Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: Sobieh, S.S., & Eid Eliwa, N. (2024). Physiological, genetical changes and *cdc2* gene expression for osmotic stressed *Vicia faba* reveal the alleviation effect of gamma radiation and putrescine. *Caryologia* 77(1): 39-56. doi: 10.36253/caryologia-2338

Received: October 19, 2023

Accepted: May 18, 2024

Published: July 8, 2024

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

ORCID
SSS: 0000-0002-1286-7352

Physiological, genetical changes and *cdc2* gene expression for osmotic stressed *Vicia faba* reveal the alleviation effect of gamma radiation and putrescine

Shaimaa S. Sobieh[1,*], Noha Eid Eliwa[2]

[1] *Botany Department, Faculty of Women for Arts, Science and Education, Ain Shams University, Cairo, Egypt*
[2] *Natural Products Research Dept., National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority, Egypt*
*Corresponding author. Email: shimaa.sobieh@women.asu.edu.eg

Abstract. Climate change caused increasing in soil salinity worldwide. Therefore, it is critical to enhance the capability of plant to tolerate salinity stress. For this goal, putrescine and irradiation by gamma radiation were used to improve the salt tolerance of *Vicia faba* (the most important human crop). The results indicated depression in mitotic division and all growth parameters associated with the induction of micronucleus (MN) when salinity increased to 100mM, while there was increase in mitotic aberrations. NaCl decreased total soluble sugar, while total N%, total free amino acid, proline and protein contents showed slight increase with increasing salinity stress. Putrescine and gamma radiation mitigated the effect of salinity on cell division and growth parameters. Salt stress decreased the expression of gene encoding cyclin-dependent kinase 2 (*cdc2*). Both putrescine and gamma radiation increased *cdc2* expression. The genetic diversity has been detected among control and treated *V. faba* using ISSR and SCoT markers. Ten primers had successfully generated 129 reproducible polymorphic amplicons that were suitable for studying the genetic diversity between studied genotypes. ISSR markers provided more discriminating data and were more informative than SCoT markers. Besides, cluster analysis using UPGMA and PAC successfully explained the genetic diversity within studied genotypes. These findings emphasize the efficiency of putrescine and gamma radiation for alleviating the negative impact of salt stress. Moreover, prove the importance of assessing mitotic activity, chromosomes behavior, physiological parameters, the expression level of *cdc2* and molecular diversity in *V. faba* under stress to improve the salt tolerance of it.

Keywords: *Vicia faba*, salt stress, putrescine, gamma radiation, growth parameters, cell division, mitotic aberrations, micronucleus, *cdc2* expression, ISSR& SCoT.

INTRODUCTION

The significant threats in the world today are caused by climate change. In the twenty-first century, salinity is one of the key issues brought on by cli-

mate change and one of the greatest risks to plants and crop yields globally, its negative impacts are increased under the severe changes in climate, particularly in arid and semiarid regions. According to recent statistics, salt stress has abused more than 45 million hectares of irrigated land worldwide, and this number is always rising (Isayenkov and Maathuis 2019). Additionally, salt stress generates a significant amount of reactive oxygen species, which damage some molecules such as lipids, proteins, and DNA, cause breakdown of cell membrane systems and terminate some enzymatic processes (Demiral and Türkan 2005). Plants use several mechanisms to manage salt damage and regulate cellular homeostasis and growth to overcome this problem. According to Isayenkov and Maathuis (2019), salt tolerance frequently involves the activation of cell signaling pathways that result in the production of antioxidant enzymes, and osmo-protective metabolites such amino acids and carbohydrates.

Vicia faba L. (2n = 12) or faba bean is one of the most important leguminous crops produced worldwide. Faba bean is the fourth most significant leguminous plant in the world (FAOSTAT 2018). Approximately 2.56 million hectares of land are harvested each year to yield 4.56 million tons of dry *V. faba* grains (FAOSTAT 2022). Eighty percent of the dried faba bean grains are produced in Asia and Africa (FAOSTAT 2022). In the Middle East, North Africa, the Mediterranean region, the Nile Valley, and Ethiopia, *V. faba* is regarded as a crucial food crop for human nutrition and cattle feed because mature *Vicia faba* seeds are a excellent source of protein, carbohydrates, cellulose, vitamin C, and minerals (Qahtan et al. 2021).

Polyamines (PAs) play a crucial role during environmental stress. They are low molecular weight growth regulators and present as aliphatic amines. According to FAO Statistics (2021) polyamines are multifunctional polycationic plant growth regulators that have an impact on several physiological, metabolic, and developmental processes. Wisniewski et al. (2014) reported that PAs could control DNA replication, cell division, seed germination, and development. Putrescine, spermidine, and spermine are typical polyamines found in plants (Gupta et al. 2013). One of the main polyamines, putrescine, is crucial for plant growth and differentiation as well as stress responses (Sequera-Mutiozabal et al. 2017). The positive charges of PAs can help stabilize cell membranes under environmental stress by attaching to the negatively charged phospholipids and proteins that make up the membranes (Kuznetsov and Shevyakova 2007), Additionally, PAs enhance antioxidant systems, regulate some gene expression (Matkovics et al. 1993) and cause scavenging of free radicals (Velikova et al. 2000).

Useful mutations are the modifications of the genotypic structure to improve the species' variability and help them to respond better to different range of stresses (Spencer et al. 2018). Physical mutagenic agents including ultraviolet light, protons, neutrons, alpha and beta particles, and ionizing radiation (X-rays and gamma rays) can cause useful mutations. Gamma radiation can directly cause physical, biological, and chemical changes in cells (Ludovici et al. 2020). It can indirectly affect free radical production and directly trigger specific alterations in the genome (Caplin and Willey 2018).

Molecular markers are considered an effective method for analyzing and identifying genetic variability within and/or between genotypes. The degree of polymorphism affects how discriminatory power, which in turn determine how markers are categorized. To ascertain the genetic diversity among species, cultivars, and treatments, polymorphism is used. In recent decades, research on the genetic diversity of genotypes has been used in numbers of disciplines, including genetics, ecology, botany, biology, and others (Chesnokov and Artemyeva 2015). Inter-Simple Sequence Repeat (ISSR) markers are Polymerase Chain Reaction PCR (PCR) approaches for measuring the genetic diversity in plants (Ziêtkiewicz et al. 1994). These markers amplify the inter-SSR sequences of varied sizes. Start codon targeted (SCoT) was generated to start a trend away from random DNA markers and toward gene-targeted markers based on the short on served region flanking the ATG of plant genes. Since the SCoT marker is frequently reliable, it is understood that factors other than annealing temperature and primer length can affect repeatability (Collard and Mackill 2009). Since no specific knowledge of the genome sequence was required for the SCoT markers design, it was possible to apply it to plants without genome references (Xiong et al. 2011). In *V. faba* and many other plant species, both markers are frequently used to assess genetic diversity (Albrifcany et al. 2022).

The most vulnerable stages of growth, germination, and seedling development are partially affected by cell cycle suppression. Salinity reduces the cell division frequency. It also causes defects in the chromosomes structure and caused induction of micronuclei (Souguir et al. 2018). Micronucleus Test (MN Test) is used to evaluate the genotoxic potential of substances.

