MAGNETIZED WATER AS AN ECO-FRIENDLY IRRIGATION ALTERNATIVE AMELIORATES CYTOGENETIC IMPAIRMENTS IN *Vicia faba* UNDER TWO FERTILIZERS

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Egyptian water resources are limited, the Nile River is the main water resource and agricultural sector is the main water consumer as it consumes about 85% of all available water resources, hence more efficient use of water in agriculture needs to be top most priority (El-Rawy *et al*., 2020). The rapid growth of the Egyptian population and the expected impacts of climate change on water resources and agriculture threaten current and future food security. Water resources are being constantly under pressure and require a scientific approach to sustain the productivity of agricultural crops. Because of the water quality problems, the use of low-quality irrigation water is gaining importance in Egyptian agriculture besides many other countries (Makanda *et al*., 2022). Hence, vital activities and modern agricultural technologies for better utilization of agricultural resources are now in search of an efficient ecofriendly production technology for improving the crop productivity without harming the environment as reusing of treated waste water, improving water use efficiency and managing groundwater resource (Asthana, 2022).

Magnetic water treatment is simple nonchemical treatment of water that does not require any filtration substitutes where the water flows through a magnetic field (MF). Exposing water to MF changing its physiochemical properties and becomes more biologically active stimulating the activity of proteins, the movement of free radicals and enhancing the overall biochemical processes inside the living cells (Mohamed *et al*., 2022).

However, non-magnetic treated water, showed loose and chaotic form of attraction predisposes binding of water to

toxins developing a large molecule that being blocked to pass through the cell membrane while some of smaller sized chaotic clusters carrying toxins can enter the cell with consequent harmful effects (Mabrouk *et al*., 2016).

The application of MF for irrigation water treatment is still an attractive and promising process to reduce the adverse effects of irrigation water hardness, soil salinity and the enhancement of crop production (Khaskhoussy *et al*., 2023). Magnetized water has desirable effects on plant ecosystem; it increases the leaching of excess soluble salts, lowers soil alkalinity, prevents harmful metals as lead from uptake by roots and increases the percentage of nutrient elements as phosphorus and potassium (Khosrojerdi *et al*., 2023). Magnetic field stimulates the initial growth stages, early sprouting of seeds and accelerates reproductive growth in plants due to an increase in energy and its distribution to biomolecules in the cell (Alakhdar *et al*., 2022).

Zinc (Zn) and Manganese (Mn) are two of essential micronutrient for plant growth and development. Both are crucial for enzyme activation and functions**.** Zn activates enzymes that are responsible for certain proteins synthesis aids in plant growth. It is used in the formation of auxins, which help with growth regulation and stem elongation. Zinc sulphate (35% zinc), least expensive form of zinc, is the most commonly zinc fertilizer used to supply the needed amount of zinc. Manganese Sulphate offers plants a readily available source of manganese, an essential micronutrient activates enzymes related to respiration, photosynthesis, and nitrogen metabolism. It is necessary for root elongation and lateral root formation (Ahmed *et al*., 2024).

Cytogenetic tests are suitable for identification of risky effects of particularly known substances in various concentrations. It is considered one of the best/sensitive, and time-consuming method to measure risky effects of any agent of interest as compared to other physical, chemical and radiological methods on genetic level (Fiket *et al*., 2020 and Rajesh *et al*., 2020). Plants are unique in their ability to serve as in situ monitors for environmental genotoxins that inflict damage to DNA and cause genotoxic stress, which can reduce plant genome stability, growth and productivity (Plaksenkova *et al*., 2020).

It is important for detection of genotoxicity and mutagenicity of various types of environmental factors as water quality of different water sources on crop plants, to understand their biological consequences and their molecular action on protein and DNA of plant cell by introducing cytogenetic, biochemical, and molecular assays (Al-Ahmadi, 2019).

Genotoxicity assessment to test alterations on the mitotic index (MI), micronuclei formation (MN), nuclear abnormalities (NA) and chromosome aberrations (CA) are important cytogenetic endpoints that are routinely used in cytotoxicity and genotoxicity evaluation (Campos *et al*., 2008).

The mitotic index (MI), characterized by the total number of dividing cells in cell cycle, has been used as a parameter to assess the cytotoxicity of several agents. The cytotoxicity levels of an agent can be determined by the increase or decrease in the MI (Lubini *et al*., 2008). MI significantly lowers than the negative control can indicate alterations, deriving from the chemical action in the growth and development of exposed organisms. Instead, MI higher than the negative control are results of an increase in cell division, which can be harmful to the cells, leading to a disordered cell proliferation (Campos *et al*., 2008).

CAs occurs upon sudden breaks or exchange of chromosomal materials can be taken as signs of genome instability in the exposed organism. MN formation, chromatin bridges, breaks and rings indicate potential disturbances in DNA or protein synthesis (Chandra *et al*., 2020). Vagrants, stickiness, and laggards are indicative of anomalies of chromatin organization or cell cycle fluctuations (Kassa, 2021). Observations are valuable in identifying environmental toxins that can affect the cytoplasm and nucleus during cell divisions (Ghosh *et al*., 2019).

DNA fingerprinting offers a useful biomarker assay in assessment of genotoxicity (Rahmanian *et al*., 2021). Molecular technique such as random amplified polymorphic DNA (RAPD), Inter simple sequence repeated (ISSR) and simple sequence repeats (SSR) has been successfully applied to detect changes in DNA fingerprint which reflect DNA variations in genome (Choudhury *et al*., 2022).

ISSRs represent easy and widely adopted molecular markers, since their use does not require any prior information about target sequences and their efficiency and reproducibility are ensured and It was DNA based markers permit detection of polymorphisms in inter-microsatellite loci, using a primer designed from dinucleotide or trinucleotide repeats and used in different plant research as they are easier to use, less expensive, faster, involve non-radioactive substances and not requires information about genomic sequences (Gemmill and Grierson, 2021). The ISSRs were reported as an effective tool for generating the genetically stable diagnostic markers for studying genetic stability of plants (Nayak *et al*., 2013; Alhasnawi, 2023).

The prospect of using cheap magnetic energy to improve the properties of soil and plant growth and development may be of great practical importance. Magnetized water technology may be considered a promising technique to improve water quality enhancing plant growth and yield. It stimulates water and nutrient uptake and has positive effects on photosynthesis, carbohyd- rates and protein metabolisms (Radhakrishn-an, 2019 and Hafeez *et al*., 2023).

Therefore, the current study was carried out to investigate the applicability of using of magnetized irrigation water to promote *Vicia faba* growth traits under zinc and manganese sulphates fertilization

and evaluate its effect on cell division, chromosome structure and DNA finger-print.

MATERIALS AND METHODS

Magnetized irrigation water preparation

Irrigation water passed through a magnetic field of 1000 Gauss magnetron unit of 0.5 inch diameter and acquire a magnetic moment for 48 hours according to Tantawy *et al*. (2019).

Field experiment and culture of the *Vicia faba* plants

The Egyptian faba bean (*Vicia faba*, Giza 716) seeds were kindly provided by Agricultural Research Center, Giza, Egypt. Prepared soil was packed in a plastic pot (5 kg. capacity) at a rate of 4Kg. *V. faba* seeds were cultivated at rate of 4 seeds/pot. Both nitrogen and potassium fertilizers were added at total rates of 0.8 g/pot and 0.8 g/pot as urea (46% N) and potassium sulphate (48% K_2O), respectively. Total rates of applied N and K were divided into three doses; the first dose was applied after germination, while the second and the third doses were applied after 15 and 30 days from the germination, respectively. 15-day old plants were fertilized with different concentrations; 50ppm (0.005%) and 100ppm (0.01%) of zinc sulphates ($ZnSO_4$) or manganese sulphates ($MnSO_4$) and irrigated every two days with non-magnetized and magnetized water as presented in Table (1) as nine treatments.

Plant growth traits

Some growth traits were recorded as germination time (GT) in days after planting (DAP), plant height in centimeter per plant (cm/plant) and flowering time (FT) in DAP.

Percentage (%) change in response to each specific treatment in relation to the control level was calculated as follow: %Change= [level maintained in response to each treatment - control level / control level] x 100 (Abdel-Aziz *et al*., 2021). All growth analysis experiments were performed in three replicates.

Means of data with standard deviation were statistically compared to the control using One-way ANOVA followed by Duncan's test by a commercial statistics package (IBM SPSS Statistics 24.0) and means were considered statistically significant when $p < 0.05$ (Ducan, 1955).

Cytotoxicity effects

Newly emerged radicals of 1.5-2.0 cm from each treatment set were cut, fixed in Carney's solution (glacial acetic acid/ethanol 1:3) and stored in refrigerator for 48 hours. According to Chattoadhyay and Sharma (1988), the root tips were washed by distilled water and hydrolyzed in 1N HCl at room temperature for five minutes, then stained using 2% aceto- orcein. One millimeter of the mitotic zones was immersed in a drop of 45% acetic acid on a clean slide and squashed under a cover glass in order to spread the cells.

