant defense system (Wu *et al*. 2022). Besides, Ren *et al*. (2022) stated that spraying with ethephon + diethyl aminoethyl hexanoate (EDAH) reduced plant height, ear height and leaf area. In addition, Shekoofa and Emam (2008) found that plants without ethephon at higher plant density had the greatest leaf area index but under different levels of ethephon at higher plant density decreased leaf area index.

Table 4. Analysis of variance for leaf area in M_1 and M_2 generations of two hybrid genotypes influenced by plant growth regulators

Source of variance	DF	M_1			M_2		
		SS	MS	Calculated F	SS	MS	Calculated F
Treatment (Hybrids x PGRs)	3	0.000359271	0.000119757	2993.93 **	0.003056	0.001	9819.41 **
Replications	2	0.000000023	0.000000012	0.29 ^{NS}	0.0000003	0.0000002	1.50 ^{NS}
Hybrids	1	0.000077400	0.00007740	1935.01 **	0.00011	0.00011	1056.90 **
PGRs	1	0.000274050	0.00027405	6851.26 **	0.0029	0.0029	27799.84 **
Hybrids x PGRs	1	0.000007820	0.00000782	195.51 **	0.00006	0.00006	601.48 **
Error (1)	6	0.000000240	0.00000004		0.000001	0.0000001	
Main plot	11	0.000359535			0.003057		
Concentrations	1	0.000005900	0.000005900	363.10 **	0.00003	0.00003	218.64 **
Hybrids x Concentrations	1	0.000003920	0.000003920	241.26 **	0.0001	0.0001	625.97 **
PGRs x Concentrations	1	0.000000004	0.000000004	0.23 ^{NS}	0.0001	0.0001	610.70 **
Hybrids x PGRs x Concentrations	1	0.000007150	0.000007150	440.03 **	0.00001	0.00001	61.28 **
Error (2)	8	0.000000130	0.000000016		0.000001	0.0000002	
Sub-plot	12	0.000017105			0.000243		
Total	23	0.000376640			0.0033		

SS, MS: Sum and mean squares, respectively. DF: Degrees of freedom. PGRs: Plant growth regulators.
NS, ** : Insignificant differences, significant at 0.01 probability level, respectively.

These effects were found before in corn by Durli (2016). The application of ethephon can enhance maize productivity using crowded stands of maize ofgenotypes having yield decreases due to stem lodging and breaking. This plant growth regulator can also exhibit positive effects for allowing greater interception of solar radiation and consequently enhanced grain yield. Both hybrid genotypes differed in their response to GA_3 concerning leaf area and plant height which increased in the H_2 genotype and decreased in the H_1 genotype. The present study was based upon the hypothesis thatethephon and GA_3 with some genotypes increase the response of maize to crowding increasing grain yield of maize. The divergent results of GA_3 among both hybrid genotypes emphasized the need for a better understanding of gene expression in both hybrids used in this study under the effect of PGRs, as well as, hybrid sensibility. Such behavior is probably due to morphophysiological traits of the genotypes (Leolato *et al*. 2017).

Thus, ethephon affected negatively on leaf area index with an associated reduction in crop growth rate. So, ethephon might be more necessary under water stress since it decline leaf area and hence improves water availability for the reproductive phase of crop under drought conditions. The decrease in leaf area was not beneficial in maize crops under the availability of moisture conditions. This is because it impaired the photosynthetic efficiency of the above-ground parts (Cox and Andrade 1988). Additionally, Shekoofa and Emam (2008) found that ethephon application reduced leaf number in maize, producing plants with lower, smaller and thicker leaves. The results are in harmony with Sulistiono *et al*. (2021), who stated that the leaf area was affected by the interaction between the PGR dose and the time of application. The number of leaves and stage of plant development are closely linked to the photosynthetic capacity of maize. Leaves located upper the main ear are relatively older and more active than that below the ear. The leaves upper the ear

contribute with carbohydrates for grain filling in the ear, whereas that below the ear primarily sustain the roots and stem. Therefore, leaves number developed upper the ear position in maize is an important element in determining overall plant productivity. Thus, the number of leaves upper the ear can lead to improved photosynthetic efficiency and greater accumulation of assimilates to the ear. This reflected, in turn, higher ear size and stimulated grain yield (Palmer *et al*. 1973). The results indicated that the genes control leaf number in maize whose expression was downregulated under the effect of PGRs leading to reduced leaf area. Meanwhile, these genes in control plants were upregulated (Li *et al*. 2024). The results obtained herein agreed with Mustafa *et al*. (2024), who found a highly significant relationship between leaf area and all studied traits, suggesting that increasing leaf area tends to increase other traits.

Chlorophyll pigment: The results presented in Table 5 showed significant differences between treatments with PGRs for chlorophyll a, chlorophyll b and total chlorophyll in the M_1 generation, as well as, chlorophyll a in the M_2 generation of H_1 genotype. Total chlorophyll significantly declined gradually among the concentrations of PGRs in the M_1 generation of the H_1 genotype. In contrast, chlorophyll a was gradually increased over the control among the concentrations of PGRs in the M_2 generation of the H_1 genotype. The results agreed with Zang *et al*. (2016), who found that foliar application of gibberellic acid increased chlorophyll content, leaf area, leaf fresh weight, leaf dry weight, individual fruit weight, the number of fertile seeds and the level of chlorophyll a, b in blueberry. Recent studies have demonstrated that PGRs can effectively regulate endogenous hormones in plant cells, enhance morphological traits and improve physiological metabolism (Burton and Kemanian 2022). The results obtained herein are also in harmony with Xu *et al*. (2024), who found that following the application of PGRs there was a notable decline in chlorophyll b by 1.1% and chlorophyll a by 11.9%. The same authors found under the same condition of another treatment increase in chlorophyll b by 16.8% and in chlorophyll a more rise by 7.3%.

Furthermore, Xu *et al*. (2024) reported that applying PGRs at the 15-leaf stage in maize increases photosynthetic pigment content, extends the leaf functional stage and increases maize productivity by facilitating greater photosynthetic product and their transportation to maize ear. Photosynthetic pigments are also affected by the antioxidant defense system (Wu *et al*. 2022). Malondialdehyde (MDA) can bind with the proteins in the cell membrane leading them inactive and disrupting membrane function and structure. Antioxidant enzymes such as peroxidase, superoxide dismutaseand catalase play a protective role via combating lipid peroxidation in the membranes of plant cells. The buildup of MDA and reactive oxygen species can determine the effects on plant cell membranes including lipid peroxidation, protein denaturation and interference with photosynthesis as shown in the gradual decline of total chlorophyll concentration in M_1 generation. This ultimately accelerating leaf senescence (Wang *et al*. 2023). The results presented in Table 6 showed significant differences between treatments with PGRs for the concentrations of chlorophyll a, chlorophyll b and total chlorophyll in the M_1 generation of H_2 genotype. The same trend of significant differences between treatments was also obtained for the concentrations of chlorophyll b and total chlorophyll in M_2 generation. The total chlorophyll was significantly decreased in the M_1 generation. In contrast, GA_3 - 100 ppm and ethephon - 1000 ppm appeared significant increase in total chlorophyll among M_2 generation. Treatments with PGRs showed significant differences between treatments for the concentration of chlorophyll a in M_2 generation. These results agreed with Li-sha *et al*. (2021), who found that treatments with PGRs significantly increased the relative content of chlorophyll in maize. Zhou *et al*. (2004) found that foliar spraying with DA-6 enhanced grain yield in maize by increasing chlorophyll concentration and photosynthetic rate. Li-sha *et al*. (2021) found that treatment with DA-6 improved the photosynthetic performance of ear leaves in maize. Treatment with DA-6 can prolong the functional stage of maize leaves by delaying the senescence rate, as well as, reducing the abscisic acid (ABA) content of maize ear leaves.Extension of the functional stage of maize leaves leads to a longer time of highly active period, which contributes to the production and transport of more values of carbohydrates to the ear (Gao *et al*. 2017). Therefore, chlorophyll content and leaf area are two important traits that are related directly to the photosynthesis process.

Table 5. Mean values of chlorophyll concentrations (mg/g FW) at 20 days plant old in single hybrid 3062 (H_1) influenced by plant growth regulators

Treatments (ppm)		M_1			M_2		
		Chl a	Chl b	Total	Chl a	Chl b	Total
0		1.26	4.41	5.67	0.397	0.747	1.144
GA_3 - 100		1.31	4.01	5.32	0.400	0.747	1.147
GA_3 - 500		1.19	3.68	4.87	0.402	0.752	1.154
Ethephon - 1000		1.19	3.52	4.71	0.402	0.755	1.157
Ethephon - 3000		1.18	3.42	4.61	0.405	0.753	1.158
F-test		**	**	**	*	NS	*
LSD	0.05	0.06	0.11	0.07	0.005	0.010	0.010
	0.01	0.09	0.16	0.10	0.007	0.014	0.014

NS, *, **: Insignificant differences, significant at 0.05 and 0.01 probability levels, respectively.

Table 6. Mean values of chlorophyll concentrations (mg/g FW) at 20 days plant old in single hybrid 3198 (H_2) influenced by plant growth regulators

Treatments (ppm)		M_1			M_2		
		Chl a	Chl b	Total	Chl a	Chl b	Total
0		1.40	4.30	5.70	0.400	0.749	1.149
GA_3 - 100		1.20	3.39	4.59	0.400	0.755	1.155
GA_3 - 500		1.29	3.90	5.18	0.398	0.746	1.143
Ethephon - 1000		1.22	3.80	5.02	0.399	0.832	1.231
Ethephon - 3000		1.20	3.35	4.55	0.401	0.751	1.152
F-test		**	**	**	NS	**	**
LSD	0.05	0.04	0.07	0.09	0.004	0.014	0.012
	0.01	0.06	0.10	0.13	0.006	0.020	0.018

NS, **: Insignificant differences, significant at 0.01 probability level, respectively.

Thus, chlorophyll fluorescent is a relatively new biotechnology to study the effects of PGRs on the photosynthetic efficiency of leaves in the farm. The results indicated that chlorophyll concentration may decrease in leaves of the susceptible genotype to PGRs, but increase in the resistant genotype. The loss of chlorophyll concentration in genotypes sensitive to PGRs is caused to decrease photosynthetic activity.