Biotic and abiotic stress cause cell cycle inhibition which in turn causes harmful effect on plant growth. Although the molecular interactions that link the cell cycle machinery to perception of stress are not fully understood, recent studies indicated the involvement of cyclin dependent kinases (cdcs) in the plant response

machinery (Kitsios and Doonan 2011). Cell divisions in eukaryotic cells are controlled by a family of protein kinases, the cyclin-dependent kinases (cdcs). The activity of cdcs is regulated by cyclins, or to the cdc inhibitors. The activity and localization of different cdc complexes regulate many of the actions during the cell cycle (Nigg 1995). In plants and animals many cdcs are present and regulate the G_1/S and the G_2/M transitions (Magyar et al. 1997). Different plant species contain one or two cdc gene, which contains a fully conserved PSTAIRE sequence motif (Hirt and Heberle-Bors 1994). Mutant cdc gene in *Arabidopsis (cdc2a-At)* arrest cell cycle at G_1/S and/or G_2/M points, demonstrating its ability to control both checkpoints (Hemerly et al. 1995).

From the previous, plants must develop mechanisms to adapt to the variable environment conditions. Therefore, this study was conducted to declare the role of gamma radiation and putrescine in the alleviation of the harmful effect of salinity on *V. faba* plants by tracking the physiological, genetical, and molecular changes and affirm the involvement of *cdc2* in the signaling control of stress tolerance.

MATERIALS AND METHODS

Seeds of bean (*Vicia faba*) (cv. Giza 2) were obtained from the Agriculture Research Center, Ministry of Agriculture, Giza, Egypt.

Radiation treatments

Dry seeds irradiation was performed at National Center for Radiation Research and Technology (NCRRT). Cairo, Egypt. By using (Co60) as source of γ-rays at dose levels 10 Gy with dose rate of 0.623 rad/sec.

Putrescine treatments

Putrescine solutions were prepared at levels of the solutions (5 mMol) were sprayed on leaves of the seedlings after the appearance of first leave and repeated every seven days until harvest after 50 days.

Experimental design

A pot experiment was conducted under field conditions at a wire house at NCRRT, Cairo, Egypt. The seeds were irradiated with 10 Gy radiation dose and then three seeds were grown in each plastic pot, (40 × 35 cm, height × diameter), containing equal quantities of sandy loam soil, commercial peat and clay. The seeds were left to grow inside the greenhouse under natural lighting. A full concentration of Hoagland's nutrient solution was used to irrigate pots (Hoagland and Arnon, 1950).

Randomized complete block design with three replications was used for the experimental design. Salt solution (250 ml) was applied at 15 days after seedling emergence twice per weak. Foliar spray of putrescine was applied after 2day from salinity treatments. It repeated twice a week.

Either:

1) Full strength Hoagland's solution (control).
2) Full strength Hoagland's solution +50 mMNaCl.
3) Full strength Hoagland's solution + 100 mM NaCl.
4) Full strength Hoagland's solution + 5 mM putrescine (foliar spray).
5) Full strength Hoagland's solution + 5 mM putrescine (foliar spray) + 50 mM NaCl.
6) Full strength Hoagland's solution + 5 m M putrescine (foliar spray) + 100 mM NaCl.
7) Full strength Hoagland's solution + 10 Gy.
8) Full strength Hoagland's solution + 10 Gy + 50 mM NaCl.
9) Full strength Hoagland's solution + 10 Gy +100 mM NaCl.

After 50 days seven growth parameters were measured.

Physiological analysis

Extraction of plant extract. Fresh grinded leaves (0.1g) were accurately weighted then extracted by in 80 % aqueous ethanol for 6 h (AOAC 1984). The extract was filtered and completed to 50 ml in a measuring flask with ethanol 80% for further experiments.

Determination of total soluble sugar. Total soluble sugar of the fresh leaves was quantified using a modified phenol-sulfuric acid assay (Zhang 1993).

Determination of total free amino acid. Total free amino acid of fresh leaves was measured according to Rosen (1957).

Determination of proline content. Proline content was evaluated using the method of Bates et al. (1973).

Determination of Nitrogen (N) %. The leaf N concentration (LNC) was assessed according to the method Wild et al. (1985).

Determination of total soluble protein content. Total soluble proteins were estimated according to Lowery et al. (1951).

Genetical analysis

Quantitative analysis of mitotic division and chromosomal aberrations. To get rid of the soil remnants, the roots of each sample group were taken and rinsed under running water. Root tips (3 to 4 cm in length) were stored in 70% ethyl alcohol at 4°C until usage after being treated in Carnoy's solution for 24 hours. After being hydrolyzed in 1N HCl, the roots were stained for one hour with basic Fuchsin stain. The root tips that were darkly discolored were crushed in a drop of 45% acetic acid. For each treatment group and control group, 1000 cells on 10 slides were used to score and compute the percentage of mitotic division (MI) and frequency of mitotic abnormalities (Xavier et al., 2023).

Quantitative analysis of micronucleus. Cell with micronuclei (MN) reveals the genotoxicity effect of treatment. Cells with intact cell wall that contains MN alongside the nucleus were scored. The frequency of MN was scored in 5000 cells and expressed as the percentage of the number of cells with micronuclei per 1000 cells per slide (Xavier et al. 2023). MN was scored to prove genotoxic effect of salinity stress and the mitigating effect of both putrescine and gamma radiation.

*Cyclin dependent kinase 2 (*cdc2*) gene expression quantification using qReal-Time PCR.* Pure RNA was extracted from *V. faba* root for all experiment groups using total RNA Purification Kit (Qiagen, Rneasy Kit) following the manufacturer protocol. RNA input for all samples was adjusted to 1000 µg and cDNA was synthesized using Reverse Transcription kit (Thermo Fisher Scientific, RevertAid RT Reverse Transcription Kit). Specific primers for *cdc2* gene F 5'ACTCTCATAGGGTTCTCC3' (Tm 54°C); R 5' CTCGGTACCAGAGAGTAA3' (Tm 54°C) were used. The amplification protocol was as follow, 1 cycle at 95°C for 10 min followed by 40 twostep cycles 95 °C for 15 sec, and 60 °C for 60 sec. Results were normalized by housekeeping *actin* gene using the following primer F 5' CTTCCCAAGATAGTAGGAG3' (Tm 55°C) and R 5' CTTAGACTGTGCCTCATC3' (Tm 54°C). The expression level of genes was calculated in relation to $2^{-\Delta\Delta ct}$ according to Livak and Schmittgen (2001).