The slides were viewed under the

electric microscope (Olympus CX 40) using oil immersion objective lens. At least 3000 cells from about 40 slides of each treatment were examined. The cells were recorded as normal or aberrant in different stages of mitotic division namely: interphase, prophase, metaphase, anaphase and telophase.

All cells with aberration were counted and the most representative ones for each abnormality were photographed using digital microscope camera. Different phases of mitosis were counted and chromosomal aberrations were observed to calculate mitotic index, phase indices, interphase and total abnormalities percentages based on Deogade and Nasare, (2016) equations;

Mitotic index (MI)%
=[Number of divided cells /
Total cell scored] x100

Phase index (PI)%

=[Number of particular mitoticphase/
Number of divided cells] x100

Total abnormalities ($T_{abn.}$)% ==[number of abnormal cells /
Total cell scored] x100

One way ANOVA was carried out for statistical analysis of data with the help of SPSS software program followed by Duncan's test (Ducan, 1955). Differences between the control and treatments were considered statistically significant at $p<0.05$. The data was expressed as Mean ± S.E. (standard error) for each treatment.

Molecular markers

a) DNA extraction and ISSRs assay

Total DNA was extracted from fresh leaves using DNeasy Plant Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. Ten ISSR primers were used in the detection of polymorphism (Table 2).The amplification reaction was carried out in 25 µl reaction volume containing 12.5 µl Master Mix (sigma), 2.5 µl primer (10 pcmol), 3 µl template DNA (10 ng) and 7 µl dH_2O thenpolymerase chain reaction program conditions for ISSRs were done as described by Ibrahim *et al*. (2019) then PCR products were separated on 2% agarose gel and visualized by ethidium bromide on UV light.

b) Data analysis and calculations

PCR products were photographed using a Gel Documentation System (BIO-RAD 2000). Clear and unambiguous ISSR bands were visually scored as either present (1) or absent (0) for all samples. The binary data produced were used to estimate levels of polymorphism by dividing the polymorphic bands by the total number of recorded bands. The amplification profiles of ten ISSR primers were used to determine genome template stability percentage (GTS%) as a qualitative parameter used to evaluate genetic variations shown between treated *V. faba* and the control, GTS%= (100-100a/n), where (a) is the average number of polymorphic bands detected in each treated sample and (n) is

the number of total ba nds in the control (Osman *et al*., 2020).

RESULTS AND DISCUSSION

Plant growth traits

In general, the studied *V*. *faba* growth traits showed significant improvements in response to irrigation by NW or MW under zinc or manganese sulphates fertilization throughout the entire experiment period if compared to the control as presented in Table (3) and Fig. (1).

GT was significantly improved by about 30.77% in case of MW+0.01% $MnSO_4$ treatment above the control.

The irrigation by MW with different levels of $ZnSO_4$ or $MnSO_4$ fertilizers showed high significant values of germination of *V*. *faba* seeds rather than its counterparts of NW. Zhang *et al*. (2022) said that MW could promote cotton seed germination and seedling growth by increasing seed water absorption as it was more easily absorbed by cotton seeds than NW.

There are highly significant differences in plant shoot length; the highest improved percentage was 12.10% for MW+0.005%$MnSO_4$ treated *V*. *faba*, while it was retarded by about 14.00% in NW+0.005%$MnSO_4$ treated *V*. *faba*. In relation to the control value, there were significant changes in FT as it was accelerated in response to all treatments except for MW+0.005%$MnSO_4$ and NW+0.01%$MnSO_4$ treated *V*. *faba* they were delayed by 5.00% and 1.67%, respectively.

The same results were obtained by Ehtaiwesh *et al*. (2019) who decided that, growth parameters of faba bean were significantly increased if irrigated with MW compared with the NW. Maheshwari and Grewal (2009) studied the beneficial effects of magnetic treatment of different irrigation water types on water productivity and yield of celery plant and found that the magnetic treatment of recycled water in- creased celery yield by 12% and water productivity by 12%.

Cytotoxicity effects

To assess the influence of NW and MW under $ZnSO_4$ or $MnSO_4$ fertilization, the present study focused on *V*. *faba* mitotic cell division. Tables (4-6) listed the percentage of mitotic index (MI%) and frequency of different mitotic phases; phase indices (PI%) in mitotic cells of treated *V*. *faba* and the control at exposure period of 15 days of germination. In general, all treatments led to significant increases in the MI% at $p \leq 0.05$, particularly compared to the control. It is obvious that the treatment of magnetized irrigation water under $ZnSO_4$ or $MnSO_4$ fertilization induced more significant increases in MI% of *V*. *faba* mitotic cells rather than its corresponding treatments of non-magnetized irrigation water and it may be the reason of the increasing of *V*. *faba* shoot length of these treatments. The highest MI% (18.67%) was observed in *V*. *faba* mitotic cells in response to MW+0.005%$MnSO_4$ treatment, while the lowest MI% (6.70%)

was in response NW+0.01%$MnSO_4$ treatment.

Also, it is obvious that MI% readings were fertilizer dose dependent as it decreased in most treatments by increasing fertilizer concentration regardless of whether it was applied with NW or MW as seen in Table (4) and Fig. (2). This result was in line with data reported by Antonin and Neumann (2016) who explained that the increase in mitotic phase index of *V. faba* perhaps indicated the meristematic cells were actively dividing and high proliferation potential and cell cycle phase transitions.

Mitotic index means the percentage of cells to go through division, any alteration in this index is considered as a cytotoxicity parameter (Salah *et al*., 2022). The mitosis can be evaluated via parameters, such as mitotic index, phase index and percentages of abnormal mitosis at each phase. Regarding the effect of tested preparations on the mitotic phase indices as indicator for the direct effect on the cell cycle, the results in Table (4) showed significant variations in the frequencies of different mitotic indices in treated plants when compared to the control. Prophase frequency reached a maximum value of 55.81% in response to MW treatment and a minimum value of 4.95% in response to NW+0.005% $MnSO_4$ treatment while the control had 22.89%.

Increases in prophase index reflect a delay in chromosome condensation (Binarova *et al*., 1993), hence the MW treatment without fertilizers could cause cell cycle arrest at prophase inhibits the progression into metaphase. However, MW with 0.01% of either $MnSO_4$ or $ZnSO_4$ recorded decreased prophase frequences (16.33% and 14.88%, respectively) compared to the control and this is good evidence for the enhanced *V. faba* growth traits recorded of either zinc or manganese in this study. Metaphase showed significant decreased frequency in *V. faba* mitotic cells of all treatments except the NW+0.005%$MnSO_4$ treatment, it was increased by a percentage of 22.69% over the control.

For anaphase and telophase frequencies, both showed significant increases in response to all treatments except the MW treatment, it was lowered. In *V. faba* mitotic cells, anaphase recorded its highest frequency of 22.38% in response to MW+0.01%$ZnSO_4$ treatment and its lowest frequency of 10.53% in response to MW treatment while telophase recorded its highest frequency of 34.64% in response to NW+0.01%$ZnSO_4$ treatment and its lowest frequency of 8.05% in response to MW treatment. Prolonged metaphase could be due to delayed kinetochore cleavage while prolonged anaphase and telophase could be due to non-functional spindle fiber and so chromosomes fail in proper segregation or deconensation (Binarova *et al*., 1993).

Chromosomal berrations; the chang-es in the structure of chromosomes, were considered a good indicator to estimate the mutagenicity of chemical (Wilhelm *et al*., 2020). CAs can arise from

various sources, such as DNA strand breaks, inhibition of DNA synthesis, errors in replication mechanisms or abnormal chromosome segregation (Krupina *et al*., 2021).

Microscopic study revealed the presence of, disturbance, stickiness in metaphase and anaphase with broken chromosome and bridges which confirmed that chromosomal aberration has occurred. Here different kind of chromosomal aberrations induced at different stages of mitosis besides interphase in root meristem cells are variable.

Various chromosomal irregularities in metaphase and anaphase are because of the shifting of poles by depolymerization of spindle fibers (Soliman *et al*., 2021). Changed cellular division phase indices and enhanced percentages of different types of chromosome aberrations were recorded in most treatments, especially after irrigation with NW under 0.01% of $ZnSO_4$ or $MnSO_4$ fertilization resulting in the highest percentages of chromosomal abnormalities (32.02% and 30.42%, respectively) in *V*. *faba* mitotic cells (Table 5). The total abnormalities produced by treatments were observed to be water source dependent, overall, total abnormalities percentage showed much significant increases in mitotic cells of *V*. *faba* treated with non-magnetized irrigation water under $ZnSO_4$ or $MnSO_4$ fertilization rather than its counterparts of magnetized irrigation water.