This caused chlorosis or yellowing plants, leading to decreased growth and yield (Khosh and Ando 1995). The decline obtained in chlorophyll concentration may be due to chlorophyll degradation under the effect of PGRs. This agrees with Zaeifizade and Goliov (2009), who reported that resistant genotypes of durum wheat landraces to environmental stresses have more chlorophyll concentration. Besides, chlorophyll is a significant photosynthetic pigment component in maize highly determining photosynthetic capacity, plant growth and their productivity (Tak *et al*. 2018). A significant increase of total chlorophyll in M_2 generation under the effect of lower doses of GA_3 and ethephon was agreed with Kavita and Kumar (2020). The same authors found that the application of GA_3 + cytokinin at 10 ppm significantly increased chlorophyll content in two genotypes of maize. Generally, an increase in chlorophyll content is considered a trait corresponds to an increase in photosynthesis and consequently increases plants vigor and their production potential (Bashan *et al*. 2006). It can be assumed that reducing photosynthetic capacity may result in cell death (Rymen *et al*. 2007).

was significantly influenced by hybrid genotypes, PGRs and concentrations of PGRs among M_1 and M_2 generations. Therefore, new works are important to evaluate the viability of PGRs as a management tool to enhance the maize tolerance to lodging and crowding.Selfing M_1 plants was mainly caused by decreased kernel number per ear, associated with a decline in ear weight, ear size, as well as, 100-kernel weight. Lower ear size is associated with lower kernel numbers that decreased ear weight. Thus, PGRs showed a negative effect on the yield performance of maize because they may decreased the number of kernels per ear and kernel filling.This may be due to pollen abortion resulting from chromosomal anomalies in meiosis affected by PGRs that lead to the reduced number of kernels per ear.Therefore, much research attention should be done on the cytological effects of PGRs on the meiotic cells before using PGRs for yield improvement of maize. The results obtained in this study agreed with Cao *et al*. (2015), who found in low lodging years that the application of PGRs decreased the grain yield of maize by 4.58 - 6.74%. Furthermore, Gaska and Oplinger (1988) decided thatethephon applied may reduce yields if lodging is not a problem in

Table 7. Analysis of variance for total chlorophyll in M_1 and M_2 generations of two hybrid genotypes influenced by plant growth regulators

Source of variance	DF	M_1			M_2		
		SS	MS	Calculated F	SS	MS	Calculated F
Treatment (Hybrids x PGRs)	3	0.616	0.2052	518.41 **	0.00700	0.00233	109.42 **
Replications	2	0.002	0.0012	3.06 ^{NS}	0.00007	0.00003	1.62 ^{NS}
Hybrids	1	0.009	0.0092	23.25 **	0.00158	0.00158	74.27 **
PGRs	1	0.435	0.4347	1098.20 **	0.00353	0.00353	165.39 **
Hybrids x PGRs	1	0.172	0.1717	433.78 **	0.00189	0.00189	88.61 **
Error (1)	6	0.002	0.0004		0.00013	0.00002	
Main plot	11	0.620			0.00720		
Concentrations	1	0.069	0.0693	31.76 **	0.00258	0.00258	54.29 **
Hybrids x Concentrations	1	0.168	0.1683	77.10 **	0.00363	0.00363	76.20 **
PGRs x Concentrations	1	0.193	0.1926	88.22 **	0.00200	0.00200	42.00 **
Hybrids x PGRs x Concentrations	1	0.753	0.7526	344.70 **	0.00137	0.00137	28.69 **
Error (2)	8	0.017	0.0022		0.00038	0.00005	
Sub-plot	12	1.200			0.00995		
Total	23	1.821			0.01715		

SS, MS: Sum and mean squares, respectively. DF: Degrees of freedom. PGRs: Plant growth regulators.
NS, ** : Insignificant differences, significant at 0.01 probability level, respectively.

Table 8. Mean values of ear weight (g) influenced by plant growth regulators among two maize genotypes

Treatments (ppm)		Single hybrid 3062 (H_1)		Single hybrid 3198 (H_2)	
		M_1	M_2	M_1	M_2
0		235.79	125.55	259.22	127.09
GA_3 - 100		176.48	69.27	172.84	124.44
GA_3 - 500		147.85	56.93	203.90	142.88
Ethephon - 1000		111.95	27.13	100.35	45.84
Ethephon - 3000		98.14	25.94	83.62	23.16
F-test		**	**	**	**
LSD	0.05	3.85	2.43	3.87	4.44
	0.01	5.60	3.54	5.63	6.46

** : Significance at 0.01 probability level.

Thus, reduced chlorophyll content in response to some doses of PGRs, as well as, leaf area per plant are closely related with leaf senescence (Wang *et al*. 2009). Chlorophyll degradation reduces photosynthetic efficiency (Yong *et al*. 2015). Recently, some studies reported that the decline in chlorophyll concentration at the later stages of the plant life cycle was due to the increase in leaf senescence in younger plants (Ahmed *et al*. 2019). So, the loss of leaf greenness due to chlorophyll concentration decline resulting from chloroplast degradation was the first symptom of senescence (Ye *et al*. 2020). Therefore, the leaf chlorophyll concentration is the primary factor that maintains leaf photosynthesis (Cairns *et al*. 2012). The leaves provide up to 50 – 80 percent of the photosynthesis products to kernels (Ye *et al*. 2020). Lower chlorophyll concentrations obtained in this study under the effect of PGRs are primarily due to damage to chloroplast structure and down regulation of their enzymatic activities. All of these adverse alterations reduced photosynthesis and decreased dry matter accumulation, as a consequence ultimately decreased kernel productivity (Ye *et al*. 2020). The results obtained in Table 7 appeared that total chlorophyll in maize subjected to PGRs

maize. Confirming the results tabularized in Table 9, the ear weight was significantly affected by hybrid genotypes, PGRs, the interaction between hybrids by PGRs, concentrations of PGRs, the interaction between hybrids by concentrations of PGRs, PGRs by their concentrations, as well as, hybrid genotypes by PGRs by their concentrations among M_1 and M_2 generations. The results agreed with Shekoofa and Emam (2008), who found a significant interaction between ethephon treatment and irrigation levels for grain production in maize. The same authors found that yield and yield component traits could be influenced by the foliar ethephon application at the 6 - leaf stage. The results obtained herein agreed with Earley and Slife (1969), who stated that the application rate of ethephon was associated with increases in yield reduction of maize. Meanwhile, Gao *et al*. (2009) observed that ethephon has a negative regulator impact on kernel development or grain production in maize, because of its negative impacts on crop growth rate, leaf area development and reduced photoassimilate. Thus, more physiological studies are necessary to explore the role of ethephon in managing kernel abortion. The results are supported by Su *et al*. (2020), who found

that excessive N application with maize may cause slight reductions in grain production due to adverse impacts on root development though the early stages of plant growth leading to a shortage in relative nitrogen absorbed during the reproductive stage. Spitzer *et al*. (2015) found the grain yield in maize was reduced in all treatments with PGRs. Moreover, Langan and Oplinger (1987) found that ethephon had a highly significant effect on ear height, plant height, grain yield and brace root development in maize.Xu *et al*. (2024) noted that spraying PGRs at 15 - leaf stage led to enhanced ear length, number of kernels per ear and thousand-grain weightalong with a decline in the grain abortion rate. Meanwhile, Xu *et al*. (2024) stated that spraying PGRs at 10 - leaf stage reduced ear number, ear length, 1000-grain weight, number of kernels per ear, and increased grain abortion rate. These ultimately impacting on grain filling and result in decreased yield.

Table 9. Analysis of variance for ear weight in M_1 and M_2 generations of two hybrid genotypes influenced by plant growth regulators.

Source of variance	DF	M_1			M_2		
		SS	MS	Calculated F	SS	MS	Calculated F
Treatment (Hybrids x PGRs)	3	37919.79	12639.93	4267.97 **	42758.97	14252.99	3671.30 **
Replications	2	1.40	0.70	0.24 ^{NS}	1.55	0.77	0.20 ^{NS}
Hybrids	1	259.19	259.19	87.52 **	9249.79	9249.79	2382.57 **
PGRs	1	35347.21	35347.21	11935.26 **	27632.82	27632.82	7117.68 **
Hybrids x PGRs	1	2313.40	2313.40	781.14 **	5876.37	5876.37	1513.64 **
Error (1)	6	17.77	2.96		23.29	3.88	
Main plot	11	37938.96			42783.81		
Concentrations	1	296.46	296.46	72.08 **	118.36	118.36	40.46 **
Hybrids x Concentrations	1	1209.13	1209.13	294.01 **	32.49	32.49	11.11 *
PGRs x Concentrations	1	407.47	407.47	99.08 **	336.93	336.93	115.19 **
Hybrids x PGRs x Concentrations	1	1470.00	1470.00	357.44 **	1024.73	1024.73	350.33 **
Error (2)	8	32.90	4.11		23.40	2.93	
Sub-plot	12	3415.96			1535.90		
Total	23	41354.92			44319.71		

SS, MS: Sum and mean squares, respectively. DF: Degrees of freedom. PGRs: Plant growth regulators.
NS, *, **: Insignificant differences, significant at 0.05 and 0.01 probability levels, respectively.

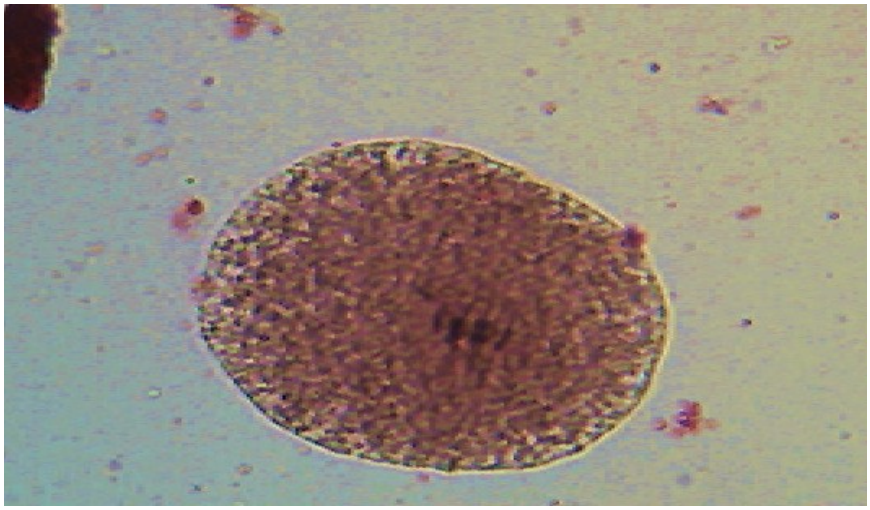

Figure 1. Meiotic abnormalities in M_1 generation of Zea mays H_1 genotype treated with GA_3 500 ppm achieved stray bivalent at metaphase I

The adverse effect of PGRs can disrupt the process of pollen formation leading to grain abortion, reducing the number of grains developed per ear, as well as, decrease in overall yield productivity (Zhang *et al*. 2021). Pollen viability is also impacted by PGRs affecting on pollination and fruit setting resulting to a higher female ear abortion rate and lower grain production (Zhang *et al*. 2018). This study was also confirmed this point.The results also agreed with Shekoofa and Emam (2008), who found that yield and yield components could be impacted by ethephon application. Furthermore, Li-sha *et al*. (2021) reported that grain production in maize was significantly influenced by PGRs. The same authors stated that kernels developed per ear were significantly reduced under the treatment with EDAH (27% ethephon + 3% DA-6). The results agreed with Sulistiono *et al*. (2021), who stated that the interaction between the dosage of PGRs and spraying time determines the weight of filled maize cob. Pollinated kernels enter the lag phase (about 15 days) with increased endosperm cell division and decreased assimilation requirements. Redundant photosynthates are stored in the stem. Then maize kernels start effective filling using photosynthates to fill the skin capacity. The carbohydrates stored in the stem are reallocated to kernels leading to reduced stem weight. Abiotic stress as PGRs affected on stored matter in the leaves and stem to be partitioned to kernels. The weight of these organs increased from pollination to 15 days after the main pollination and then declined (Gao *et al*. 2017).