Molecular fingerprint using ISSR-PCR and SCoT-PCR. DNA was isolated from *V. faba* fresh leaves according to cetyltrimethylammonium bromide (CTAB) method. The quality of DNA was checked at 260/280 nm and on 1% agarose gel. DNA was used for ISSR and SCoT techniques according to Ziêtkiewicz et al. (1994) and Collard and Mackill (2009) respectively. Ten primers (Table1) five for each technique were screened against each DNA sample to analyze molecular diversity among all treated and control groups. Polymerase chain reaction (PCR) was accomplished in an automated thermal cycle (model Techno 512, Stafford, UK system) using Dream Taq green master Mix (Thermo-Scientific). The PCR reaction volume was 25 µl including 2.5 µl of dNTPs (2.5 mM), 1.5 µl of $MgCl_2$ (25 mM), 2.5 µl of 10× buffer, 2.0 µl of primer (2.5 µM), 2.0 µl of template DNA (50 ng µl/1), 0.3 µl of Taq polymerase (5 U µl/µ1) and 14.7 µl of sterile ddH_2O. PCR reaction for both fingerprints was 1 cycle at 95°C for 5 min followed by 40 cycles of 1 min at 95°C, 1 min at annealing temperature 56°C and 2 min at 72°C, followed by 1 cycle at 72°C for 10 min. PCR products were resolved on a 1.5% agarose gel with 100 – 3000bp DNA Ladder, GeneDirex, Inc (100bp DNA Ladder H3 RTU Ready-to-Use).

Table 1. ISSR and SCoT primers nucleotide sequences.

Analysis	Primer	Nucleotide sequence of the used primer 5' to 3'
ISSR	UBC810	GTGTGTGTGTGTGTGTCA
	UBC818	CAC ACA CAC ACA CAC AG
	UBC849	GAGAGAGAGAGAGAGAT
	UBC-823	TCTCTCTCTCTCTCTCC
	UBC-817	CACACACACACACACAA
SCoT	SCoT -31	CCATGGCTACCACCGCCT
	SCoT -34	ACCATGGCTACCACCGCA
	SCoT -13	ACGACATGGCGACCATCG
	SCoT -14	ACGACATGGCGACCACGC
	SCoT -52	ACAATGGCTACCACTGCA

Data and cluster analyses

Binary data matrix for the bands (0 for absent 1 for present bands) were analyzed. Polymorphism percentage, total number of amplicons (TNA), total polymorphic amplicons (TPA), monomorphic amplicons (MA) and unique amplicon (UA) were scored. Four parameters, polymorphic information content (PIC), effective multiplex ratio (EMR), marker index, and resolving power (RP) were examined the efficiency of ISSR and SCoT markers as well as the genetic difference among genotypes under study. PIC, Rp, EMR and MI were calculated according Venkatesan et al (2021).

The genetic similarity coefficient was used to construct the phylogenetic tree. Genetic similarity coefficient between two genotypes and principal component analysis (PCA) was achevied using the Paleontological Statistics Software Package for Education and Data Analysis (PAST) version 4.03 (Hammer et al. 2001).

Statistical analysis of data

Statistical analysis was carried out according to Snedecor and Cochran (1980). The individual comparisons between the obtained data were carried using M-STAT computer software program at $p \leq 0.05$.

RESULTS

Physiological analysis

Figure 1A declared the effect of salinity, putrescine, and gamma radiation on the stem length of *V. faba* plants. Increasing NaCl concentration from 50 mM to 100 mM decreased stem length from 33.71 to 31.11 cm compared to the control (36.50 cm). Foliar spraying with putrescine increased stem length to (37.72 cm) compared to the control plants. It is obvious that gamma radiation gave the longest stem length (48.68 cm) above all treatments used especially with 10Gy + 50 mM NaCl treated plants. The same trend was observed with the root length i.e. salinity reduced root length to 2.4 cm with 100m M NaCl treated plants. Putrescine and radiation alleviated the harmful effect of salinity as they gave 4 cm with 5m M treated plants and 6.5 cm with 10 Gy + 50 m M NaCl treated plants. In Fig. 1C number of leaves decreased to 12 leaves with increasing salinity concentration to 100 mM. While putrescine and radiation had a non-significant effect on the number of leaves. On the other hand, radiation increased shoot fresh and dry weight above the control or putrescine treatments (Fig. 1 D, E). The maximum shoot fresh and dry weight was 36.8 g and 7.1 g in 10 Gy + 50 Mm NaCl treated plants respectively. The same effect of radiation appeared in root fresh weight and dry weight (Fig. 1 F, G). Figure 2A showed the effect of salinity, putrescine, and radiation on total soluble sugars (TSS) of *V. faba* plants. It is cleared that salinity reduced TSS% from 1.89% to 1.52% as salinity increased from zero to 100 Mm NaCl. Foliar spraying of putrescine has a positive effect in TSS % i.e increased to 2.64%with 5 mM put + 50 mM NaCl treated plants. The highest TSS% was observed with radiation in all treatments used especially with 10 Gy+50 mM NaCl treated plants (2.508%). Fig. 2B cleared the influence of salinity on total free amino acid. The maximum increment (0.1221g/ 100 g) reached by 100 mM NaCl treated plants. Radiation also increased the total amino acid with 10 Gy + 100 mM NaCl treated plants (0.2089 g/ 100 g).

Salinity showed positive effect on proline contents of leaves, the content of proline increased as salinity increased, it reached 7.004 mg/100g in plants treated with 100 mM NaCL compared to zero NaCl 2.8600 mg/100g. This increment is still observed with putrescine treatments. There was a decrease in proline content (7.5840) by 10Gy +100mM NaCl than with 10 Gy treated plants (9.278). Concerning N% contents in leaves of *V. faba* plant, N% increased in 50 mM NaCl treated plants and 10 Gy + 50 m M NaCl treated plants to 0.407% and 0.438% respectively. Fig. 2E illustrates that protein content increased with increasing salinity concentration, the maximum increase observed with 50 mM NaCl treated plants. Putrescine and radiation increased protein contents of *V. faba* leaves. Treatment with both 50 mM NaCl+ 5mM putrescine and 50 mM + 10 Gy showed the maximum value of protein contents (1.56 and 2.74 respectively).

Genetical analysis

Quantitative analysis of mitotic division and chromosomal aberrations

The mitotic index (MI) is a very important indicator for the rate of mitotic division. Salinity stress by 50mM and 100mM NaCl caused significant decrease in MI of *V. faba* meristematic root cells reached 5.58±0.14 and 4.98±0.20 respectively as compared with control (Fig. 3A). Alleviation effect of both putrescine and gamma radiation (at low dose 10 Gy) was obvious specifically with concentration of 50mM NaCl. Foliar application of salt stressed *V. faba* with 5mM putrescine caused highly significant increase in MI as compared with salinity stressed *V. faba,* but MI was still less than control. While gamma radiation mitigated the harmful effect of NaCl and enhanced the mitotic division and caused highly significant increase in MI as compared with control and salt stressed plants. The maximum MI was 7.84±0.21, which has attained after treatment with 10 Gy + 50mMNaCl.

The changes in mitotic index frequencies were associated with significantly increment in mitotic aberrations percentage under salinity stress. The frequency of mitotic abnormality after treatment with 50mM NaCl was 20.64±0.75, while the ratio of mitotic abnormality after treatment with 100mM NaCl was 31.21±0.26. As well as all treatments with gamma radiation and putrescine significantly increased the ratio of mitotic abnormality but less than those of salt stress treatment (Fig. 3B). Only one exception, treatment with 10 Gy with 100mM NaCl caused higher frequency of mitotic aberration reached 32.44±0.39 (the maximum ratio), while the minimum ratio was produced after the foliar application by putrescine only.