Magnetized irrigation water as a single treatment noticed non-significant change at $p \leq 0.05$ in total abnormalities percentage if compared with the control as seen in Table (5) and Fig. (3). The various types of CAs observed in mitotic cells of *V*. *faba* in response to irrigation by NW or MW under $ZnSO_4$ or $MnSO_4$ fertilization are shown in Table (6) and Plate (1). These aberrations are micronucleus at interphase and prophase, disturbance, micronucleus, non-congression, oblique, stickiness, chromosome ring and two- groups at metaphase besides bridge, diagonal, disturbance, laggard and late separation at anaphase and telophase as well.

Micronuclei are fragments of genetic material that are not incorporated into the main nucleus and can result from chromosome breakage or disruption of the mitotic apparatus. MN of plant roots are appropriate and efficient cytogenetic materials for the detection of cytotoxicity potential of environmental pollutants, especially for aquatic systems including surface water, lakes, wastewater and landfill leachate. Plant assays have been integrated as a cytotoxicity component in risk assessment for detection of environment mutagens because of the simple, quick, inexpensive, efficient and reliable characters (Alias *et al*., 2023).

In Table (6), the highest frequency of interphase-micronucleus (Plate 1; A) (0.40%) was observed in *V*. *faba* mitotic cells of NW+0.01%$ZnSO_4$ treatment; while its lowest frequency (0.07%) was observed in response to NW+0.01%$MnSO_4$ and MW+0.01%$ZnSO_4$ treatments. Micronuclei appeared only in *V*. *faba* mitotic-

prophase (Plate 1; B) in response to NW+0.01%$ZnSO_4$ and MW+0.01%$MnSO_4$ treatments with percentages of 0.29% and 0.25%, respectively. MN appears during cell division by breakage of a part of the chromosome leading to a small chromosome fragment or by failure of the whole chromosome migration during anaphase. Chromosome breakage correlated with the formation of chromosome fragments and micronucleus cells (Abdel-Khalek *et al*., 2021).

Different types of mitotic metaphase chromosomal abnormalities were observed in treated *V. faba* mitotic cells as disturbed metaphase (Plate 1; C) that was noticed with the highest percentage (7.49%) in response to NW+ 0.005% $MnSO_4$ treatment and the lowest value (2.11%) in response to MW treatment. Micronucleus mitotic metaphase abnormality (Plate 1; D) was a restricted abnormality (0.21%) observed only in root tip cells of magnetized irrigation water treated *V. faba* plants. Non- congression (Plate 1; E) showed its highest percentages of 2.05% and 1.82% in response to irrigation with NW and NW+ 0.005% $ZnSO_4$ fertilization, respectively, while its lowest percentage (0.38%) was in response to NW+ 0.005% $MnSO_4$ treatment.

Mitotic cells of all treated *V. faba* showed the appearance of oblique metaphase (Plate 1; F) and sticky metaphase (Plate 1; G) abnormalities. Oblique and sticky metaphase recorded their highest values (5.97% and 5.47%; respectively) in response to NW+0.01%$MnSO_4$ treatment and lowest values (1.47% and 1.69%; respectively) in response to MW treatment.

Chromosome ring (Plate 1; H) as a mitotic metaphase abnormality recorded its highest value (3.04%) in *V. faba* mitotic cells as responses to irrigation with NW+0.005%$ZnSO_4$ and lowest value (1.05%) in response to

Two-groups is an obvious mitotic metaphase chromosomal abnormality (Plate 1; I-J) appeared with a maximum percentage of 4.10% in case of *V. faba* mitotic cells of NW, while recorded a minimum percentage of 0.75% in case of mitotic cells of *V. faba* irrigated with NW+ 0.005% $MnSO_4$.

The presence of disturbance in metaphase, anaphase and telophase poles may be due to the altered direction of chromosomes during different stages of mitotic division through the interaction chemical fertilizers with mitotic spindle apparatus, centrioles or their associated proteins leading to the loss or gain of chromosomes in daughter cells (Daphedar *et al*., 2021). The chromosomal adherence is another common sign of toxic effects on the genetic material and may cause irreversible effects on the cell triggering the cell death process (Wickrama and Wijeyaratne, 2020). It is also associated with the formation of chromosomal bridges and eventually leads to chromosomal breaks (Sheikh *et al*., 2020).

The chromosomal bridges resulted from adherence which can multiply and

persist until telophase stage (Kamal *et al*., 2021). Ihegboro *et al*. (2020) attributed chromosomes stickiness to the formation of complexes of toxic agents with phosphate groups in DNA, on DNA condensation, or formation of inter- and intra-chromatid cross-links. Stickiness formation involves the matrix of chromatin material that makes the chromosome stick or clump (Dhara *et al*., 2021).

Additionally, the late separation of chromosome and multipolar anaphases suggest the effect on microtubule assembly. The microtubules perform a central role during the growth and mitotic cycle as chromosome migration, cell structure, and formation of cell wall (Vladimirovich *et al*., 2021). In this study, various CAs were shown at anaphase and telophase stages as bridges, diagonal, disturbed, laggard and late separation as seen in Table (6) and Plate (1; K-T). According to anaphase, bridges (Plate 1; K) induced in a significant high percentage of 3.68% in *V*. *faba* mitotic cells of MW+0.01%$ZnSO_4$ treatment, while the treatment of NW+0.01%$MnSO_4$ induced the lowest percentage of 0.50%.

Diagonal anaphase (Plate 1; L) percentage showed its highest value (2.10%) in response to MW+0.01% $ZnSO_4$ treatment and the lowest value (0.25%) was due to MW+ 0.01%$MnSO_4$ treatment. Disturbed ana-phase (Plate 1; M) presented its highest value (3.04%) due to NW+ 0.005%$ZnSO_4$ treatment and lowest value (0.57%) in response to NW+ 0.01% $ZnSO_4$ treatment. Percentage of laggard chromosome (Plate 1; N) noticed in *V*. *faba* mitotic cells with a highest significant percent of 1.47% in response to irrigation with magnetized irrigation water and a lowest percent of 0.53% in response to MW+ 0.01% $ZnSO_4$ treatment.

Moreover, late separation (Plate 1; O) showed its highest value (1.53%) in mitotic cells of *V*. *faba* plants irrigated with MW+0.01% $MnSO_4$ and its lowest value of 0.50% in mitotic cells of *V*. *faba* irrigated with NW+ 0.01% $MnSO_4$.

For telophase, bridges (Plate 1; P) that appeared in *V*. *faba* mitotic cells with a high significant value of 1.14% as a response to NW+ 0.01% $ZnSO_4$ treatment and a lowest value of 0.21% was due to magnetized irrigation water treatment. Diagonal (Plate 1; Q) showed its highest percent 3.13% was noticed in response to NW+ 0.01% $ZnSO_4$ and its lowest percent 0.49% was recorded in response to MW+ 0.005%$ZnSO_4$ treatment. Disturbance (Plate 1; R) in mitotic telophase was noticed in response to all treatments and the control with a highest percentage of 2.80% in case of *V*. *faba* plants in response to MW+ 0.01%$MnSO_4$ treatment and the lowest percentage of 0.71% in response to MW+0.005%$MnSO_4$ treatment.

Furthermore, laggard chromosome (Plate 1; S) showed its highest percentage (1.14%) in mitotic cells of *V*. *faba* plants treated with NW+ 0.01% $ZnSO_4$, while its lowest percentage (0.43%) showed due to MW treatment. For late separation (Plate 1; T), the highest percentage (3.98%) was recorded in *V*. *faba* mitotic cells in re-

sponse to NW+ 0.01%$MnSO_4$ treatment, while the lowest percentage (1.05%) was noted in response to MW treatment.

The results of this research were well-matched to the results of Rizk *et al*. (2015) who assessed the cytotoxicity of normal irrigation water collected from different districts along Dakahlia Governorate on *Ipomoea carnea* Jacq. Such irrigation water induced a significant increased mitotic index ranged from 2.33% in mitotic cells of *I*. *carnea* irrigated with NW collected from Belgay drainage canal to 38.45% in mitotic cells of *I*. *carnea* irrigated with NW collected from Shawa irrigation canal and produced number of chromosomal anomalies as micronucleus at interphase and stickiness at metaphase by NM collected from Meet-Khamis drainage canal, oblique at metaphase by NM collected from El-Sallab irrigation canal and chromosome ring at metaphase by NM collected from Shawa irrigation canal. Also, NW induced increased total anomalies percentage to 5.88% in case of mitotic cells of *I*. *carnea* collected from Rezka irrigation canal above the control.

ISSRs molecular evaluation

Recently, advances in molecular biology succeed in developing a number of selective and sensitive assays to demonstrate the variations at the DNA level, and differences can clearly be shown when comparing DNA fingerprints from individuals exposed and/or nonexposed to genotoxic agents (Vassoler *et al*., 2021). Advantages of measuring the genotoxicity in plants at DNA level are mainly related to sensitivity and short response time.

Molecular markers have advent- ages over morphological, cytological, and biological markers and have been applied extensively in the life sciences as they provide new tools for detection of genetic alteration in response to toxic chemicals at the level of DNA sequence and structure (Agrawal *et al*., 2020).