Cytogenetic analysis: Meiosis was perfectly normal in the control plants that appeared with ten bivalents in diakinesis and metaphase I, in addition, 10: 10 chromosomes separated at anaphase I. Meanwhile, the plants treated with PGRs displayed varying degrees of chromosomal anomalies distributed at all stages of meiotic division. A dose-based increase in meiotic anomalies was observed among both PGRs sets. The dose of GA_3 500 ppm achieved stray bivalent at metaphase I (Figure 1). The abnormal chromosomal behavior was increased along with increasing doses of PGRs. This led to sterile pollen grains which was significantly correlated with meiotic irregularities and kernel abortion. The application of PGRs on plants revealed a greater decrease in pollen fertility as compared in control sets. The abnormalities induced differed between treatments. Therefore, the meiotic cells are a great tool as it appears the cytogenetic damage that is passed on to the next generation. Disorientation of bivalents was achieved in the M_1 generation of the H_1 genotype treated with ethephon 1000 ppm (Figure 2). This may be due to the inhibition of spindle development or to the destruction of spindle fibers formed. The behavior of this stage and the laggard chromosome generally lead to micronucleus formation. This criterion was useful in detecting anomalies in seeds derived from plants

sprayed with PGRs. The chromosomal aberrations induced in pollen mother cells of grains indicated that plants subjected to PGRs somehow alter the normal function and structure of chromosomes. These results agreed with D'Amato (1951), who reported that chromosomal aberrations were increased along with an increase in the storage periods of *Pisum sativum* L. seeds and maize grains. Accumulated chromosomal abnormalities affect gamete formation and lead to non-viable gametes, as a consequence leads to non-viable pollen grains, which considerably reduce plant fertility and increase kernel abortion.

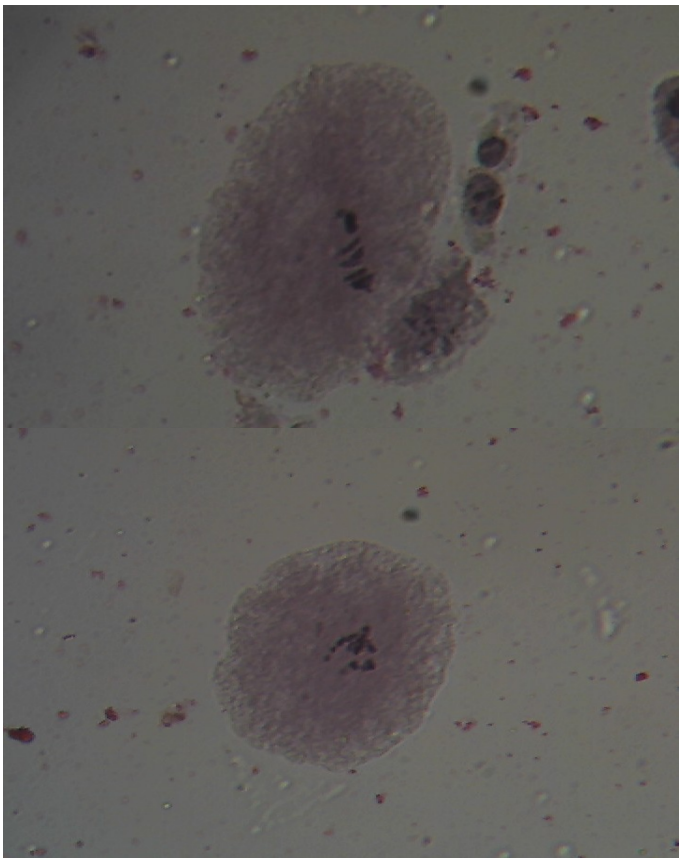

Figure 2. Meiotic abnormalities in M_1generation of Zea mays H_1genotype treated with ethephon 1000 ppm showing disorientation at metaphase I

The cytological investigation presented in Figure 3 showing stray bivalent at metaphase I in the M_2 generation of the H_1 genotype treated with GA_3 100 ppm. This is supposed to be one of the reliable indices to estimate the cytogenetic effects of any chemical agent used in the agriculture sector. The assessment of chromosomal anomalies in the present study clearly appeared that PGRs have a great potency in altering the genetic architecture of maize. In most cases, the stray bivalent fails to get the equatorial plate during metaphase and subsequently constitutes micronuclei. These results are in harmony with Khah and Verma (2017), who found that micronuclei arise from abnormal behavior of multivalents, univalents, unoriented chromosomes and fragments. This leads to decreased pollen fertility (Kolar *et al*. 2013). The frequency of meiotic anomalies observed in the present study became a significant cause of decreased pollen fertility that led to kernel abortion in maize. The cytogenetic changes brought about by PGRs provide better scope for the future avoid of these synthetic compounds from application on maize. The most common meiotic anomalies were related to irregular chromosome segregation that could be divided into two classes, (a) precocious chromosome migration to the poles and laggards as seen in Figure 4, (b) non-oriented bivalents at the equatorial plates. Stray bivalent showing here at metaphase I appeared in the M_2 generation of the H_1 genotype treated with ethephon 1000 ppm. The inability of chromosomes to congregate on the equatorial plate may be related to the kinetochore (Utsunomiya *et al*. 2002). The same authors decided that stray bivalent may resulted from the late chiasma terminalization. This may produce micronuclei if they still fail to reach the equatorial plate or reachthe poles. Micronuclei were generated when the chromosome set was divided into more than two groups reaching the opposite poles at telophase. Micronucleus producing microcytes or remaining as micronucleus in the tetrad. In maize, the micronucleus generally remains in the microspores of the tetrad (Utsunomiya *et al*. 2002).

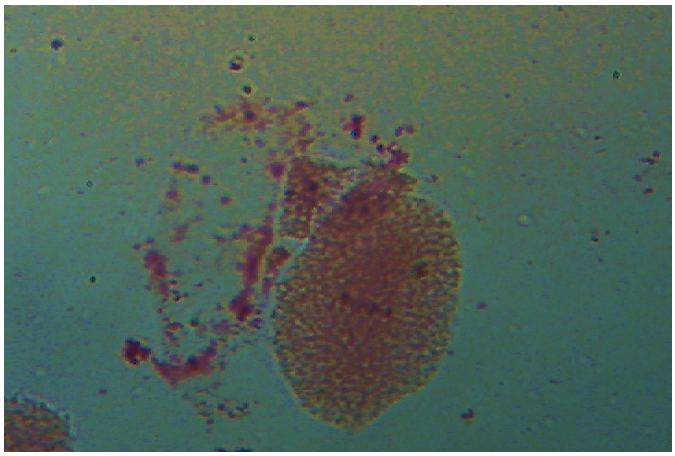

Figure 3. Meiotic abnormalities in M_2 generation of Zea mays H_1 genotype treated with GA_3 100 ppm showing stray bivalent at metaphase I

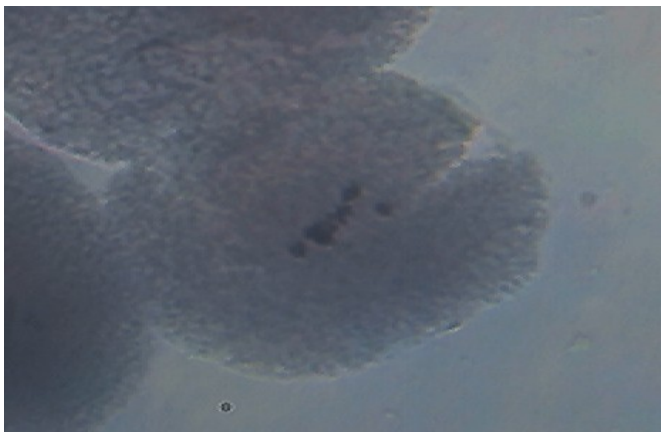

Figure 4. Meiotic abnormalities in M_2 generation of Zea mays H_1 genotype treated with ethephon 1000 ppm showing stray bivalent at metaphase I

The results that appeared in Figure 5 focused on the epigenetic effects of ethephon 3000 ppm that appeared in the M_2 generation of H_1 genotype stray bivalent at metaphase I. However, meiosis is a highly coherent and genetically programmed process. Like any other biological pathway, all sequential stages involved in meiosis are controlled by a large array of genes (Sosnikhina*et al*. 2005). Mutations arise in any of these genes that govern micro – or megasporogenesis from pre-meiotic to post-meiotic events leading to serious anomalies, resulting in genetically aberrant end products that have an adverse impact on fertility and reproductive efficiency of maize (Kaul and Murthy 1985). Pollen genesis was an important stage toward successful pollination in maize. It is extremely vulnerable to environmental stresses. Pollen viability is dramatically decreased under the application of chemicals such as PGRs (Tang *et al*. 2011). Pollen viability is a key factor in determining pollination efficiency (Wang *et al*. 2010). The results obtained herein agreed with Jain (1957), who found that heat treatment induced failure of co-orientation at metaphase I of bivalents in *Lolium*. Henderson *et al*. (1970) found abnormal orientation of bivalents during alternating cold and room temperature treatment. In addition, Maguire (1974) found an abnormality in metaphase I after maize was treated with ethylene oxide-treatment starch and its extracts. The possibility of the

same author indicated that ethylene glycol, as well as, polyethylene glycols may disrupt meiotic centromere spindle interactions.

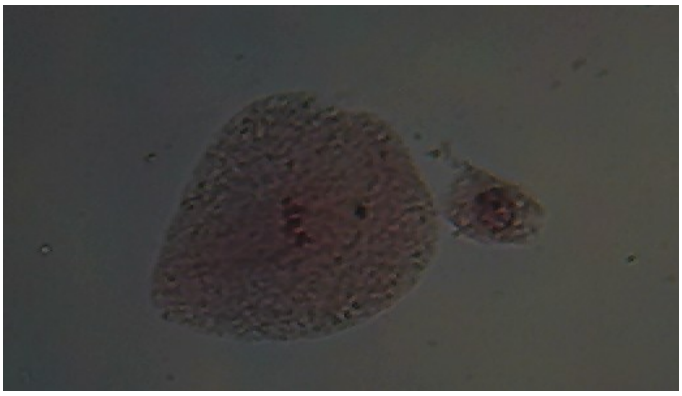

Figure 5. Meiotic abnormalities in M_2 generation of Zea mays H_1 genotype treated with ethephon 3000 ppm showing stray bivalent at metaphase I

The results presented in Figure 6 achieved multivalent formation at metaphase I resulting in M_1 generation of H_1 genotype treated with GA_3 500 ppm. Here in the present case, the presence of multivalents could lead to disturbed polarity in the cells. In disturbed polarity, multivalents could lead to the development of tripolarity due to the failure of chromosome disjunction at anaphase in meiosis I. The cells showed chromosomes are distributed at multiple poles leading to the development of individual nuclei at later stages in meiosis. The micronuclei as cytogenetic marks obtained in this study may be attributed as a result of various meiotic abnormalities at metaphase and anaphase induced by PGRs. This leads to an increase in pollen sterility with the increasing doses of PGRs. The cumulative effects of different chromosomal abnormalities due to PGRs have been a possible cause of decreasing pollen fertility (Khah and Verma 2017).