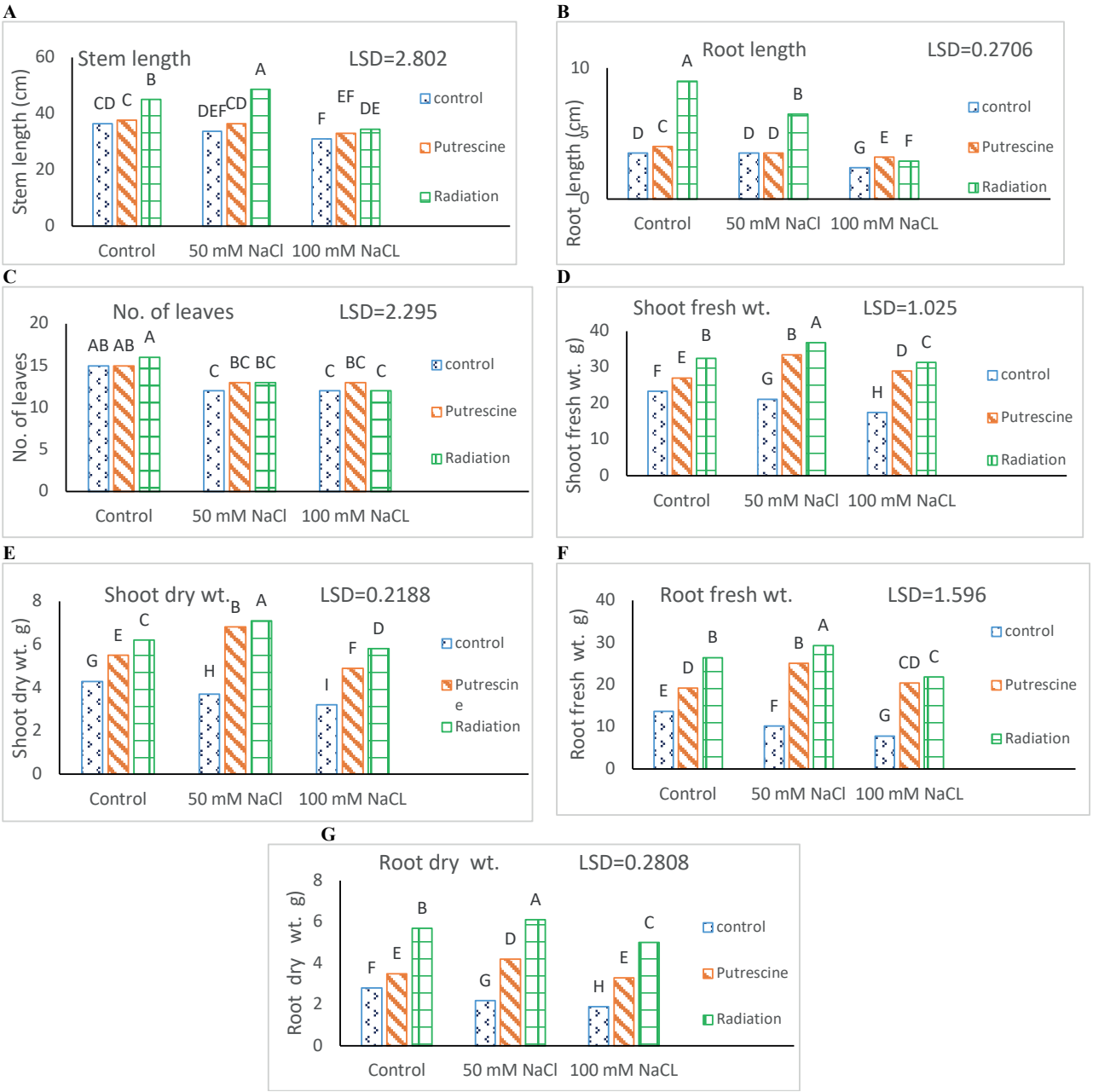


Figure 1. Effect of salinity, putrecine and gamma radiation on growth of bean plant (Non identical letters indicate significant difference).

Additionally, the reduction of MI after salinity stress was accompanied with significant increase in prophase index (Fig. 3C). The maximum prophase accumulation was 48.16±0.14 achieved by treatment with 100mMNaCl. Concerning treatment with putrescine, phase indices showed non-significant accumulation of prophase and displayed normal contribution of phases as compared with control. While treatment with gamma radiation reflected general accumulation in metaphase. The maximum metaphase accumulation was 42.97±0.23 achieved by treatment with 10 Gy.

Several types of chromosomal aberrations were observed after all treatments as compared with control. There were three main classes of chromosomal abnormalities (Fig. 4i-xii). The first class was chromosome stickiness, the second class was clastogenic aberrations including breakage, bridge, and ring chromosome, and finally chromosome disturbance including disturbance,