Missing bands, the appearance of new bands or change in band intensities were the aspects of variations in the resulting DNA profiles, hence the scored bands can be used as an index for evaluation of the genetic simi- larities or dissimilarities among treatments (Thakur *et al*., 2021). It is considered as a powerful molecular technique used to assess the effect of chemicals in terms of DNA damage by genomic template stability (GTS) evaluation in plants (Coşkun, 2023; Soliman *et al*., 2023). Based on this fact, in the present study, ten ISSR-primers were used to assess whether it can uncover variations in DNA fingerprints of *V*. *faba* in response to irrigation with NW and MW under $ZnSO_4$ or $MnSO_4$ fertilization (Tables 7-9 and Plate 2).

For ISSR-PCR analysis (Table 7), the size of the amplified DNA bands varied from 75 to 1600 base pair (bp), a total of 117 DNA bands (76 monomorphic bands and 41 polymorphic bands of 13 unique bands) were distinguished and the percentage of polymer- phism fluctuated between 8.33% and 12.50% in ISSR-2 besides ISSR-5, respectively and 66.67% in ISSR-4 with a mean of 33.29%.

ISSR-4 primer produced the highest level of polymorphism, detecting 10 distinct polymorphic amplification products; while ISSR-2 and ISSR-5 primers produced the fewest amplified polymorphic fragments of 1 fragment. The band frequency means ranged from 0.70 (ISSR-4), 0.80 in ISSR- 3, ISSR-7 and ISSR-8 and 0.90 in the remaining ISSR-markers with an average around 0.85.

These DNA polymorphisms may result from DNA structural changes within base-pair sequences of DNA as a nucleotide substitution within a target site, base pair insertion or deletion of a DNA fragment, inversions or translocations which result in the loss or gain of DNA bands resulting in different DNA lengths and consequently highly level polymorphisms (Mohamed and Alrashd, 2020). The events were observed in the PCR pat- terns to occur subsequent to non- magnetized and magnetized irrigation water treatments under different levels of ZnO_4 or $MnSO_4$ fertilizers: loss of normal bands, and appearance of new bands in comparison with the control plants.

The ten ISSR-markers gave 13 molecular markers (six positive and seven negative) linked to irrigation with NW and MW of different levels of studied fertilizers (Table 8), which might be potentially im- plicated with certain genes unique for the treated plants as a response to certain interaction of its genome and the treatments. For example, as seen in Plate (2), the band of 610 bp was observed with ISSR-8 could be considered as a positive-molecular marker for plants treated with MW+0.005% $MnSO_4$ and the band of 860 bp generated by ISSR-1 may be a negative molecular marker for plants treated with MW+0.01%$ZnSO_4$. Therefore, unique mole- cular marker pattern either negative or positive can be relied upon in distinguishing among studied treated plants.

The same conclusion was adopted by El-kholy *et al*. (2023) who used ten ISSRs to demonstrate the genetic variations in band- ing pattern profile of ISSR fingerprinting in *Allium cepa* treated with three concentration of waste water (25%, 50% and 100%) as appearance of new markers in the used ISSRs in the treated plants that were absent in the control as 3 bands formed by UBC 809 with sizes 210, 344 and 450 bp. Instead, bands appeared in control and were absent in the other treatments as 2 bands formed by UBC 857 with sizes 286 and 395.

In Table (9), the ISSR patterns showed notable variations between all treatments and the control with apparent changes in the number of amplified DNA fragments; the highest polymorphic bands noted are 22 bands in DNA profiles of *V. faba* irrigated with NW under 0.01%$MnSO_4$ or 0.01%$ZnSO_4$ fertilization, while the lowest polymorphic bands noted are 11 bands in DNA profiles of *V. faba* irrigated with MW as compared with the control. In addition to appearance and disappearance of bands in ISSR profiles, a decrease in GTS% was recorded in all treatments. GTS, a qualitative measure of

genotoxic effect, is directly related to the degree of DNA damage and to the competence of DNA repair and replication (Sicińska *et al*., 2021).

In this study, changes in the ISSR patterns are expressed as decreases in genome template stability as seen in Fig. (4); the highest GTS% (89.42%) was recorded in ISSR profiles of *V*. *faba* irrigated with MW, while the lowest GTS% (78.85%) was observed in case of ISSR profiles of *V*. *faba* irrigated with NW of either 0.01% $MnSO_4$ or 0.01% $ZnSO_4$; these treatments lower the GTS% by about 21.15%.

GTS% is related to the level of DNA damage, the efficiency of DNA repair and replication, so high GTS% indicate that the genome is less disposed to alterations in its DNA, where- as low GTS% indicate greater chances of DNA alteration (Abdelmigid and El Rab, 2016). Overall, the results indicated the general tendency of decrease in GTS% with exposure to NW rather than MW.

The result obtained with GTS% values are compatible with that of cytotoxicity results. The cytotoxicity of treatments indicated by their great changes in mitotic indices and development of chromosomal aberrations in *V*. *faba* mitotic cells was confirmed by their effect on DNA profiles through appearance and disappearance of some bands. Similar results were observed by **Altwaty *et al*. (2016)** who recorded changes in mitotic activity and appearance of different types of CAs in *V*. *faba* root tips and the appearance or disappearance of RAPD bands which could be regarded as modifications in genomic template stability induced by chemicals from extract of *Dipterygium glaucum*.

New PCR products appearance could be referred to the presence of priming sites which become accessible to primers after structural alternation in DNA sequence that occurred due to mutations (resulting in new annealing events) or large deletions (bringing two pre-existing annealing sites closer); while the disappearance of bands may be attributed to the presence of DNA adducts, which can act to block or reduce the polymerization of DNA in the PCR-reaction (Amiteye, 2021).

ISSRs could be widely applicable to study the effect of different irrigation water sources (NW and MW) on a population genetics. DNA polymorphism detected using ISSR analysis due to induced or disappearance of bands in different treatments as compared with the control could be used as an investigation tool for environmental toxicology (El-Kholy *et al*., 2023).

Changes in ISSR profile induced by the treatments can be regarded as changes in genomic DNA template stability and these genotoxic effects can be directly compared with alteration in other parameters. The results obtained in this study indicated that both assays (*V*. *faba* and ISSRs) can lead to the same conclusion, indicated that *V*. *faba* assay can be used as an initial screening step and then followed by extra analysis for DNA. Plant assay is an efficient and reliable test system for

dicated that both assays (*V. faba* and ISSRs) can lead to the same conclusion, indicated that *V. faba* assay can be used as an initial screening step and then followed by extra analysis for DNA. Plant assay is an efficient and reliable test system for chemicals mutagenicity monitoring (Farizan *et al*., 2021).

ISSR analysis proved a highly sensitive technique for detection of DNA change and the evaluation of genotoxic effect and genomic instability induced in *V. faba* irrigated by NW or MW under $ZnSO_4$ or $MnSO_4$ fertilization.

Perusal of data presented in (Table (10), Fig.5) revealed significant moderate positive correlations appear, in general, to exist between the GTS-ISSR of all treated *V. faba* and the changes in growth traits (germination time, shoot length and flowering time) Thus, it is worthy to conclude that the irrigation of *V. faba* with magnetized irrigation water under zinc and manganese sulphates fertilization induced less significant impairments to *V. faba* plant genome, produced tallest plant; accelerated its germination and flowering time; in comparison to other treatments and the control.

SUMMARY

This study investigates how non-magnetized and magnetized irrigation water influence *Vicia faba* (Giza 716*)* growth traits, chromosomal structure and DNA markers (ISSR). Irrigation water passed through a magnetic field of 1000-**g**auss magnetron unit of 0.5 inch diameter. Fertilized *V. faba* with different levels (0.005% and 0.01%) of zinc or manganese sulphates were irrigated every two days with the two different water sources. Significant acceleration in seed germination and flowering times besides increased shoot length of plants irrigated by magnetized irrigation water under zinc or manganese sulphates fertilization compared to their counterpart treatments of non-magnetized irrigation water were observed. Cytotoxicity parameters as mitotic index, phase indices and total abnormalities percentages developed in *V. faba* mitotic cells were evaluated.

As a result, all treatments showed significant increases in the mitotic index and produces several chromosomal abnormalities mostly at the concentration of 0.01% of the fertilizers as micronucleus at interphase and prophase, disturbance, micronucleus and two-groups at metaphase, bridge, diagonal, and late separation at anaphase and telophase.

For ISSR-PCR analysis, a total of 117 DNA bands appeared whereases 35.04% of these bands were polymorphic. The results indicated significant decreases of GTS% with exposure to treatments of non-magnetized irrigation water rather than magnetized irrigation water. Irrigation with non-magnetized water of 0.01% fertilizers lowers the GTS by about 21.15%. Thus, it was concluded that non-magnetized water showed cytotoxic activities and genome template instability more than magnetized irrigation water that could be used as an alternative irrigation water

source that occupy simple, cost effective and ecofriendly preparation method.