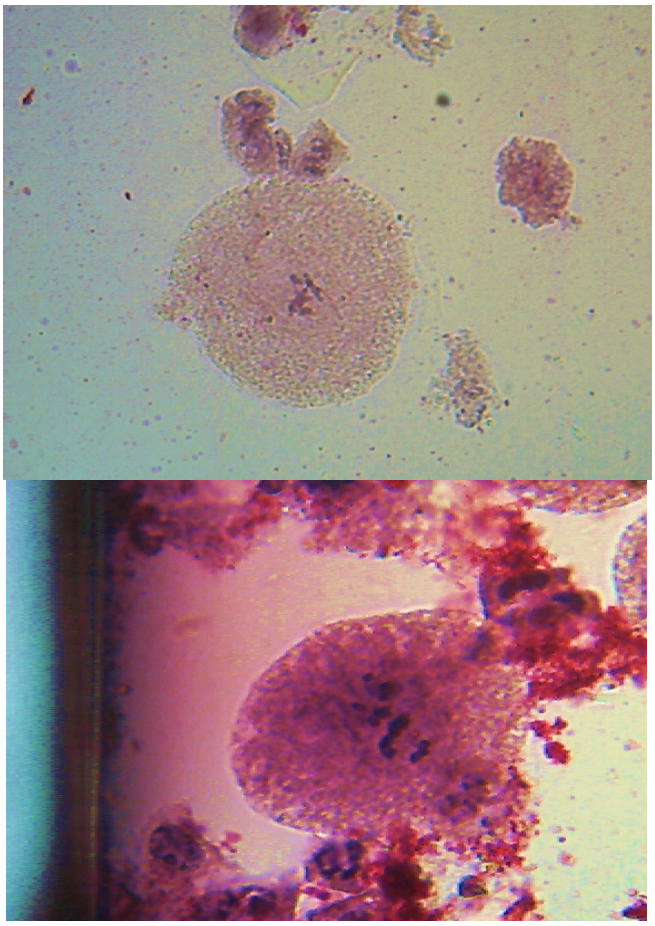

Figure 6. Meiotic abnormalities in M_1 generation of Zea mays H_1 genotype treated with GA_3 500 ppm showing multivalent formation at metaphase I

The cytological studies that appeared in Figure 7 achieved multivalent formation with non-oriented on the equatorial plate in the M_1 generation of the H_1 genotype treated with ethephon 1000 ppm. Chromosome disorientation was due to the destruction of spindle fibers formed or to the inhibition of spindle formation. These generally lead to micronucleus formation. The results obtained herein agreed with Kumar and Rai (2009), who observed that gamma rays and ageing treatments induced a number of chromosomal anomalies independently in the gametic cells of maize. Meanwhile, Khah *et al*. (2018) found that gamma-irradiated seeds in maize displayed a wide spectrum of meiotic anomalies including lagging chromosomes, chromosome stickiness, multivalents, unoriented bivalents, micronuclei and bridges. In addition, other aberrations were induced as univalent precocious chromosome movement, disturbed polarity and chromosome scattering. The same authors investigated that the spectrum of these meiotic anomalies was observed higher at metaphase than anaphase and telophase as seen in this study.

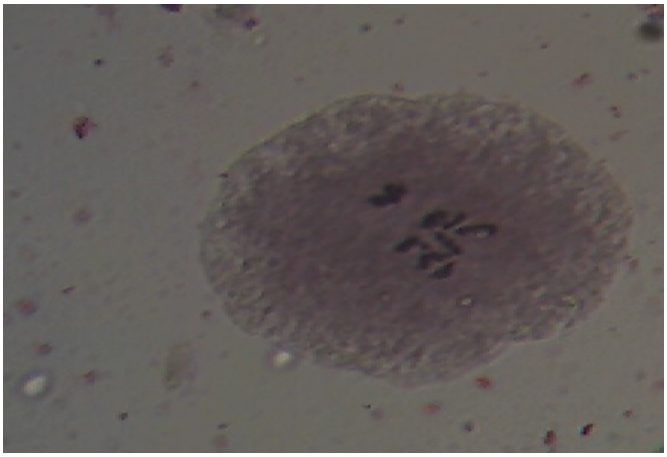

Figure 7. Meiotic abnormalities in M_1generation of Zea mays H_1genotype treated with ethephon 1000 ppm showing multivalent formation with non-oriented on the equatorial plate at metaphase I

The cytogenetic study observed in Figure 8 appeared chromosome scattering at metaphase I in the M_1 generation of the H_1 genotype treated with ethephon 1000 ppm. The scattering of chromosomes that appeared herein may resulted from the inhibition of spindle development or to the destruction of spindle fibers formed. These abnormalities lastly affect the fertility of pollen grains, as a consequence productivity of maize. The results obtained in Figure 9 achieved chromosome scattering at metaphase I in the M_1 generation of the H_1 genotype treated with ethephon 3000 ppm. These results are in harmony with Tabur and Demir (2009), who found that exogenous application of 24-epibrassinolide on barley declined approximately 50% the mitotic index with an induced higher number of chromosomal anomalies. In addition, Kartal *et al*. (2009) observed that homobrassinolide application on barely increased mitotic activity and mitotic abnormalities. Recently, much interest has been focused on the cytogenetic effects of PGRs. It is presented evidence that the mitotic index was decreased by all PGRs but chromosomal aberrations were increased (Kartal *et al*. 2009). This indicated that many stimulators inhibit cell division, as well as, can produce abnormalities in chromosome structure and behaviors. From the results obtained in this study, it is evident that there is no need to add exogenously any PGRs on maize to avoid cytogenetic abnormalities that decrease pollen fertility, as well as, decline plant productivity. The cytogenetic study presented in Figure 10 achieved chromosome scattering at metaphase II in the M_2 generation of the H_2 genotype treated with GA_3 100 ppm. The results presented in Figure 11 appeared the same trend of chromosome scattering obtained at metaphase I in the M_2 generation of the H_2 genotype treated with GA_3 500 ppm. These results indicated that both concentrations of GA_3 induced scattering of chromosomes that may be due to the destruction of spindle fibers formed or to the inhibition of spindle formation (Kumar and Rai 2009). This is in line with Petronczki *et al*. (2003), who observed that chromosome misorientation and missegregation

occur once cohesion is interfered with. The inner kinetochore proteins were essential for kinetochore assembly and functioning (Cheeseman and Desai 2008). Kinetochore protein MIS 12 is required for co-orientation of sister kinetochores during meiosis I in maize (Li and Dawe 2009). Therefore, the weakness in kinetochore protein activity leads to scattering chromosomes as seen in this study.

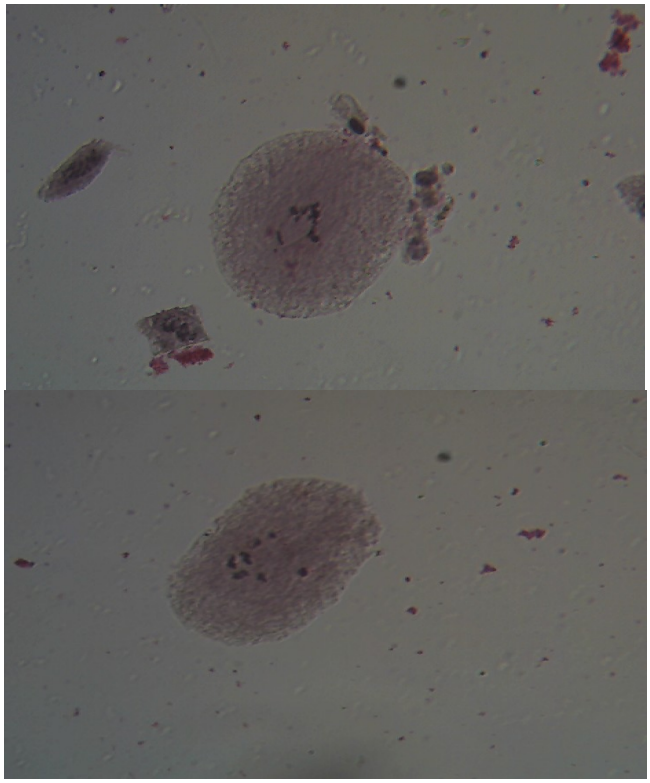

Figure 8. Meiotic abnormalities in M_1generation of Zea mays H_1genotype treated with ethephon 1000 ppm showing chromosome scattering at metaphase I

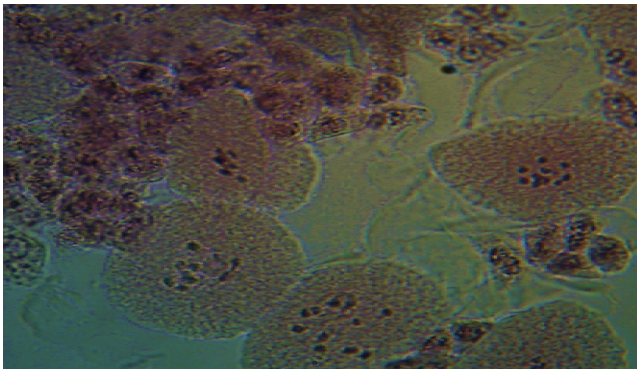

Figure 9. Meiotic abnormalities in M_1 generation of Zea mays H_1 genotype treated with ethephon 3000 ppm showing chromosome scattering at metaphase I

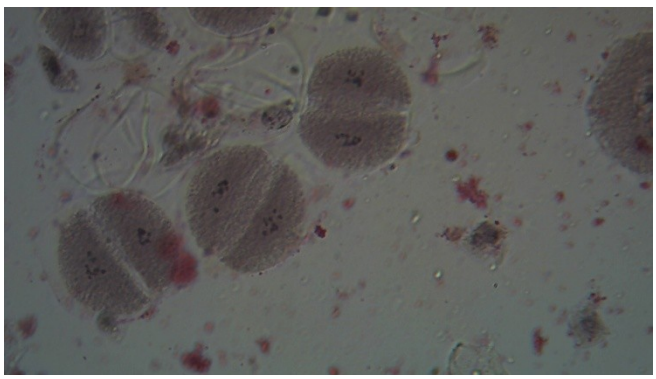

Figure 10. Meiotic abnormalities in M_2 generation of Zea mays H_2 genotype treated with GA_3 100 ppm showing chromosome scattering at metaphase II

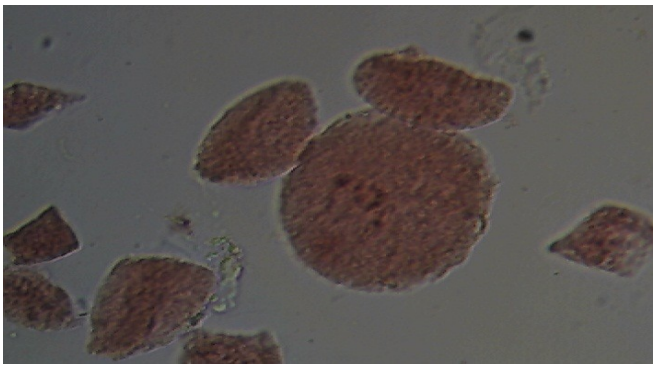

Figure 11. Meiotic abnormalities in M_2generation of Zea mays H_2genotype treated with GA_3 500 ppm showing chromosome scattering at metaphase I

Zheng *et al*. (2017) decided that *Ndc* 80 is an essential regulator of chromosome orientation in maize and spindle attachment which had a four-fold lower expression level in the case of misorientation. Cytogenetic defects obtained in this study under the application of PGRs on maize are the most fundamental factors affecting pollen germination and their viability (Sanchez-Moran *et al*. 2004). The frequency of cytogenetic disorders causes an extremely high frequently of reproductive abortion (Handel and Schimenti 2010). Moreover, Zheng *et al*. (2017) observed that some cells containing micronuclei may die out before developing into mature pollen grains, as well as, some of them may fail to germinate as pollen grains or eventually abort at later developmental stages. So, the lower rate of pollen grain germination was partially, at least, due to numerous tetrad cells containing micronuclei under the application of PGRs (Zheng *et al*. 2017). The results of genotoxicity assays in maize treated with GA_3 500 ppm showed late movement of chromosomes toward the opposite poles in the M_1 generation of the H_1 genotype (Figure 12). This concentration of GA_3 showed a strong depressive on the dynamic of chromosomes toward the opposite poles. Notably, the meiosis stage is an extremely complicated cytogenetic process (Cheeseman and Desai 2008). This indicated that GA_3 500 ppm somehow modified the normal structure and the function of the kinetochore. This concentration induced down-regulation in kinetochore dynamic orientation toward the opposite poles, and interrupted cell cycle progression that led to anaphase arrest. These abnormalities could impair pollen viability. Thus, the application of PGRs decline maize productivity. These abnormalities decreased the grain number developed per ear. The number of kernels developed per ear is the most significant factor affecting yield productivity (Prasad *et al*. 2011). The results agreed with Lima-de-Faria (1958), who reported that the occurrence of rearrangements in the kinetochore structure leads to forming kinetochores with different genetic constitutions. Due to these disturbances in the functioning of the kinetochore, the anaphase segregation of dyads behavior is affected. Further detailed studies are necessary to evaluate the cytogenetic effects of PGRs. These changes have affected the genetic expression and increased chromosomal aberrations, as well as, the frequency of spontaneous mutations in general.