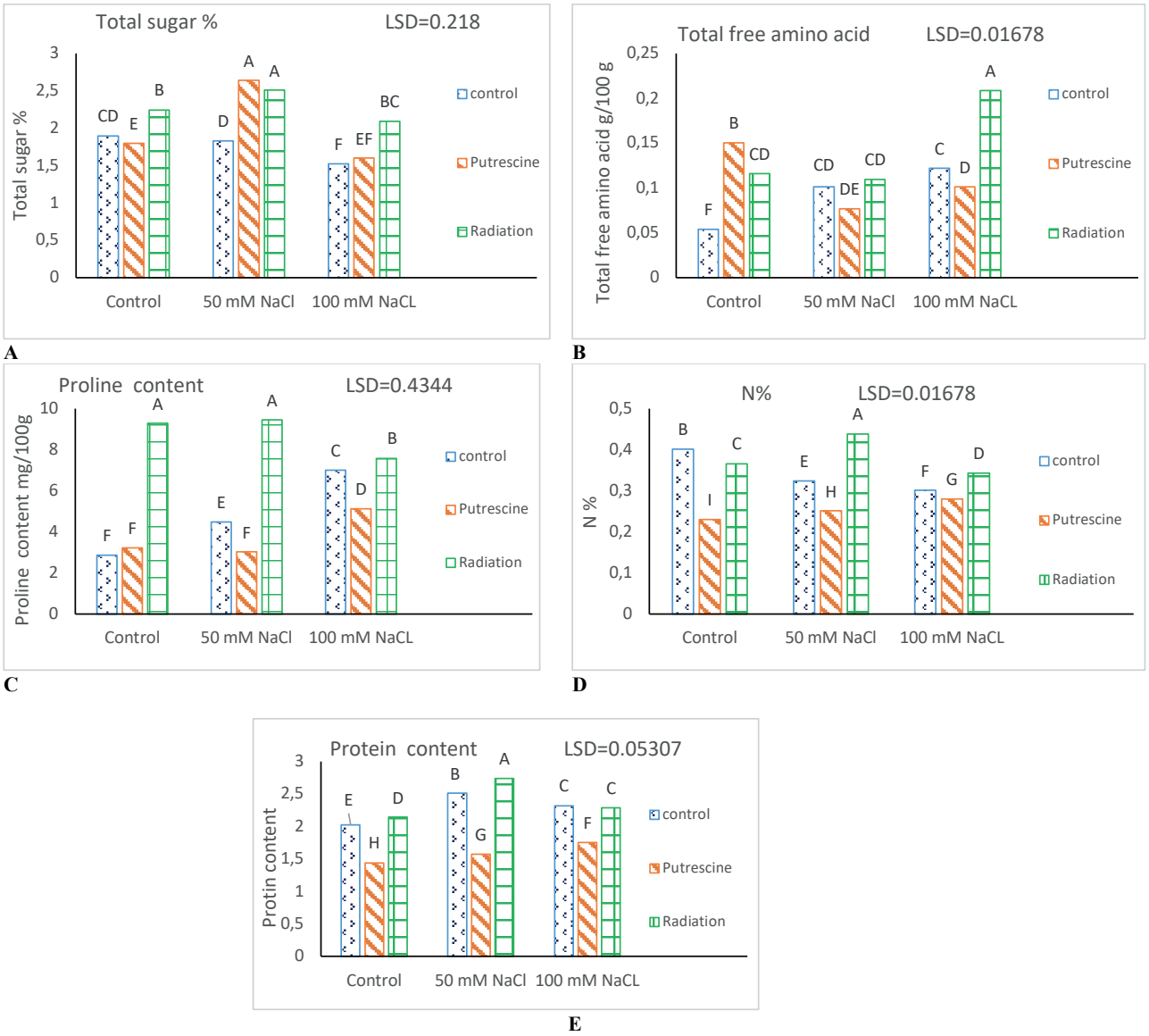


Figure 2. Effect of salinity, putrescine and gamma radiation on physiological contents of bean plants (Non identical letters indicate significant difference).

multipolarity, diagonal. Chromosome stickiness was more pronounced by salt stress. Although the alleviation effect of gamma radiation on MI, clastogenic aberration was more obvious after gamma radiation treatment.

Quantitative analysis of micronucleus

Meristematic cells of *V. faba* under salt stress and gamma radiation showed significant production of MN. The formation of MN was significant at level $p \leq 0.001$. Micronucleus was observed in interphase cells and in several phases (Fig. 4 xiii-xvi). The percentage of MN formation was concentration dependent. Treatment with 10 Gy+100mM NaCl exhibited the highest frequency of MN (18.46±0.11). Foliar application of putrescine alleviated the toxic effect of NaCl and produced the least MN percentage 3.47±0.26 after treatment with 5mM putrescine (Fig. 5).

Cyclin dependent kinase 2 (*cdc2*) gene expression quantification using qReal-Time PCR

Significant changes were recognized in the expression level of *cdc2* gene in *V. faba* root. Marked sig-

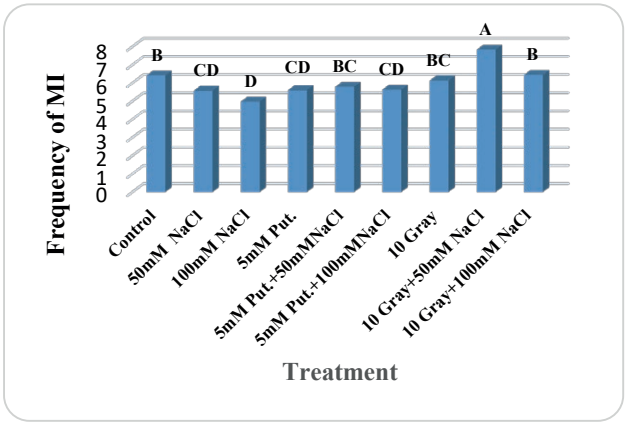


Figure 3A. Change in mitotic index of osmotic stressed *V. faba* treated by gamma radiation and putrescine. Non identical letters indicate significant difference at $p \leq 0.001$ LSD= 0.6671.

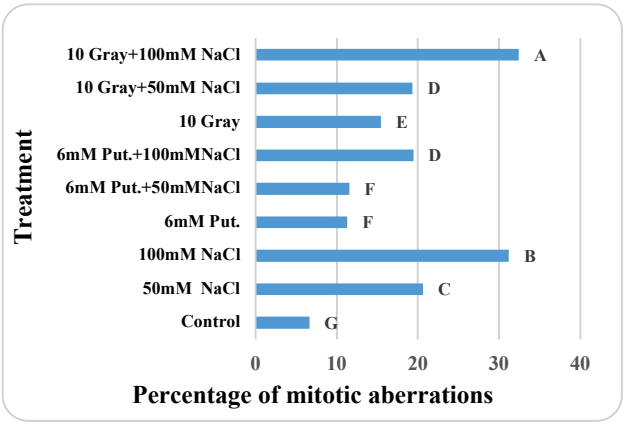
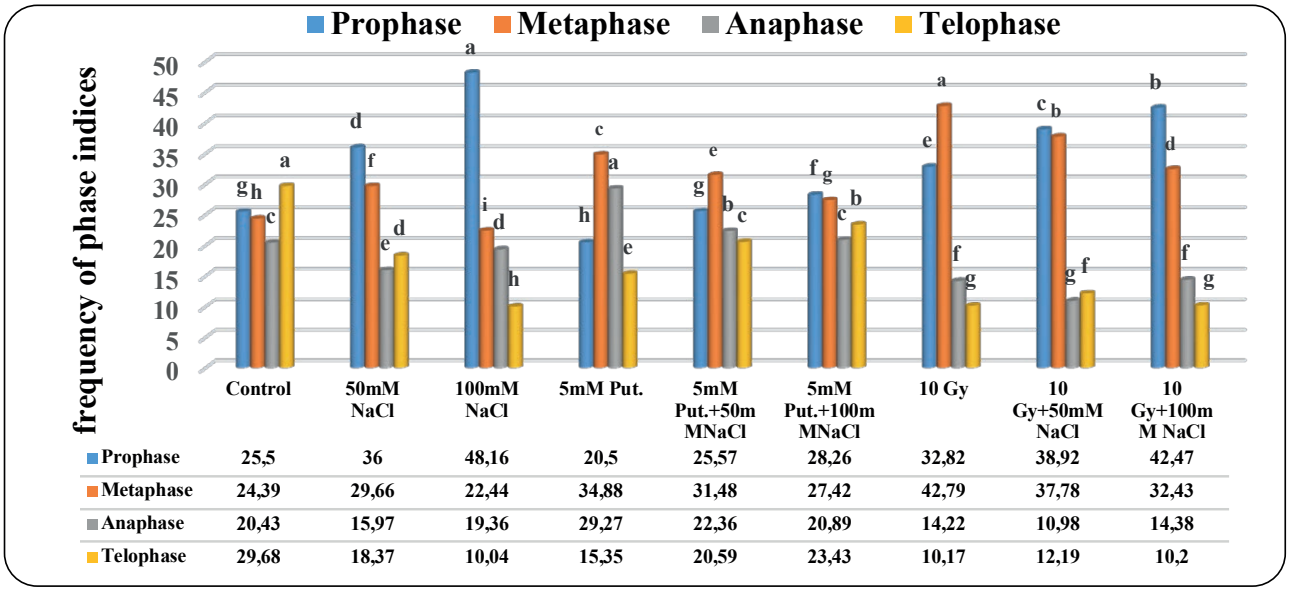


Figure 3B. Frequency of mitotic abnormalities of osmotic stressed V. faba treated by gamma radiation and putrescine. Non identical letters indicate significant difference at $p \leq 0.001$ LSD=0.7180.