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Table (1): Experimental treatments with non-magnetized (NM) and magnetized (MW) irrigation water under $ZnSO_4$ or $MnSO_4$ fertilization in *Vicia faba Giza* 716.

Treatment		Details	Irrigation water source	Fertilizer concentration (ppm)	
				[$ZnSO_4$]	[$MnSO_4$]
T0	Control		Non-magnetized water	0 .00	0.00
T1	MW		Magnetized water	0.00	0.00
T2	MW+0.005%$MnSO_4$		Magnetized water	0.00	50.00
T3	MW+0.005%$ZnSO_4$		Magnetized water	50.00	0.00
T4	MW+0.01%$MnSO_4$		Magnetized water	0.00	100.00
T5	MW+0.01%$ZnSO_4$		Magnetized water	100.00	0.00
T6	NW+0.005%$MnSO_4$		Non-magnetized water	0.00	50.00
T7	NW+0.005%$ZnSO_4$		Non-magnetized water	50.00	0.00
T8	NW+0.01%$MnSO_4$		Non-magnetized water	0.00	100.00
T9	NW+0.01%$ZnSO_4$		Non-magnetized water	100.00	0.00

MW: Magnetized water, NW Non-magnetized water,

Table (2): List of ISSR primers and their nucleotide sequence used in this study.

Primer	Sequence 5'to 3'
ISSR-1	5'-AGAGAGAGAGAGAGAGC-3'
ISSR-2	5'-AGAGAGAGAGAGAGAGG-3'
ISSR-3	5'-ACACACACACACACACT-3'
ISSR-4	5'-ACACACACACACACACG-3'
ISSR-5	5'-GTGTGTGTGTGTGTGTG-3'
ISSR-6	5'-CGCGATAGATAGATAGATA-3'
ISSR-7	5'-GACGATAGATAGATAGATA-3'
ISSR-8	5'-AGACAGACAGACAGACGC-3'
ISSR-9	5'-GATAGATAGATAGATAGC-3'
ISSR-10	5'-GACAGACAGACAGACAAT-3'

Table (3): *Vicia faba* growth traits in response to irrigation with non-magnetized and magnetized irrigation under $ZnSO_4$ or $MnSO_4$ fertilization.

T	GT (Days after planting)	Change%	SL (cm/plant)	Change%	FT (Days after planting)	Change %
C	13.00^{e}	0.00	62.00^{d}	0.00	60.00^{c}	0.00
T1	13.00^{e}	0.00	63.50^{e}	2.42	60.00^{c}	0.00
T2	10.00^{b}	-23.08	69.50^{i}	12.10	63.00^{e}	5.00
T3	12.00^{d}	-7.69	67.00^{g}	8.06	60.00^{c}	0.00
T4	9.00^{a}	-30.77	68.50^{h}	10.50	58.00^{a}	-3.33
T5	10.00^{b}	-23.08	58.50^{b}	-5.60	59.00^{ab}	-1.67
T6	12.00^{d}	-7.69	53.50^{a}	-14.00	59.00^{ab}	-1.67
T7	12.00^{d}	-7.69	64.50^{f}	4.03	58.00^{a}	-3.33
T8	10.00^{b}	-23.08	59.00^{bc}	-4.80	61.00^{d}	1.67
T9	11.00^{c}	-15.38	58.50^{b}	-5.60	59.00^{ab}	-1.67

T; Treatments, C; Control, GT; Germination time, SL; shoot length, FT; Flowering time. Means, in each column, followed by similar letter are not significantly different at the 5% probability level-using Post Hoc. Duncan test (Ducan, 1955).

Table (4): Mitotic index and phase indices percentages in *Vicia faba* mitotic cells in response to irrigation with non-magnetized and magnetized irrigation water under $ZnSO_4$ or $MnSO_4$ fertilization.

T	Mitotic index %		Phase indices %			
	NDC	MI% (Mean± S.D.)	Prophase	Metaphase	Anaphase	Telophase
C	195	6.50^{a}±4.70	22.89^{cd}±31.63	45.57^{c}±34.67	12.05^{ab}±17.52	19.49^{b}±26.44
T1	475	15.83^{de}±8.67	55.81^{c}±20.19	25.61^{a}±19.65	10.53^{a}±11.38	8.05^{a}±11.85
T2	560	18.67^{e}±15.19	27.08^{d}±23.99	32.83^{ab}±20.04	17.78^{abc}±18.32	22.31^{bc}±18.84
T3	412	13.73^{cd}±9.60	27.75^{d}±19.83	33.76^{ab}±18.88	16.56^{abc}±14.25	21.93^{bc}±19.74
T4	393	13.10^{cd}±10.39	16.33^{bc}±19.18	38.58^{bc}±22.37	19.96^{bc}±14.33	25.13^{bc}±19.17
T5	380	12.67^{c}±9.33	14.88^{bc}±20.24	37.71^{bc}±20.97	22.75^{c}±20.73	24.66^{bc}±20.58
T6	267	8.90^{ab}±6.72	4.95^{a}±14.00	55.91^{d}±34.92	17.13^{abc}±20.00	22.01^{bc}±27.86
T7	329	10.97^{bc}±8.58	8.57^{ab}±16.40	45.46^{c}±27.15	22.38^{c}±30.76	23.59^{bc}±19.04
T8	201	6.70^{ab}±9.74	12.43^{ab}±24.25	36.03^{abc}±29.43	22.38^{c}±20.16	29.16^{cd}±28.07
T9	351	11.70^{bc}±7.99	10.75^{ab}±17.67	38.67^{bc}±28.86	15.94^{abc}±18.81	34.64^{d}±27.45

T; Treatments, C; Control, NDC; Number of dividing cells. Mitotic index was scored in a total of 3000 cells. Means, in each column, followed by similar letter are not significantly different at the 5% probability level-using Post Hoc. Duncan test (Ducan, 1955).

Table (5): Abnormal phase indices in non- dividing and dividing cells and total abnormalities in *Vicia faba* mitotic cells in response to irrigation with non-magnetized and magnetized irrigation water under $ZnSO_4$ or $MnSO_4$ fertilization.

T	Interphase Abn. %	Prophase Abn. %	Metaphase Abn. %	Anaphase Abn. %	Telophase Abn. %	$T_{abn.}$ %
C	0.00^{a}±0.00	0.00^{a}±0.00	14.36^{bcd}±29.66	1.54^{a}±8.51	4.62^{ab}±15.08	20.52^{b}±3.57
T1	0.13^{ab}±0.71	0.00^{a}±0.00	7.79^{a}±11.65	5.05^{ab}±8.22	3.37^{a}±7.58	16.34^{a}±1.43
T2	0.13^{ab}±0.62	0.00^{a}±0.00	11.43^{abc}±15.99	6.96^{bc}±13.62	4.29^{ab}±12.36	22.81^{b}±2.08
T3	0.00^{a}±0.00	0.00^{a}±0.00	12.14^{abc}±13.61	4.85^{ab}±13.41	5.83^{bc}±12.30	22.82^{ab}±1.4
T4	0.13^{ab}±0.46	0.25^{b}±2.41	12.47^{abc}±24.33	6.11^{bc}±11.64	6.62^{c}±15.86	25.58^{b}±2.63
T5	0.07^{ab}±0.27	0.00^{a}±0.00	9.47^{ab}±15.87	7.89^{c}±19.75	5.53^{b}±9.48	22.96^{b}±2.11
T6	0.23^{bc}±1.07	0.00^{a}±0.00	16.11^{abc}±26.62	4.87^{ab}±12.01	5.61^{b}±14.02	26.82^{b}±27.6
T7	0.00^{a}±0.00	0.00^{a}±0.00	18.24^{cd}±27.48	3.65^{a}±14.66	5.47^{b}±6.86	27.36^{b}±2.86
T8	0.07^{ab}±0.43	0.00^{a}±0.00	18.41^{bcd}±28.12	3.48^{a}±9.93	8.46^{d}±15.99	30.42^{b}±2.68
T9	0.40^{c}±1.11	0.29^{b}±3.34	18.23^{d}±49.87	4.27^{a}±7.06	8.83^{e}±24.68	32.02^{c}±5.99

T; Treatments, C; Control. Abn.; abnormalities. Means, in each column, followed by similar letter are not significantly different at the 5% probability level-using Post Hoc. Duncan test (Ducan, 1955).

Table (6): Types of scored chromosome abnormalities and its percentage at each phase in *Vicia faba* mitotic cells in response to irrigation with non-magnetized and magnetized irrigation water under $ZnSO_4$ or $MnSO_4$ fertilization.