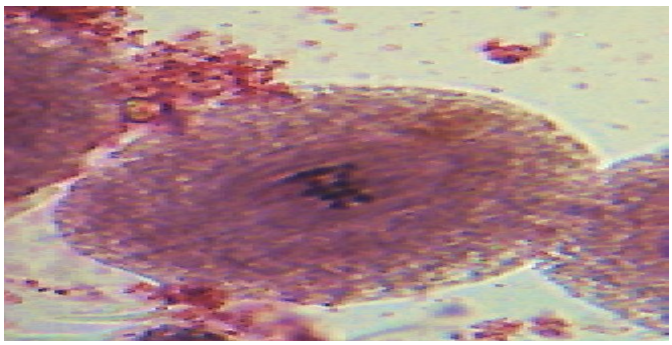

Figure 12. Meiotic abnormalities in M_1generation of Zea mays H_1genotype treated with GA_3 500 ppm showing late movement of chromosomes toward the opposite poles at anaphase I

As shown from the results presented in Figure 13, ethephon 1000 ppm induced laggard chromosomes at anaphase I in the M_1 generation of the H_1 genotype. The behavior of this laggard chromosome generally leads to micronucleus development. Laggards and disturbed polarity may be due to importer spindle functioning. In most cases, laggard chromosomes and unoriented chromatin material fail to get the poles during anaphase and subsequently form micronuclei. The disturbed polarity may result from spindle disturbances (Khah and Verma 2017).

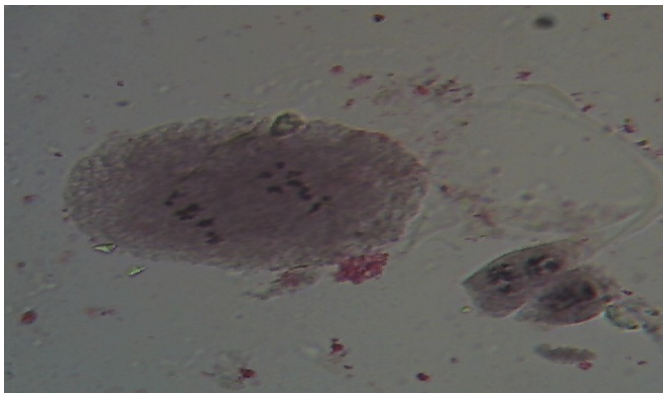

Figure 13. Meiotic abnormalities in M_1generation of Zea mays H_1genotype treated with ethephon 1000 ppm showing laggards chromosomes at anaphase I

The cytogenetic studies recorded in Figure 14 show stickiness chromosomes at telophase I in the M_1 generation of the H_2 genotype treated with GA_3 500 ppm. Koernicke (1905) identified earlier chromosome stickiness as an effect of ionizing radiation. Gaulden (1987) suggested that chromosome stickiness resulted from the changes in specific non-histone proteins (topoisomerase II and peripheral proteins) that are integral components of the chromosome and their function which is required for the separation and segregation of chromatids. The alteration was caused either by a mutation in structural genes for the proteins (heritable stickiness) or by the direct action of mutagens on the proteins (induced stickiness). According to Gaulden (1987), who demonstrated that stickiness occurs at various degrees including slight, moderate, severe and extreme. The degree of stickiness was associated with the number of target protein molecules affected. In this Figure, chromosomal stickiness appeared was classified as severe because most or many of the chromosomes were clumped.

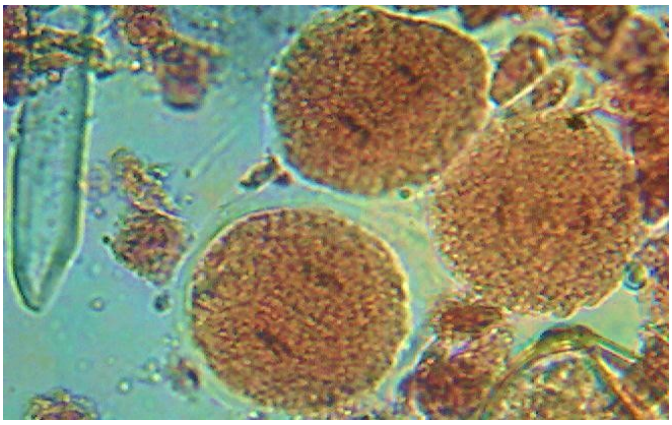

Figure 14. Meiotic abnormalities in M_1generation of Zea mays H_2genotype treated with GA_3 500 ppm showing stickiness at telophase I

The results obtained in Figure 15 appeared extreme stickiness at metaphase I resulting from the application with ethephon 1000 ppm in the M_1 generation of the H_1 genotype. All the bivalents in this Figure were highly clumped. These results are in line with Gaulden (1987), who suggested that chromosome stickiness induced chromosomal aberrations through the physical stretching and breaking of chromatids at the sticky sites. According to Golubovskaya (1979) chromosome stickiness was controlled by a single recessive gene in maize. In rye populations chromosome stickiness was independently obtained through spontaneous mutations (Sosnikhina*et al*. 2003). These abnormalities cause male sterility which is beneficial in plant breeding programs (Tsvetova and Elkonin 2003).

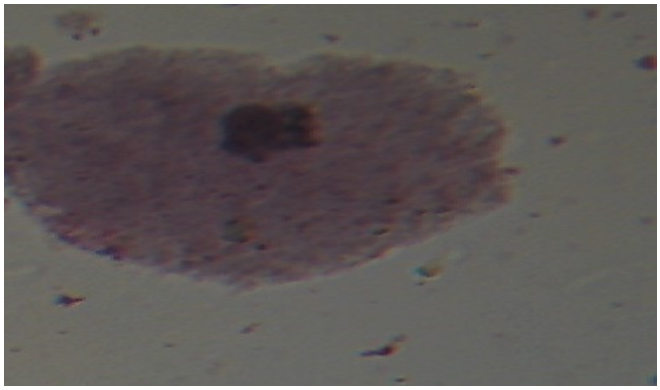

Figure 15. Meiotic abnormalities in M_1generation of Zea mays H_1genotype treated with ethephon 1000 ppm showing chromosome stickiness at metaphase I

The results obtained in Figure 16 appeared severe stickiness at metaphase I in the M_1 generation of the H_1 genotype treated with ethephon 3000 ppm. In this Figure many or most of the chromosomes were clumped. This phenomenon was earlier identified by Koernicke (1905), who characterized the stickiness of chromosomes by intense chromosome clustering through any phase of the cell cycle. Stickiness was first employed by Beadle (1932) when he described the sticky chromosomes in maize cells that had suffered from a mutation. The results are in harmony with Gaulden (1987), who suggested that sticky chromosomes might induced from the defective functioning of one or two types of specific non-histone proteins leading to chromosome organization which is required for chromatid segregationandseparation.

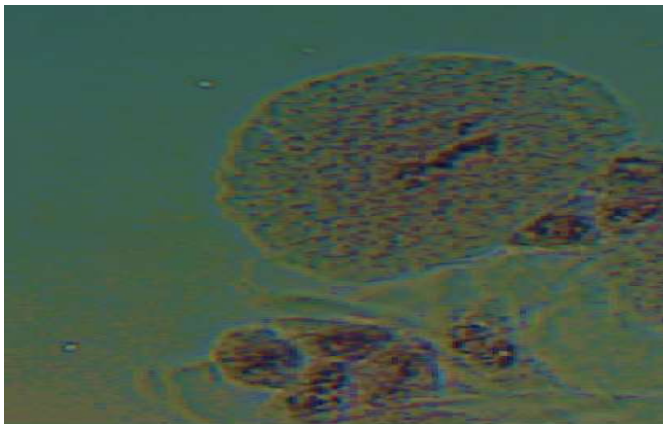

Figure 16. Meiotic abnormalities in M_1 generation of Zea mays H_1 genotype treated with ethephon 3000 ppm showing stickiness at metaphase I

The results of cytogenetic studies obtained in **Figure 17** appeared chromosomes to moderate stickiness at metaphase I in the M_2 generation of the H_1 genotype treated with GA_3 100 ppm. In this case, some of the bivalent chromosomes were clumped. The results obtained in Figure 18, 19, 20, 21, 22 and 23 showed chromosome stickiness at metaphase II, metaphase I, pachytene, metaphase I and metaphase I, respectively. Thesestickiness were resulted from the treatment with GA_3 500, GA_3 500, GA_3 100, ethephon 1000, ethephon 3000 and GA_3 500 ppm, respectively. Chromosomal stickiness is one of the abnormal phenomenonof chromosomal behavior that are categorized by intense chromosome clustering during any phase of the cell cycle.

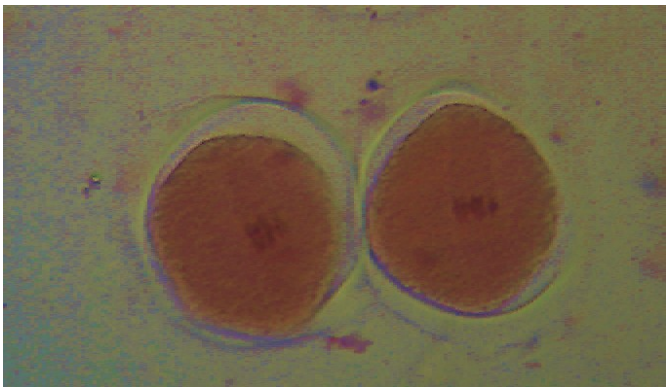

Figure 17. Meiotic abnormalities in M_2 generation of Zea mays H_1 genotype treated with GA_3 100 ppm showing chromosome stickiness at metaphase I

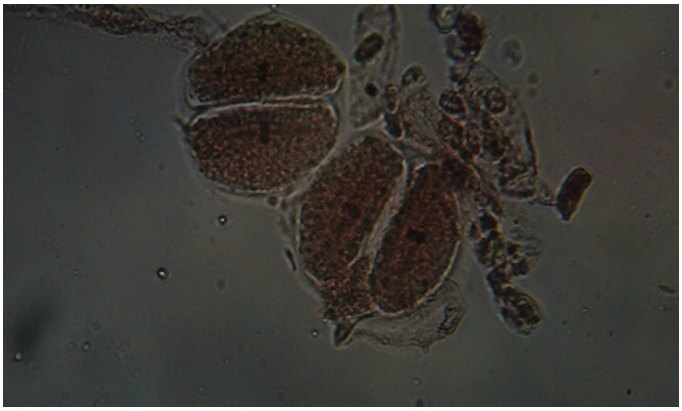

Figure 18. Meiotic abnormalities in M_2generation of Zea mays H_1genotype treated with GA_3 500 ppm showing chromosome stickiness at metaphase II