	Control	50mM NaCl	100mM NaCl	5mM Put.	5mM Put.+50mMNaCl	5mM Put.+100mMNaCl	10 Gy	10 Gy+50mM NaCl	10 Gy+100mM NaCl
Prophase	25,5	36	48,16	20,5	25,57	28,26	32,82	38,92	42,47
Metaphase	24,39	29,66	22,44	34,88	31,48	27,42	42,79	37,78	32,43
Anaphase	20,43	15,97	19,36	29,27	22,36	20,89	14,22	10,98	14,38
Telophase	29,68	18,37	10,04	15,35	20,59	23,43	10,17	12,19	10,2

Figure 3C. Change in mitotic phases of osmotic stressed *V. faba* treated by putrescine and gamma radiation. Non identical letters for each phase indicate significant difference at $p \leq 0.001$.

nificant down regulation was recorded after treatment with 50 and100mM NaCl (Fig. 6). Conversely, marked upregulation in *cdc2* expression level was noticed after the combination between NaCl and putrescine or with10Gy radiation. Exposure of *V. faba* to 5mM putrescine+50mM NaCl and 10Gy+ 50mM NaCl increased the cdc2 expression level 2-folds and 4-fold respectively. In contrast, both 5mMputrescine and 10Gy with 100mM NaCl caused downregulation of *cdc2* gene expression level (0.965 and 1.04 respectively), which were more than those of salt stressed only.

Molecular fingerprint using ISSR-PCR and SCoT-PCR)

ISSR and SCoT markers were used to investigate the molecular variations in osmotic stressed *V. faba* treated with putrescine as well as gamma radiation. Ten ISSR and SCoT-primers (5 for each analysis) succeeded in amplifying 126 amplicons with a range between 2840 to 135 bp (Fig.7). Eighty amplicons of 126 were reproducible polymorphic amplicons, 25 amplicons of 80 were unique amplicons, while 46 were reproducible monomorphic amplicons. The maximum total number of

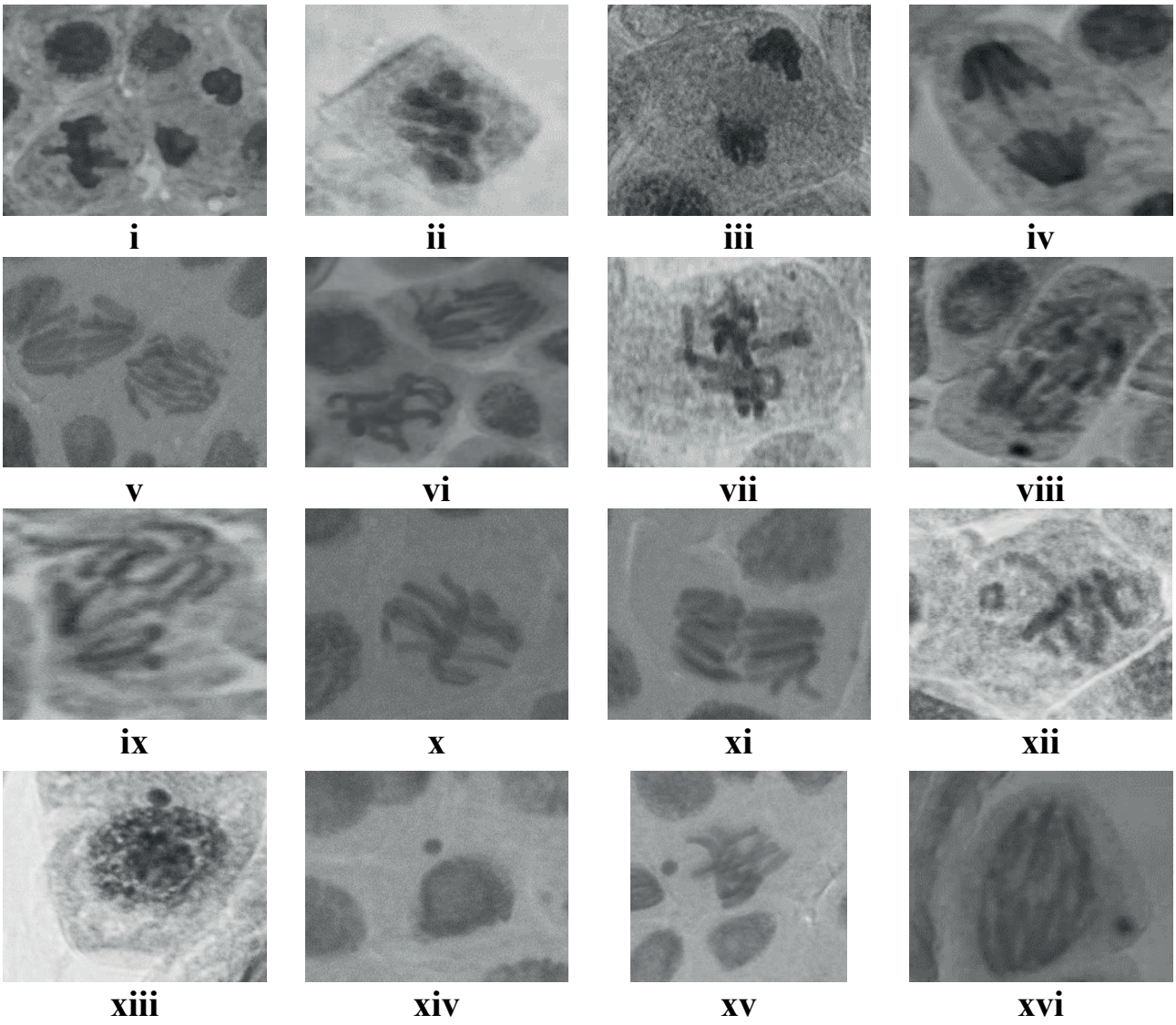


Figure 4. Types of chromosomal aberrations formed in *V. faba* by different treatments. i: Sticky metaphase and anaphase, ii: Sticky metaphase, iii: Sticky anaphase, iv: Sticky anaphase with bridge, v: Disturbance anaphase with multibridge, vi: Disturbance anaphase with laggard, vii: Disturbance metaphase, viii: C-anaphase, ix: Multipolarity, x: Metaphase with laggard, xi: Anaphase with laggard, xii: Metaphase with ring chromosome, xiii-xiv: Interphase with micronucleus, xv: Metaphase with micronucleus, xvi: Anaphase with micronucleus.