T / Abn	Interphase	Prophase	Metaphase						
	MN	MN	D	MN	NC	O	S	CR	TGs
C	0.00^{a}	0.00^{a}	2.56^{d}	0.00^{a}	2.05^{j}	2.05^{d}	3.60^{g}	0.00^{a}	4.10^{j}
T1	0.13^{ab}	0.00^{a}	2.11^{a}	0.21^{b}	0.42^{c}	1.47^{a}	1.69^{a}	1.05^{b}	0.84^{c}
T2	0.13^{ab}	0.00^{a}	2.50^{c}	0.00^{a}	1.07^{g}	2.14^{e}	1.97^{c}	2.32^{g}	1.43^{e}
T3	0.00^{a}	0.00^{a}	2.91^{e}	0.00^{a}	0.97^{e}	1.94^{c}	2.19^{d}	1.94^{f}	2.19^{g}
T4	0.13^{ab}	0.25^{b}	3.05^{f}	0.00^{a}	0.51^{d}	2.54^{f}	2.04^{c}	2.29^{g}	2.04^{f}
T5	0.07^{ab}	0.00^{a}	2.37^{b}	0.00^{a}	1.05^{f}	1.84^{b}	2.89^{f}	1.32^{c}	0.00^{a}
T6	0.23^{bc}	0.00^{a}	7.49^{j}	0.00^{a}	0.38^{b}	3.75^{h}	1.87^{b}	1.87^{e}	0.75^{b}
T7	0.00^{a}	0.00^{a}	3.95^{g}	0.00^{a}	1.82^{i}	3.04^{g}	2.74^{e}	3.04^{h}	3.65^{i}
T8	0.07^{ab}	0.00^{a}	4.48^{h}	0.00^{a}	0.00^{a}	5.97^{i}	5.47^{i}	0.00^{a}	2.49^{h}
T9	0.40^{c}	0.29^{b}	5.98^{i}	0.00^{a}	1.71^{h}	3.70^{h}	3.99^{h}	1.71^{d}	1.14^{d}

Table (6): Continued;

T / Abn.	Anaphase					Telophase				
	B	DIA	D	LC	LS	B	DIA	D	LC	LS
C	0.51^{b}	0.00^{a}	1.03^{bc}	0.00^{a}	0.00^{a}	0.51^{c}	1.03^{e}	1.03^{d}	0.51^{c}	1.54^{b}
T1	1.90^{d}	0.42^{c}	1.26^{d}	1.47^{f}	0.00^{a}	0.21^{b}	0.84^{d}	0.84^{bc}	0.43^{b}	1.05^{a}
T2	2.86^{g}	1.25^{g}	1.07^{c}	0.71^{c}	1.07^{d}	0.00^{a}	0.89^{d}	0.71^{a}	0.00^{a}	2.69^{g}
T3	1.45^{c}	0.97^{f}	1.70^{e}	0.73^{c}	0.00^{a}	0.97^{e}	0.49^{b}	1.94^{g}	0.00^{a}	2.43^{e}
T4	2.04^{e}	0.25^{b}	2.29^{f}	0.00^{a}	1.53^{e}	0.00^{a}	0.76^{c}	2.80^{i}	0.00^{a}	3.06^{h}
T5	3.68^{h}	2.10^{h}	1.05^{c}	0.53^{b}	0.53^{c}	0.26^{b}	1.84^{g}	0.79^{b}	0.53^{c}	2.11^{c}
T6	1.87^{d}	0.00^{a}	3.00^{g}	0.00^{a}	0.00^{a}	0.75^{d}	0.00^{a}	2.62^{h}	0.00^{a}	2.24^{d}
T7	0.00^{a}	0.61^{e}	3.04^{g}	0.00^{a}	0.00^{a}	0.00^{a}	1.22^{f}	1.82^{f}	0.00^{a}	2.43^{e}
T8	0.50^{b}	0.50^{d}	0.99^{b}	0.99^{e}	0.50^{b}	0.00^{a}	2.49^{h}	1.49^{e}	0.50^{c}	3.98^{i}
T9	2.28^{f}	0.57^{e}	0.57^{a}	0.85^{d}	0.00^{a}	1.14^{f}	3.13^{i}	0.86^{c}	1.14^{d}	2.56^{f}

T; Treatments, C; Control, Abn.; Abnormality, MN; Micronucleus, D; Disturbed, NC; Non-Congression, O; Oblique, CR; chromosome ring, TGs; Two-groups, B; Bridge, DIA; diagonal, LC; Laggard chromosome, LS; Late separation. Means, in each column, followed by similar letter are not significantly different at the 5% probability level-using Post Hoc. Duncan test (Ducan, 1955).

Table (7): ISSR-PCR polymorphic bands of *Vicia faba* in response to irrigation with non-magnetized and magnetized irrigation water under $ZnSO_4$ or $MnSO_4$ fertilization.

Primer	PCR products range (bp)	Mono-morphic Bands	Polymorphic Bands		Total Bands	Polymorphism %	Mean of band frequency
			Unique bands	Non-unique bands			
ISSR-1	220-1600	10	3	0	13	23.07	0.90
ISSR-2	170-1150	11	1	0	12	8.33	0.90
ISSR-3	180-900	6	0	5	11	45.45	0.80
ISSR-4	160-730	5	4	6	15	66.66	0.70
ISSR-5	120-600	7	0	1	8	12.50	0.90
ISSR-6	250-1000	10	0	2	12	16.66	0.90
ISSR-7	140-1050	7	0	6	13	46.15	0.80
ISSR-8	210-1050	5	1	1	7	28.57	0.80
ISSR-9	75-1270	9	2	4	15	40.00	0.90
ISSR-10	104-997	6	2	3	11	45.45	0.90
Total bands		76	13	28	117		

Table (8): Positive unique markers (PUM) and negative unique markers (NUM) of the ten ISSR molecular markers used to distinguish the genetic variations among *Vicia faba* in response to irrigation with non-magnetized and magnetized irrigation water under $ZnSO_4$ or $MnSO_4$ fertilization.

Primer	PUM (base pair)	NUM (base pair)
ISSR-1	1250, 1450	860
ISSR-2	340	---
ISSR-3	---	---
ISSR-4	480	220, 280, 420
ISSR-5	---	---
ISSR-6	---	---
ISSR-7	---	---
ISSR-8	610	---
ISSR-9	---	75, 131
ISSR-10	139	104
Total	6	7

Table (9): Number of bands in control with all ISSR primers in *Vicia faba* in response to irrigation with non-magnetized and magnetized irrigation water under $ZnSO_4$ or $MnSO_4$ fertilization

Primers	Control (TB)	1		2		3		4		5		6		7		8		9	
		a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
ISSR-1	12	1	1	0	1	0	1	0	1	0	2	0	1	0	1	0	1	0	1
ISSR-2	12	0	1	0	1	0	1	0	1	0	`1	0	1	0	1	0	1	0	1
ISSR-3	11	0	0	0	2	0	3	0	4	0	5	0	3	0	3	0	2	0	2
ISSR-4	9	1	1	0	1	4	0	3	2	3	1	0	1	2	1	3	1	4	1
ISSR-5	8	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0
ISSR-6	11	1	0	1	0	1	0	0	0	1	0	1	1	1	1	1	1	1	1
ISSR-7	12	1	0	0	2	0	2	0	2	1	4	0	1	1	0	1	4	1	4
ISSR-8	6	0	0	1	0	0	1	0	1	0	0	0	0	0	1	0	0	0	1
ISSR-9	13	0	1	0	1	0	2	1	0	0	0	1	3	2	0	2	2	2	2
ISSR-10	9	2	0	1	0	1	0	0	0	1	1	1	3	1	2	1	1	1	0
TB	103	6	5	3	9	6	10	4	11	6	13	3	14	7	11	8	14	9	13
a + b		11		12		16		15		20		17		18		22		22	
GTS%	100	89.42		88.46		84.62		85.58		80.77		83.65		82.69		78.85		78.85	

TB; total bands, a; indicates number of new appeared bands, b; indicates number of disappeared normal bands, a + b; indicates number of polymorphic bands and GTS%; indicates genome template stability percentages.

Table (10): Pearson's simple correlations coefficient of genome template stability percentages derived from ten ISSRs; GTS-ISSRs, and associated changes in *Vicia faba* growth traits in response to irrigation with non- magnetized and magnetized irrigation water under $ZnSO_4$ or $MnSO_4$ fertilization.