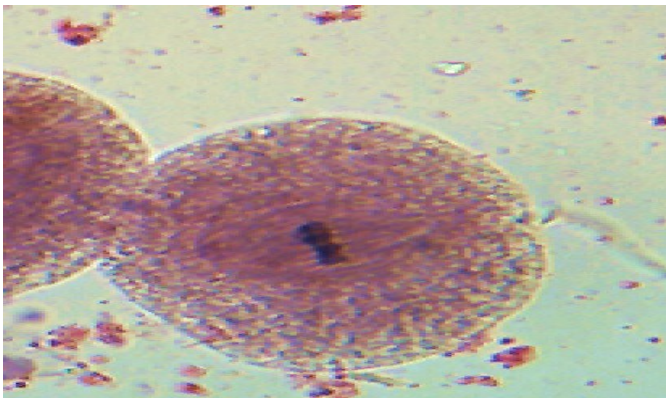

Figure 19. Meiotic abnormalities in M1generation of Zea mays H1genotype treated with GA3 500 ppm showing chromosome stickiness at metaphase I

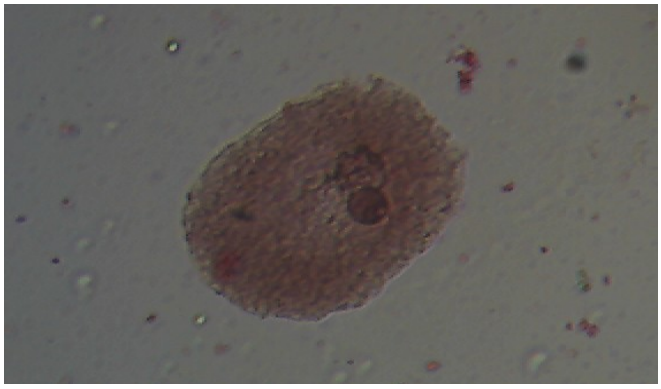

Figure 20. Meiotic abnormalities in M_1generation of Zea mays H_2genotype treated with GA_3 100 ppm showing stickiness at pachytene

This leading chromosomes in the pollen mother cells of maize had suffered from mutation. These results agreed with Kumar and Rai (2009), who obtained chromosomal abnormalities in maize through the treatment with gamma rays. In addition, Nilan and Gunthardt (1953) found that the germinability of wheat seeds decline and the frequency of chromosomal abnormalities increases with the age and x-ray dose. The induction of cytological anomalies in the meiotic cells leading to genetic damage that is passed to the next offspring.

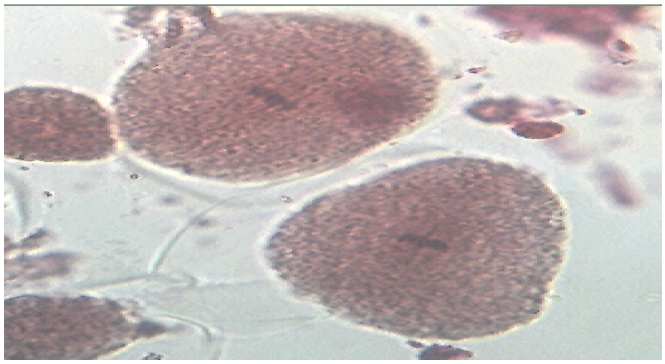

Figure 21. Meiotic abnormalities in M_2generation of Zea mays H_1genotype treated with ethephon 1000 ppm showing stickiness at metaphase I

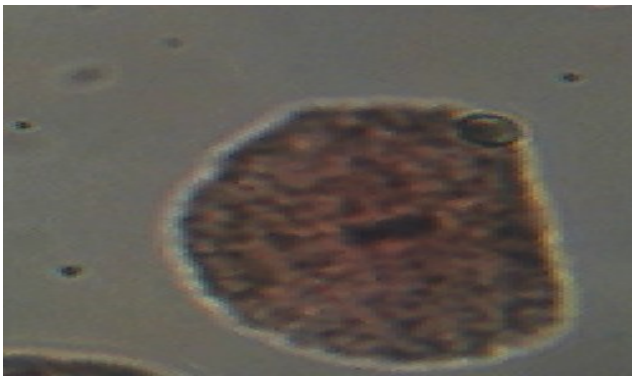

Figure 22. Meiotic abnormalities in M_2generation of Zea mays H_1genotype treated with ethephon 3000 ppm showing chromosome stickiness at metaphase I

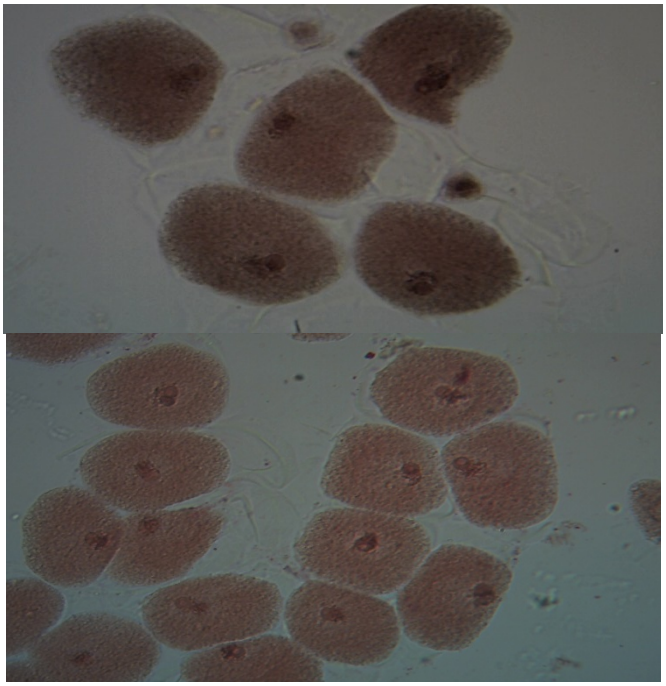

Figure 23. Meiotic abnormalities in M_2 generation of Zea mays H_2 genotype treated with GA_3 500 ppm showing stickiness chromosomes at pachytene

Cytological results obtained in Figure 24 appeared chromosome scattering at metaphase I in M_1 generation of H_1 genotype treated with ethephon 3000 ppm. The phenomena may either be due to the inhibition of spindle fibers development or to the destruction of spindle fibers developed. This lead to micronucleus formation. These result agreed with Khah *et al*. (2018), who observed that gamma irradiated seed progenies in maize displayed a wide spectrum of meiotic abnormalities including lagging chromosomes, chromosome stickiness, multivalents, chromosome scattering, univalents, unoriented bivalents,precocious chromosome movement, disturbed polarity, bridgesand micronuclei. The same authors found that the spectrum of these meiotic abnormalities was higher at metaphase than anaphase and telophase stages.

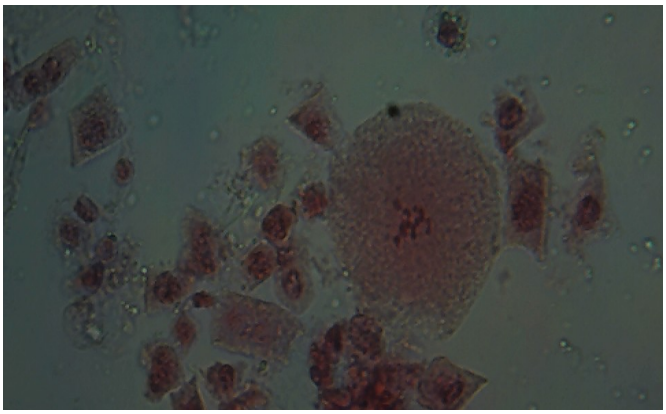

Figure 24. Meiotic abnormalities in M_1 generation of Zea mays H_1 genotype treated with ethephon 3000 ppm showing chromosome scattering at metaphase I

The results obtained in Figure 25 showing disturbed polarity at anaphase I in M_1 generation of H_1 genotype treated with ethephon 3000 ppm. In the present case, stickiness and multivalents could leads to disturbed polarity in the cells. The disturbed polarity could lead to the development of tripolarity due to failure of chromosome disjunction at anaphase in meiosis I. In some cases, the cells showed chromosomes at multiple poles that led to individual nuclei formation at the later stages. In most cases, unoriented chromosome fail to get at the poles during anaphase and telophase stages and subsequently forming micronuclei. The multivalent formation obtained in this study may be occurred as a result of chromosome breakage followed by the exchange of chromosome segments between non-homologous chromosomes (Khah and Verma 2017). Moreover, chromosome stickiness had been found to form multivalent formation (Khah and Verma 2017). The clumping of chromosomes has been also supposed resulted from depolymerization of nucleic acids, in addition due to partial deattachment of histone protein and their regular reorganization after repairing mechanisms (Evans 1962).

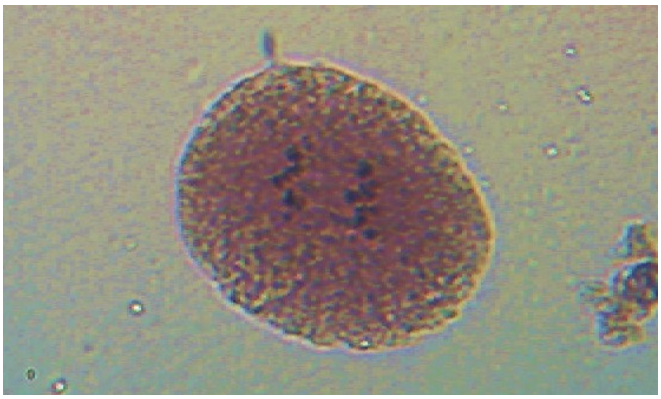

Figure 25. Meiotic abnormalities in M_1 generation of Zea mays H_1 genotype treated with ethephon 3000 ppm showing disturbed polarity at anaphase I

Cytological results appeared in Figure 26 reflected that ethephon 3000 ppm induced cytoplasmic attachment between two of tetrad cells in M_1 generation of H_1 genotype. The absence of complete cytokinesis was another abnormality found among meiotic products. This agreed with Rhoades and Dempsey (1966), who reported that there were at least two genes have disrupted cytokinesis in maize. The phenotypic expression of the absence of cytokinesis was conditioned by the *va* gene (Utsunomiya *et al*. 2002). The results obtained herein are in harmony with Utsunomiya *et al*. (2002), who obtained different kinds of meiotic abnormalities including absence of cytokinesis, cell fusion, bridges and chromosome segregation. Although, meiocyte is a highly specialized cell enable to producing four haploid cells. Environmental stress may alter the gene expression that act during meiosis resulting in abnormal microspores (Utsunomiya *et al*. 2002). Meiotic productshaveaffected by certain kind of abnormality that impairs gamete viability in different ways.

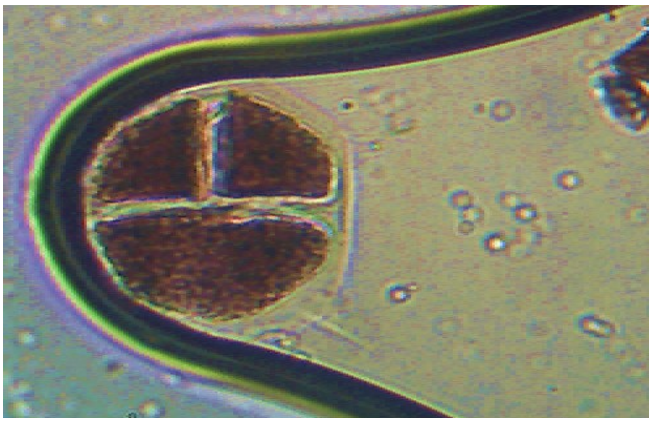

Figure 26. Meiotic abnormalities in M_1 generation of Zea mays H_1 genotype treated with ethephon 3000 ppm showing cytoplasmic attachment between two of tetrad cells

Cytological analysis presented in Figure 27 showing non-oriented bivalent on the plate at metaphase I resulted in M_1 generation of H_2 genotype treated with ethephon 1000 ppm. This case might have resulted from disturbed the spindle function and led to abnormal alignments of chromosomes at metaphase I (Khah and Verma 2017). These results agreed with Gulfishan *et al*. (2012), who found precocious chromosomal movement that might have originated from spindle dysfunction. The estimates of clastogenic potential of plant growth regulators by scrutinizing the induced meiotic abnormalities signifies an efficient technique to quantify the cytotoxicity of PGRs. Therefore, investigations on cytogenetic abnormalities and their consequences on the genotype of species become a fundamental part of mutation studies (Khah and Verma 2017).