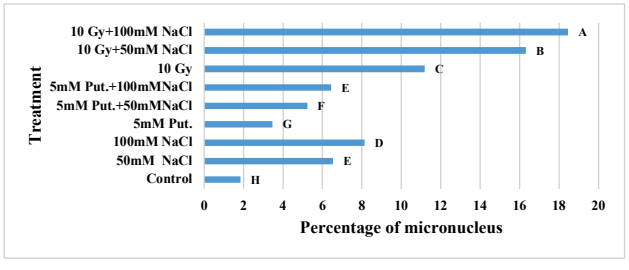


Figure 5. Frequency of micronucleus in osmotic stressed *V. faba* treated by putrescine and gamma radiation. Non identical letters indicate significant difference at $p \leq 0.001$ LSD=0.1914.

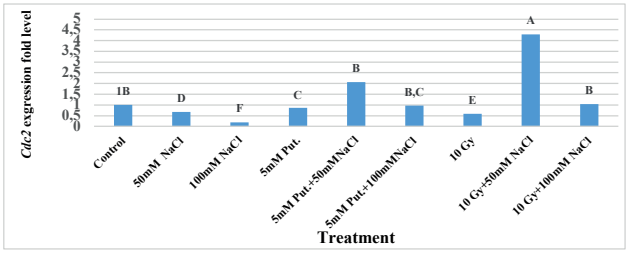


Figure 6. Change in *cdc2* gene expression level of osmotic stressed *V. faba* treated by putrescine and gamma radiation. Non identical letters indicate significant difference at $p \leq 0.001$ LSD=0.9360.

Table 2. ISSR-PCR and SCoT-PCR amplicons from osmotic stressed *V. faba* treated by putrescine and gamma radiation.

Analysis	Primer	Range of amplicon molecular size (bp)	TNA	MN	TPA	UA	Polymorphism %	PIC	RP	EMR	Marker index
	UBC810	1480-170	9	3	6	2	66.66%	0.61	2.64	4	2.5
	UBC818	2050-188	10	4	6	2	60.00%	0.10	4.22	3.6	0.36
	UBC849	1600-155	10	5	5	3	50.00%	0.80	1.82	2.5	2
ISSR	UBC-823	1960-160	14	6	8	4	57.14%	0.10	4.70	4.9	0.45
	UBC-817	2250-275	13	5	8	4	61.53%	0.64	2.96	4.9	3.15
		Total	56	23	33	15		2.25	16.34	19.9	8.46
		Mean	11.2	4.6	6.6	3	59.07%	0.45	2.27	3.98	1.69
	SCoT -31	2840-135	17	8	9	0	52.94	0.16	6.16	4.76	0.76
	SCoT -34	2050-233	18	3	15	0	83.33	0.20	10.55	12.5	2.5
	SCoT -13	2090-320	13	6	7	4	53.84	0.65	2.72	3.77	2.45
SCoT	SCoT -14	1935-310	13	3	10	3	76.92	0.15	4.68	8.10	1.22
	SCoT -52	2100-240	9	3	6	3	66.66	0.09	5.14	7.69	0.69
		Total	70	23	47	10		1.25	29.25	36.82	7.62
		Mean	14	4.6	9.4	2	66.74	0.25	5.85	7.36	1.52

amplicons was 18 amplicons for primer SCoT-34 with 83.33 % percentage of polymorphism, while the minimum number of amplicons was 9 produced by both primers UBC810 and SCoT-52 with 66.6% polymorphism for the two primers. The minimum percentage of polymorphism was 50% achieved by UBC849 primer (Table 2).

ISSR and SCoT markers performance

The polymorphism information contents (PIC), or heterozygosity index displays the capability of each marker for revealing the frequency of polymorphism or genetic diversity between the different genotypes. It was calculated for each locus depending on the number of alleles and the allele frequency. Table 2 revealed that PIC values of ISSR markers was ranged from 0.10 to 0.80 with average of 0.45, which reflected intermediate level of polymorphisms for the used ISSR sites in the different treated genotypes of *V. faba*. Three primers (UBC810, UBC849 and UBC-817) were highly informative, PIC value > 0.5 and two primers (UBC818 and UBC-817) were low informative, PIC value < 0.25. While The PIC value of SCoT markers ranged from 0.09 to 0.65 with average of 0.25, which reflected low level of polymorphisms. Four primers were low informative, PIC value < 0.25 and only one primer SCoT -13 was highly informative, PIC value > 0.5 reached 0.65.

The resolving power (Rp) is the most effective parameter used to discriminate effectiveness of the primer to reveal genetic diversity level among individuals. Rp value of ISSR was ranged from 1.82 (UBC849) to 4.70 (UBC-823), Rp value of SCoT was ranged from 2.75(SCoT-13) to 10.55 (SCoT-34).

Table 2 shows that UBC-823 generated the highest number of amplicon (14) and highest RP value (4.70) with 57.14% polymorphism frequency, as well primers SCoT-34 targeted the highest number of amplicons (18) with a polymorphism percent of 83.33% and highest RP value (10.55). This demonstrates that these two primers (UBC-823 and SCoT-34) had high-edifying and discriminative abilities in determining genetic diversity.

EMR was calculated as the total number of polymorphic loci for each primer. Marker is more efficient when the EMR value was higher. SCoT-34 gave the highest EMR value (12.5) among all used ISSR and SCoT primers (Table 2).

Marker index is a statistical tool to describe the capability of each primer to discriminate polymorphic loci among the used genotypes. The maximum marker index value was 3.15 achieved by ISSR marker UBC-817 (Table 2).

Treatment specific ISSR and SCoT primer

Twenty positive marker amplicons were produced by the 10 loci of ISSR and SCoT. All ISSR primers were successful in generating 13 amplicons extending from 260 to 1600 pb, while 3 SCoT primers (SCoT-13, SCoT-14, and SCoT-52) were able to target 7 amplicons ranging from 515 to 2090 (Table 3). These primers could be considered as marker related specific treatments.