	Germination time	**Shoot length**	**Flowering time**	**GTS-ISSRs**
Germination time	1.00	-0.18^{**}	-0.14^{**}	0.43^{**}
Shoot length	-0.18^{**}	1.00	0.34^{**}	0.38^{**}
Flowering time	-0.14^{**}	0.34^{**}	1.00	0.32^{**}
GTS-ISSRs	0.43^{**}	0.38^{**}	0.32^{**}	1.00

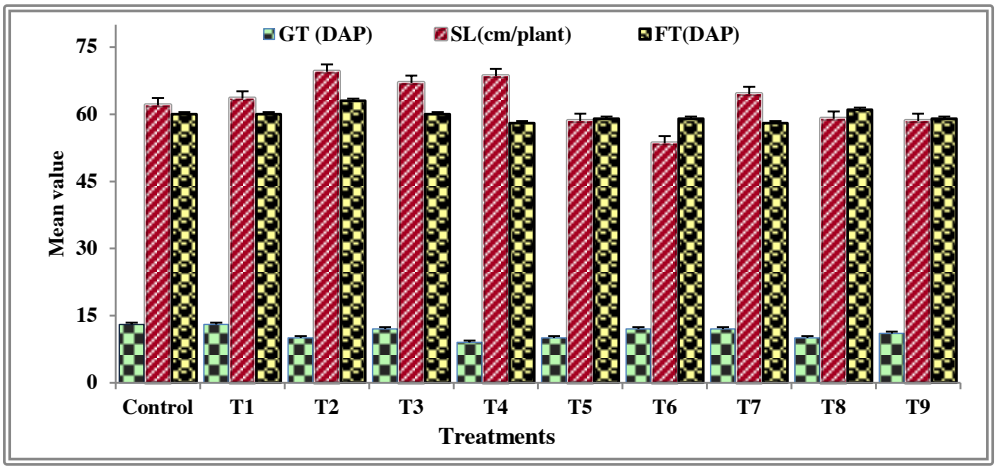

Fig. (1): Growth traits [germination time; GT, in day after planting (DAP), shoot length; SL, in cm/ plant and flowering time; FT in DAP] of *Vicia faba* plants in response to irrigation with non-magnetized and magnetized irrigation water under $ZnSO_4$ or $MnSO_4$ fertilization. Vertical bars represent growth traits ± S.E.

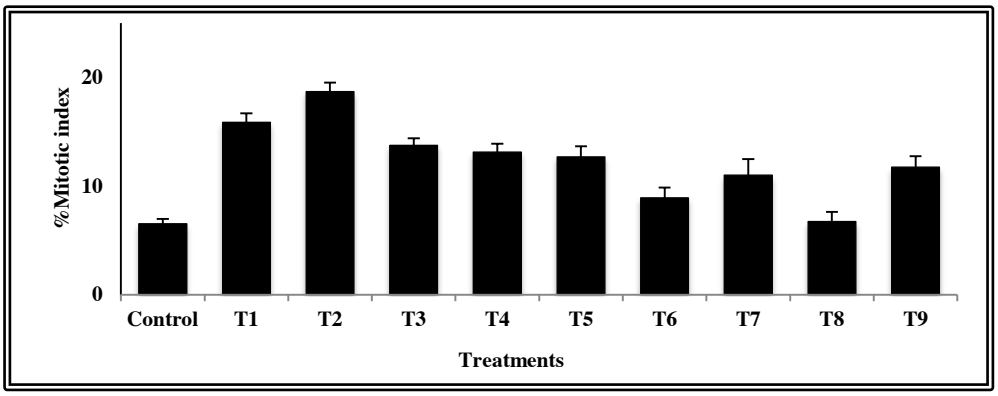

Fig. (2): Mitotic index percentages recorded in *Vicia faba* mitotic cells in response to irrigation with non-magnetized and magnetized irrigation water under $ZnSO_4$ or $MnSO_4$ fertilization. Vertical bars represent MI % ±S.E. $MnSO_4$ fertilization. Vertical bars represent MI % ±S.E.

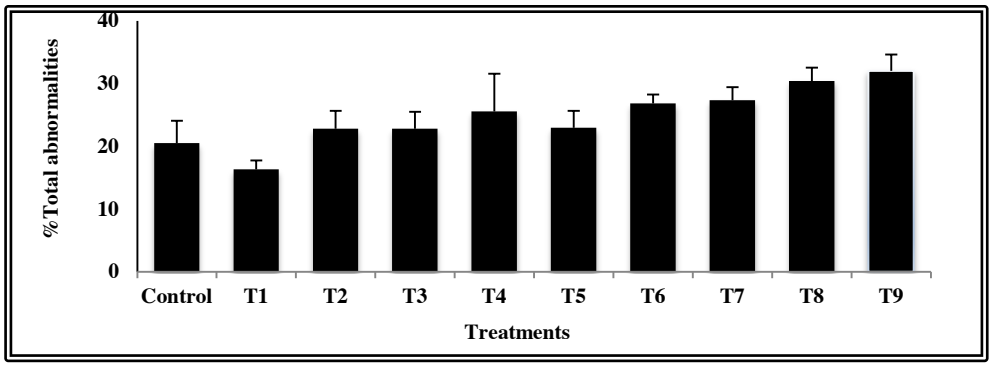

Fig. (3): Total chromosomal abnormalities percentages recorded in *Vicia faba* mitotic cells in response to irrigation with non-magnetized and magnetized irrigation water under $ZnSO_4$ or $MnSO_4$ fertilization. Vertical bars represent $T_{abn.}$ ±S.E.

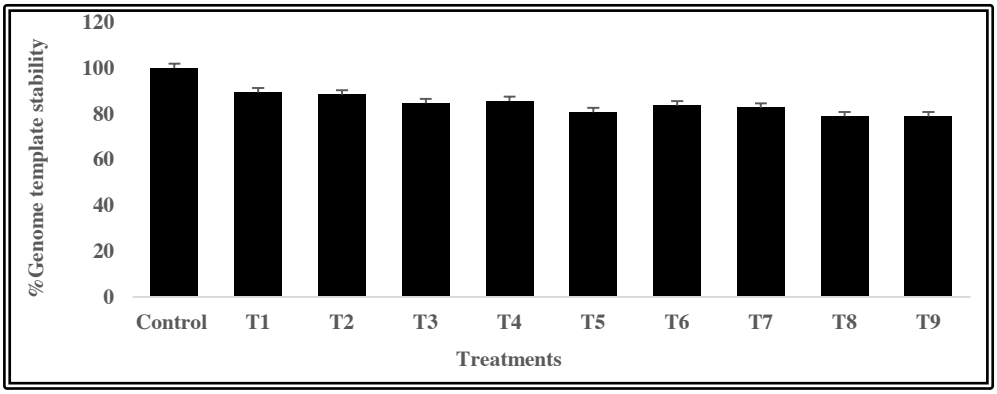

Fig. (4): Comparison of genome template stability percentages derived from *Vicia faba* DNA amplification profiles using ten ISSRs in response to irrigation with non- magnetized and magnetized irrigation water under $ZnSO_4$ or $MnSO_4$ fertilization. Vertical bars represent GTS% ±S.E.

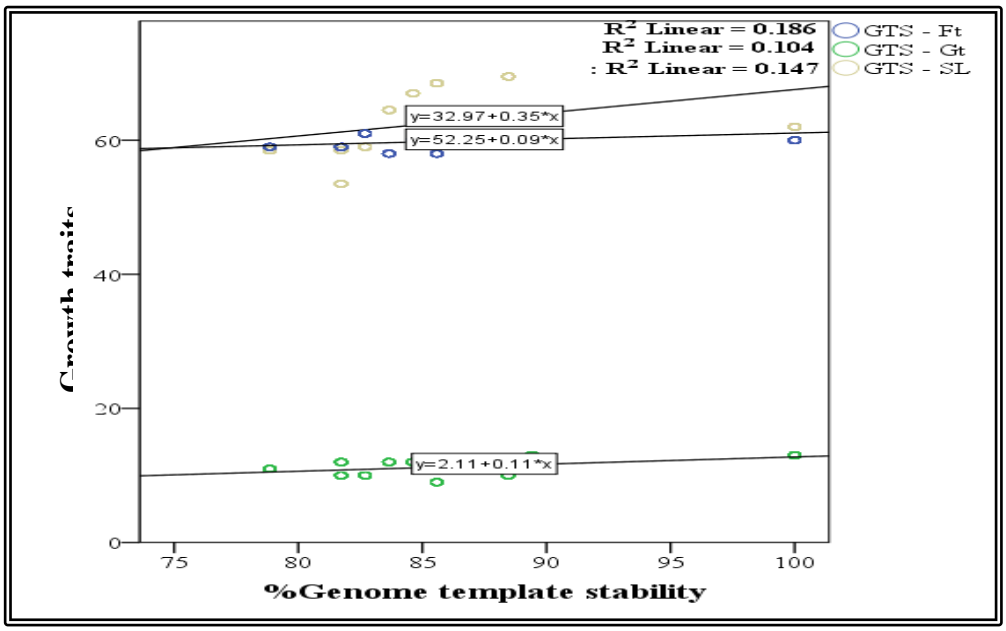

Fig. (5): Pearson's simple correlations in between genome template stability percentage and associated *Vicia faba* growth traits in response to irrigation with non-magnetized and magnetized irrigation water under zinc or manganese sulphates fertilizations.