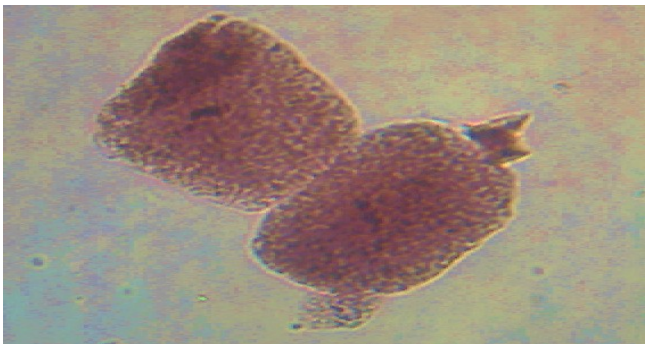

Figure 27. Meiotic abnormalities in M_1 generation of Zea mays H_2 genotype treated with ethephon 1000 ppm showing non-oriented bivalent on the plate at metaphase I.

The effect of PGRs on chromosomal behavior is still less understood. The inability of chromosomes to regulate on the equatorial plate may be related to the weakness of kinetochore. The results obtained herein are in harmony with Nicklas and Ward (1994), who decided some

factors may impair the attachment of kinetochores to the spindle fibers. In many cases the non-oriented bivalents showed its own spindle fibers with the formation of a minispinde (Utsunomiya *et al*. 2002). Non-oriented bivalents may form micronuclei if they are fail to reach the poles at the time to be included in the main telophase nucleus. The cytogenetic studies presented in Figure 28 showed varied asynchronization at meiosis II in M_1 generation of H_2 genotype treated with GA_3 100 ppm. In this case, one cell in metaphase II and the other in anaphase II. This phenomenon being one cell may taken a longest duration in meiosis II and then may taken more cellular energy. This case may be not induce meiotic abnormalities in the end products of gametes. However, pre-meiosis, meiosis and post-meiosis are genetically controlled and coordinated by numerous diverse genes. Meiosis, is controlled by a higher number of genes than other stages (Kaul and Murthy 1985). This reflected that the cell delayed in one stage of meiosis II may carried some mutations in the genes controlled and coordinated meiosis II (Golubovskaya 1989).

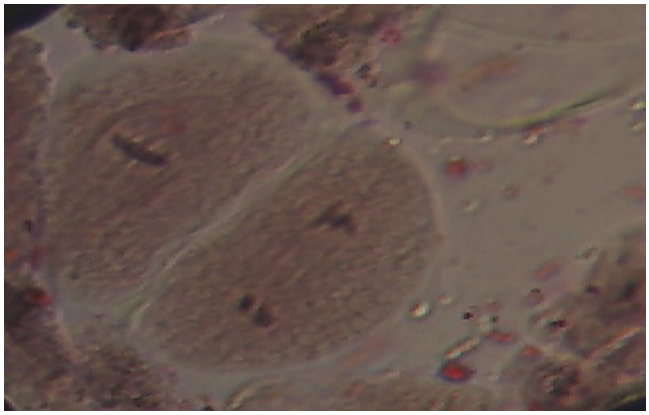

Figure 28. Meiotic abnormalities in M_1 generation of Zea mays H_2 genotype treated with GA_3 100 ppm showing varied asynchronization in meiosis II including metaphase and anaphase in two different cells

Figure 29 indicating the same cytological case of varied asynchronization at meiosis II in M_2 generation of H_1 genotype treated with GA_3 100 ppm. In this case one cell at metaphase II and the other cell in anaphase II. These results indicated that varied asynchronization was obtained from the treatments with GA_3 100 ppm among both maize genotypes, as well as, among both generations. This reflected that GA_3 100 ppm was affected on the several diverse genes that are controlled and coordinated meiosis stages. Thus, environmental stress as the application of PGRs on maize may alter the orientation of genes expression that act during meiosis, suggesting independent hierarchical gene control at each step (Golubovskaya 1989).

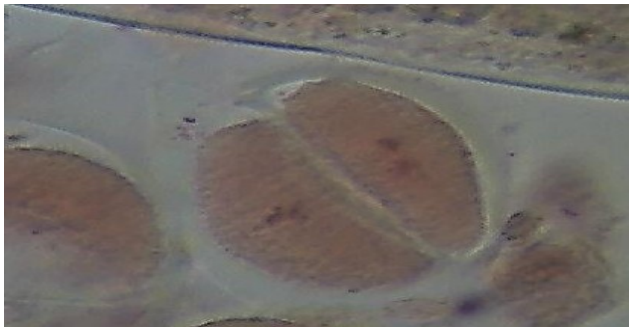

Figure 29. Meiotic abnormalities in M_2generation of Zea mays H_1genotype treated with GA_3 100 ppm showing varied asynchronization in meiosis II including metaphase and anaphase in two different cells

Cytological analysis appeared in Figure 30 achieved chromatin bridge at anaphase I in M_2 generation of H_1 genotype treated with ethephon 1000 ppm. These result agreed with Utsunomiya *et al*. (2002), who found chromosome bridges in maize of different thickness that recorded in 0.03% of the meiocytes. This case was very thin and with fragments. It was resulted from chromosomal rearrangements and sometimes from chromosome stickiness (Defani-Scoarize*et al*. 1996). The formation of chromosomal bridges were also obtained by Khah*et al*. (2018) in gamma irradiated maize. Saylor and Smith (1966)stated that the later or inability of bivalent chiasmata to terminalize may developed chromosomal bridges at anaphase and telophase I/ II. This reflected that the higher frequency of meiotic abnormalities obtained after the application of PGRs became a significant cause of reduced the survival of pollen grains leading to reduced plant productivity. The results obtained herein agreed with Kumar and Rai (2009), who observed that maize treated with gamma rays, achieved chromosomal bridges at anaphase in about 50% of the cells, often with fragments. The same authors illustrated the origin of these bridges as it may be associated with inversions or chromosome rings, as a consequences of crossing over. In some cases, the irregular outline of bridges development through delayed separation of chiasmata, as well as, due to later replication of heterochromatin or chromatin stickiness. The use of bridges and fragments as a cytological indicators of chromosomal abnormalities has been considered to be an efficient technique for assessment the genotoxicity of PGRs on plant cells that are used at a large scale in modern agriculture today (Kumar and Rai 2009). These abnormalities cause male sterility that are considered as an important tool in the development of hybrid seeds in plant breeding programs (Atanassova 2000). The recovery from male sterility is one of the most important problems in plant genetics. It is benefit in plant breeding and in studies of sexual reproduction (Tsvetova and Elkonin 2003).

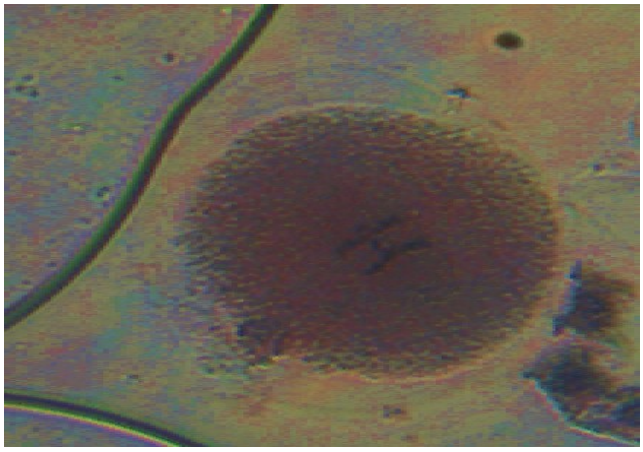

Figure 30. Meiotic abnormalities in M_2generation of Zea mays H_1genotype treated with ethephon 1000 ppm showing chromatin bridge at anaphase I

Developmental effects of PGRs: The results obtained in Figure 31 achieved complete number of rows per ear with high number of kernels were developed per row in the control plants of H_1 genotype among M_1 generation. Accordingly, the yield was determinants by the number of rows per ear and the kernel number per row. This agrees with Hütsch and Schubert (2022), who suggested that the improvement of grain yield in maize was most likely due to a combination of kernel number with an increase in cob length where the first was not significant. As shown from the results presented in Figure 32, ethephon 1000 ppm induced shorter ears with reduced kernel number developed per ear in M_1 generation of H_1 genotype. Despite the plants having shorter ears with lower seeds developed at maturity, the developing ears may have fewer florets or aborted florets that are often masculinized. Thus, phytohormones genotoxicity play crucial roles in plant growth, as well as, ear and kernels development. Meiotic abnormalities induced by PGRs were associated with a reduction in floret fertility, ear length, kernel number and grain yield. This suggested that floret fertility is critical factor for maize grain yield (Ning *et al*. 2021). These findings suggested that the role of PGRs on maize grain yield-related traits that they are more complex than in cucumber fruit size regulation (Xin *et al*. 2019).

Figure 31. Kernel - carrying cobs at maturity carrying by control plants in M_1 generation of H_1 genotype achieved longer ear with high kernels number per row

Thus, the optimization of PGRs levels for grain yield enhancement in maize needs to balance between its effect on meristem activity and floret fertility. The results obtained herein agreed with Ning *et al*. (2021), who suggested that ethylene is a key factor in inflorescence development affecting on spikelet number, floral fertility, kernel number and ear length. Maize is most productive creal crop over the world. The plants developed large ear length with hundreds of kernels developed per ear. Kernels were developed in a stereotypical pattern from fertile florets. Mature female inflorescences of maize usually carried hundreds of kernels. Kernel number per ear is one of the key breeding target (Lopez-Reynoso and Hallauer 1998). Kernels arise from female florets that they are well-developed and pollinated borne from spikelet meristems derived from inflorescence meristem (IM). Thus, the activity of IM is determined the number of florets as a consequence kernels number developed on maize inflorescence (Vollbrecht and Schmidt 2009).

Figure 32. Phenotypes of shorter ears derived from the plants treated with ethephon 1000 ppm in M_1 generation of H_1 genotype achieved lower number of kernels mis-developed per ear if compared with the control ear in the left

Ear phenotypes appeared in Figure 33 showed dwarf cob development on M_1 plants of H_1 genotype treated with ethephon 3000 ppm. Despite control that having longer ears carrying more grains at maturity, the developed ears in this Figure has significantly shorter with fewer florets producing fewer kernels. The analysis of these phenotypes indicated high abortion rate of florets in the shorter ears. Dwarfing genes are responsible on these phenotypes. Dwarfing genes in maize are mutants of genes involved in GA_3 biosynthesis and metabolism, as well as, in signaling (Sasaki *et al*. 2002). This agrees with Harberd and Freeling (1989), who found semi-dwarf mutants in maize. Dwarfing mutant phenotypes are not used in commercial production of maize. The results obtained herein are in line with Schluttenhofer*et al*. (2011), who found that uniconazole (UCZ) reduced the plant height of maize without reduced the number of nodes.

Figure 33. Dwarf ear development on M_1 plants of H_1 genotype treated with ethephon 3000 ppm achieved lower number of rows per ear, as well as, lower number of kernels developed per row and some kernels abort

Figure 34. Kernel - carrying ears at maturity carrying by control plants in M_1 generation of H_2 genotype achieved longer ears carrying high number of kernels per row and high number of rows per ear

Ear phenotypes appeared in Figure 34 achieved normal ears were developed on H_2 genotype in M_1 generation which characterized as longer ears carrying high number of kernels per row. This are in line with Ortez *et al*. (2022), who investigated that normal ears for hybrids in the US can produce of about 800 to 900 kernels per ear, normally arranged in 16 to 18 kernel cluster rows that have up 50 to 55 viable ovules per row. Normal ears as seen in this Figure do not present any major disruption in their cob, kernel, as well as, husk growth and development leading to produce higher yields (Rees *et al*. 2020). Ear phenotypes appeared in Figure 35 indicated the effect of GA_3 100 ppm in reducing the number of kernels and decreasing the number of rows developed per ear in M_1 generation of H_2 genotype. This reflected kernels abort during early kernel development. Kernel abortion resulted from chromosomal abnormalities during meiosis reduced the viability of gametes. Insufficiency the viability of pollen grains reduced fertilization rate leading to kernel abortion that mainly due to male sterility, whereas female fertility may remains unaffected (Bingham 1966). The number of grains developed per ear is the most significant factor affecting yield. Thus, reducing the viability of pollen grains resulted from chromosomal abnormalities during meiosis induced reduction in grain numbers developed per ear. The grain number is the key factor to obtaining high and stable yield (Prasad *et al*. 2011). The results obtained herein are in harmony with Dong *et al*. (2010), who reported that the grain number developed per ear depends on the total number of florets, number of fertilized florets and effective number of grains that develop from a fertilized florets.

Figure 35. Ear development on M_1 plants of H_2 genotype treated with GA_3 100 ppm achieved lower number of kernels and lower number of rows developed per ear with irregular rows

Ear phenotypes presented in Figure 36 achieved that ethephon 1000 ppm induced dwarf ear, as well as, kernels abortion in M_1 generation of H_2 genotype. Taken together, ethephon inhibited the development of some kernels, which was the main reasons for the decrease in grain number developed per ear. Grain development depends on the viability of gametes which impacted by meiotic abnormalities induced by PGRs. As a result pollen germination is the first step towards successful fertilization and development of kernels. PGRs induced insufficient viability of pollen grains that reduces grain set if female fertility remains unaffected (Saini and Aspinall 1981). This agrees with Oliver *et al*. (2005), who found that pollen sterility in rice resulting in yield losses. Under PGRs meiotic abnormalities induced are leading to a limited amount of viable pollen grains, pollen germination failure directly that reduced pollination efficiency and tends to decrease grain number developed per ear. Bollmark and Eliasson (1990) stated that ethylene resulted from ethephon may be responsible for the breakdown of cytokinins. Moreover, cytokinins are important factor for endosperm cell division and the development of kernels (Bollmark and Eliasson 1990). Therefore, ethylene derived from ethephon used in this study leading to cytokinins breakdown, as a result endosperm cell division was decline, as a consequence the development of kernels was decreased. Ning *et al*. (2021) reported that ethylene is an important signal for inflorescence development, affecting kernel number, floral fertility, spikelet number and ear length. Moreover, ethylene has been associated with fruit abortion in cotton (Guinn 1976) and wheat (Hays *et al*. 2007). The increase in ethylene was correlated with increased kernel abortion in maize and a reduction in kernel weight among the kernels retained (Ning *et al*. 2021).

Figure 36. Phenotypes of ears derived from ethephon 1000 ppm in M_1 generation of H_2 genotype achieved lower number of kernels developed per ear, as well as dwarf phenotype

Regarding to ear phenotypes appeared in Figure 37 that achieved shorter ear, complete losses of some rows and abortion of some kernels developed per ear. These phenotypes resulted in M_1 generation of H_1 genotype treated with ethephon 3000 ppm. This agrees with Ortez *et al*. (2022), who found several abnormal ear symptoms were generated by stress conditions as a responses to plant growth regulators, extreme weather and limited solar radiation. The accumulation of genetic anomalies can result in the abortion of primary ears, as well as, the development of secondary abnormal ears. The abnormal ears impacted on grain quality and yield. These anomalies were thought to result from meiotic abnormal chromosomal behavior caused by a foliar application of PGRs. Meiotic abnormalities leading to failure of pollination in the primary ear developed which correlated with the loss of some rows and kernels abortion. Furthermore, Lejeune *et al*. (1998) reported that abortion can occur if the primary ears response to extreme weather and changes in plant growth regulator. Ortez *et al*. (2022) decided that when the primary ear aborts, the secondary ear will often develop into a malformed or harvestable ear. Future research is needed to expand understanding the influence of PGRs on the physiological pathways that subjected to genetically controlled leading to abnormal ear, kernels abortion and subsequent lower yields. As shown in Figure 38, the grain abortion rate exhibited extremely higher in M_2 generation due to the dosage of 3000 ppm ethephon applied on H_1 genotype. This leading to the highest decrease in grain yield after ethephon application. However, the grain number developed per ear were depends on the number of fertilized florets,total number of florets, and effective number of grains that developed from fertilized florets. In plants under ethephon application, the floret abortion rate was

increased, pollen viability and their ability to germinate are declined. In addition, the silking time is delayed, as well as, filament viability was decreased. As a result, the pollination and fertilization processes are decreased, as well as, increases the abortion rate, as a result decline grain number developed per ear (Prasad and Djanaguiraman 2014). The results indicated that the rate of grain abortion is the key factor that determine grains number developed per ear.

Figure 37. Phenotypes of kernel rows developed per ear in M_1 generation of H_1 genotype derived from ethephon 3000 ppm achieved abortion of some kernels mis-developed per ear

Figure 38. High abortion rate of kernels mis-developed per ear after ethephon application with a dosage of 3000 ppm in M_2 plants of H_1 genotype

The greatest impact occurs in plants when they are subjected to the higher concentration of ethephon 3000 ppm. The young ear length was shorter than in the control group. Thus, an increased in grain abortion rate ultimately results in a significant reduction in the number of grains developed per ear. The results reflected that the development of kernels process is sensitive to ethephon application. The results obtained herein agreed with Bollmark and Eliasson (1990), who reported that ethylene was responsible of cytokinins breakdown. Cytokinins are important substances for endosperm cell division and the development of kernels. Therefore, the high concentration of ethylene derived from ethephon was responsible of cytokinin breakdown, as a result inhibit endosperm cell division that leading to kernels abort. The results obtained herein agreed with Kiniry and Ritchie (1985), who stated that kernels was aborted when dry weight accumulation was ceases during the early stages of kernel developments. In a recent work by Ning *et al*. (2021), who studied the influence of ethylene's on the variations in maize ear length and gains development. They identified from their molecular work the genes upregulated associated with ethylene changes. Editing that gene appeared that it was able to stimulate flower and meristem development, as well as, ethylene was declined in the developing ears. Therefore, any increase in ethylene concentration induce alteration in ears and kernels developments. Furthermore, Ning *et al*. (2021) concluded that ethylene is an essential signal affecting on floral fertility, kernel number, spikelet number, inflorescence development and ear length. Moreover, Ortez *et al*. (2022) reported that ethylene increased in embryos was correlated with increased kernel abortion and a reduction in kernel weight through the kernels retained. Therefore, ethylene derived from ethephon applied in this study is a significant factor affecting on ear length, reduced floral fertility through chromosomal abnormalities appeared in meiosis, as well as, increased kernels abort as seen in this study. The results appeared in Figure 39 achieved dwarf ears resulted from the treatment with GA_3 500 ppm in M_2 generation of H_1 genotype. In addition to dwarf ear phenotypes some kernels were aborted with irregular rows in some ears. Ear dwarf mutant displays shortened ear. This indicated that the higher concentration of GA_3 exhibited a pattern of inhibition-normality-inhibition (transient for the ear internode) (Zhang *et al*. 2019). This agrees with Lv*et al*. (2014), who reported that dwarf mutants in maize have been categorized by short internodes which have been attributed to impaired internode elongation. In addition, Guo *et al*. (2013) stated that internode elongation is mainly controlled by plant growth hormones as gibberellins and auxins, any defects in their biosynthesis can cause dwarf phenotypes. Therefore, any increase in hormones concentration can also cause dwarf mutants as seen in this Figure. Irregularities of rows developed on some ears is another mutant phenotype. GA_3 - related mutants display extremely reduced ear height.

Figure 39. Dwarf ear phenotypes developed on M_2 plants of H_1 genotype treated with GA_3 500 ppm achieved irregular rows per some ears in addition to abortion of some kernels mis-developed per ear

These mutants may be originated from a homozygous recessive alleles joined in M_2 generation, accompanied by a certain degree of yield loss. The results are in line with Zhang *et al*. (2019), who reported that *br2* allele in maize was responsible for the shortened internodes. This allele was highly expressed in dwarf mutant arose

spontaneously from the wild type inbred line. In this study, M_2 generation resulted from self crossed M_1 progenies steadily resulted uniform short ear mainly contributes dwarf phenotype. In dwarf ear phenotype, ear length, ear width, ear weight, 100-grain weight, total tassel length were altered in comparison with the wild type (Zhang *et al*. 2019). The same authors stated that the expression of *br2 - TO1* in dwarf maize phenotype was significantly lower than that of *BR2 - To1* in wild type. In addition, Multani *et al*. (2003) confirmed that *br2* mutant allele leads to decrease the auxin flow from plant top to the bottom which is responsible for the dwarf phenotype. The results indicated that in ear mutant phenotype the activity of normal auxin transport during ear development was impaired. This agrees with Zhang *et al*. (2019), who found in F_2 population of maize under excessive auxin accumulation that plant height and ear height was segregated in a 3 : 1 ratio. There are different *br2* alleles would give rise to certain defects with varying degrees (Zhang *et al*. 2019). Therefore, *br2* alleles controlled ear length appeared their phenotype in M_2 population indicating that they are recessive alleles. All *br2* mutants presented shortened ear which is closely related to dwarf ear phenotype. Overall, low auxin level is crucial for ear elongation. Chromosomal abnormalities induced by PGRs in H_1 genotype was greater than that induced in H_2 genotype indicating the effects of PGRs are genotype dependent.

Concluding remarks: Ethephon was the main compound in growth regulating effects significantly reduced plant height and ear weight. It is possible to shorten maize plants very well. Yield was generally decreasing with increasing ethephon concentration. All applications of PGRs must be made prior to the formation of female inflorescence. Shortening plant height induced by ethephon enhanced lodging resistance. This is an efficient strategy to enhance maize tolerance to crowding. Both hybrid genotypes used in this study achieved diverse results about the effects of PGRs among M_1 and M_2 generations. This indicated that the effects of PGRs on maize are strongly genotype-dependent. Meiotic activity and their chromosomal behavior wasadversely affected by exogenous application of PGRs. Chlorophyll content and leaf area are two important traits affected by PGRs that directly related to photosynthesis process. PGRs decline chlorophyll concentration which caused to decrease photosynthetic activity leading to chlorosis and yield reduction. Decreasing ear weight in response to the application of PGRs showed a dose-response. This decrease was greater in M_2 than in M_1 generation at the same dose. PGRs proved to decrease the translocation of accumulates from their source to ear leading to decline ear weight. Dwarf ear mutant was appeared in M_1 generation of H_1 genotype as a result of application by ethephon 1000 and 3000 ppm. The greatest abortion of kernels developed per ear was appeared in M_2 generation as a result of application with ethephon 3000 ppm on H_1 genotype. The frequence of meiotic aberrations obtained in this study was associated with the less viability of gametes that leading to kernels abortion.

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