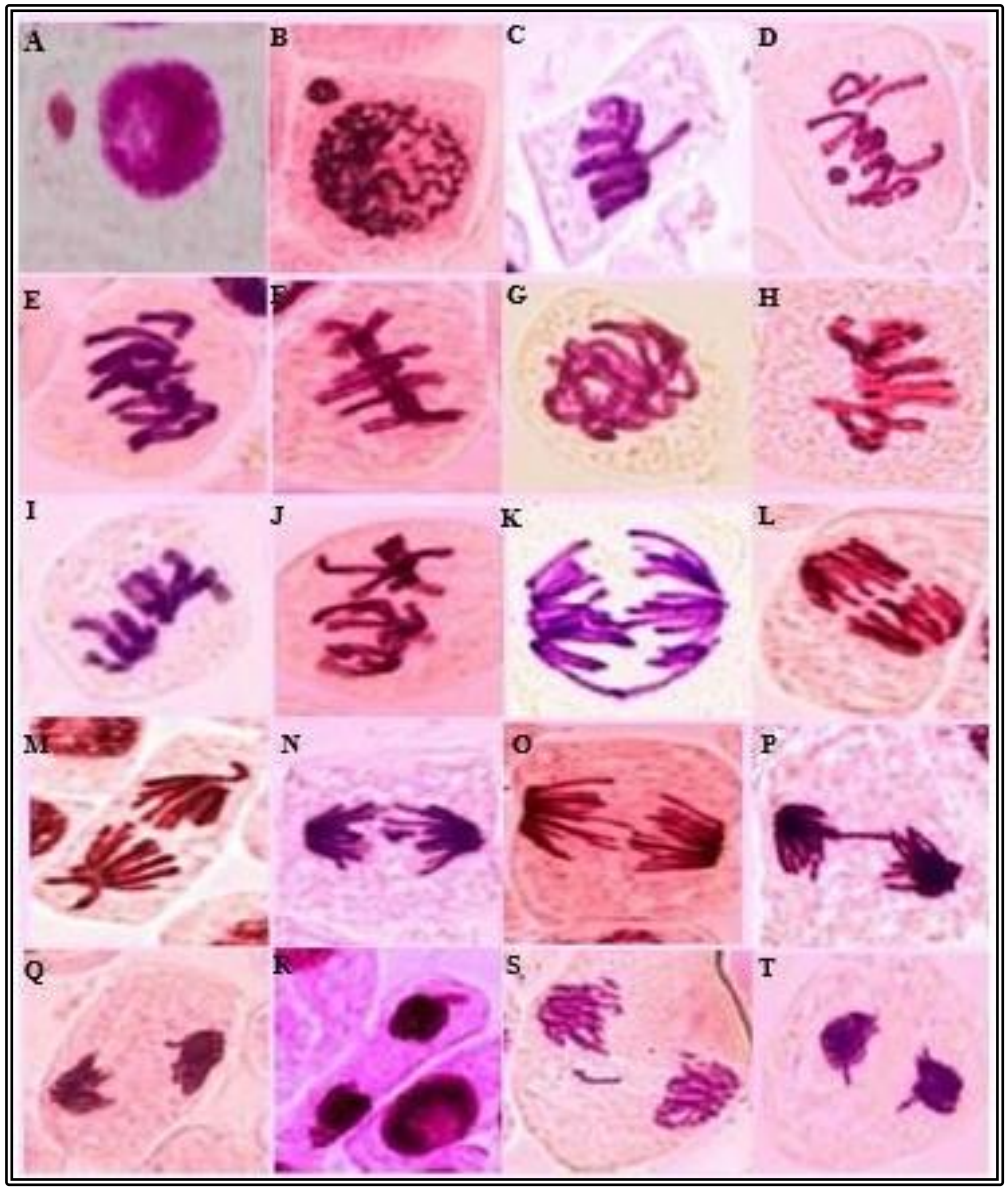

Plate (1): Chromosomal aberrations observed in *Vicia faba* root tips in response to irrigation with non-magnetized and magnetized irrigation water under $ZnSO_4$ or $MnSO_4$ fertilization; (A) Micronucleus at interphase (MW+0.01%$MnSO_4$); (B) Micronucleus at prophase (NW+0.01%$ZnSO_4$), (C) Disturbed metaphase (NW+0.005%$ZnSO_4$); (D) Micronucleus at metaphase (Magnetized irrigation water), (E) Non-Congression at metaphase (MW+0.005%$ZnSO_4$), (F) Oblique at metaphase (MW+0.005%$MnSO_4$), (G) Stickiness at metaphase (Magnetized irrigation water), (H) Chromosome ring at metaphase (Magnetized irrigation water), (I-J) Two-groups at metaphase (NW+0.005%$ZnSO_4$) and (MW+0.005%$MnSO_4$); in order, (K) Bridge at anaphase (Magnetized irrigation water), (L) Diagonal at anaphase (MW+0.01%$ZnSO_4$), (M) Disturbed anaphase (MW+0.005%$MnSO_4$), (N) Laggard at anaphase (MW+0.005%$ZnSO_4$), (O) Late separation at anaphase (MW+0.01%$ZnSO_4$), (P) Bridge at telophase (MW+0.01%$ZnSO_4$), (Q) Diagonal at telophase (MW+0.01%$ZnSO_4$), (R) Disturbed at telophase (MW+0.01%$ZnSO_4$), (S) Laggard at telophase (NW+0.01%$ZnSO_4$) and (T) Late separation at telophase (MW+0.005%$ZnSO_4$) at X= 1000.

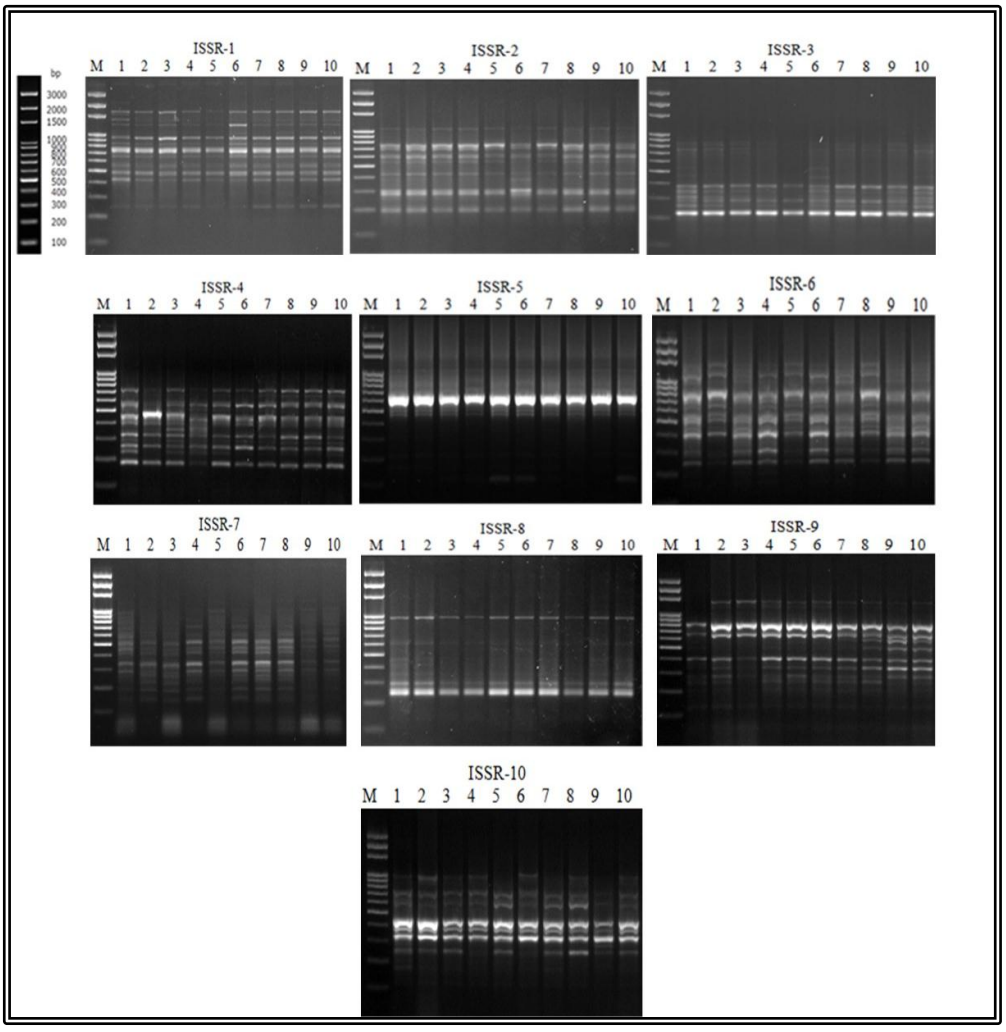

Plate (2): *Vicia faba* DNA amplification profile patterns in response to irrigation with non-magnetized and magnetized irrigation water under $ZnSO_4$ or $MnSO_4$ fertilization. generated from ten ISSR primers.1;Magnetized irrigation water (MW), 2; MW+ 0.005% $MnSO_4$, 3; MW+0.005% $ZnSO_4$, 4; MW+0.01% $MnSO_4$, 5;MW+0.01% $ZnSO_4$, 6; Control; non-magnetized irrigation water; normal water (NW), 7;NW+0.005% $MnSO_4$, 8; NW+0.005% $ZnSO_4$, 9;NW+0.01% $MnSO_4$ and 10;NW+0.01% $ZnSO_4$.