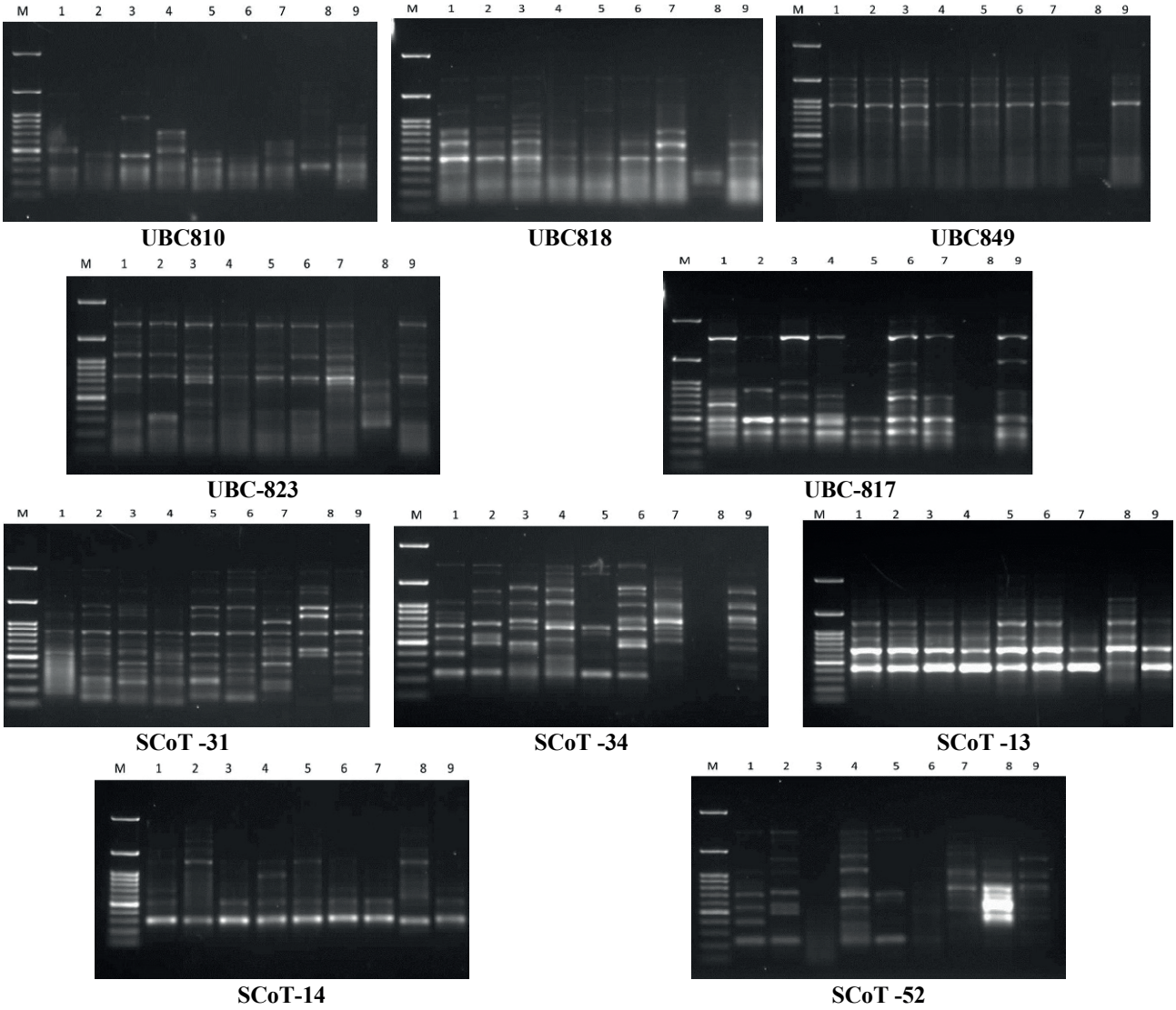


Figure 7. ISSR-PCR and SCoT-PCR profiles from osmotic stressed *V. faba* treated by putrescine and gamma radiation. M: Marker, 1:Control, 2:50mmNaCl, 3:100mM NaCl, 4:5mM Put., 5:5mM Put.+ 50mMNaCl, 6:5mM Put.+ 100mMNaCl, 7:10Gy, 8:10Gy+ 50mMNaCl, 9: 10Gy+ 100mMNaCl.

Cluster analysis

The dendrogram generated using UPGMA depends on ISSR and SCoT data revealed that all treatments and control form one cluster (cluster I), only radiated plants with 100NaCl formed a distinct cluster (cluster II) (Fig 8A). The first cluster separated into 2 subclusters. One of them included all other radiated treatments and 100 mM NaCl treatment. On the other subcluster control present in sub-subcluster alone and 50mM NaCl and all putrescine treatments are presents in other sub-subcluster. This pattern of clustering proved the genetic variability of 10Gy+100mMNaCl genotype from control and other treatments.

Principle component analysis (PCA) follows the same pattern of UPGMA dendrogram and demonstrated the genetic diversity of 10Gy+100mMNaCl genotype (Fig 8B).

DISCUSSION

Fabaceous plants are a good answer for expanding populations, improving human and animal food, and enhancing soil fertility (Castro-Guerrero et al. 2016).

Present results show that growth parameters such as stem length, root length, number of leaves, shoot fresh

Table 3. Specific positive amplicons of ISSR and SCoT loci for osmotic stressed *V. faba* treated by gamma radiation and putrescine.

Analysis	Treatment	Primer	Molecular size of positive marker amplicon (bp)
ISSR	50mM NaCl	UBC-823	1600
	100mM NaCl	UBC810	950
		UBC818	925
		UBC849	470
	5mMPut+100mMNaCl	UBC810	170
		UBC-817	1270,1055
	10Gy+50mM NaCl	UBC849	390,260
	10Gy+100mM NaCl	UBC810	641
		UBC818	188
		UBC-823	920
		UBC-817	1455
SCoT	5mMPutrescine	SCoT -52	1600
	10Gy	SCoT -52	1430
	10Gy+50mM NaCl	SCoT -13	2090,885,515
		SCoT -52	1294
	10Gy+100mM NaCl	SCoT -14	712

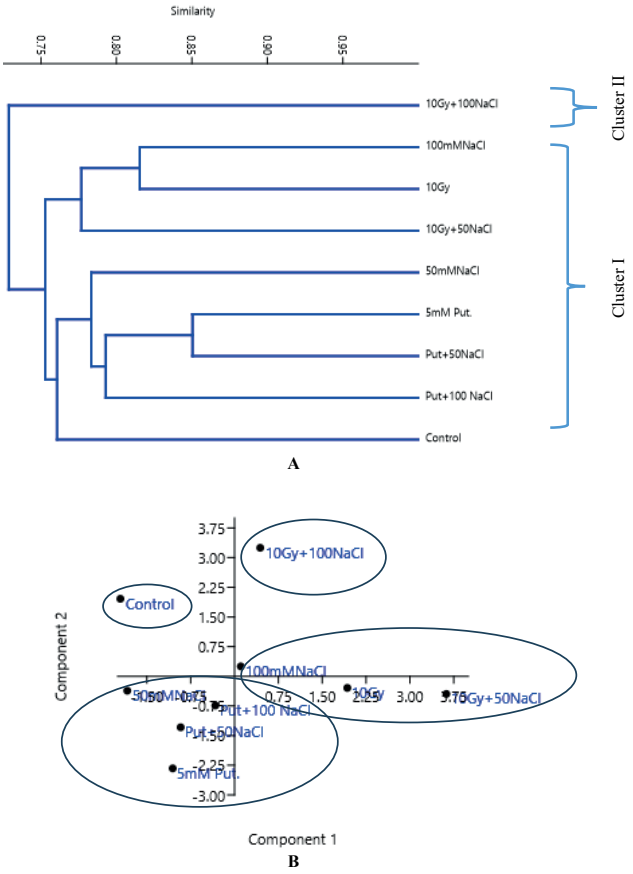


Figure 8. A) UPGMA dendrogram based on ISSR and SCoT markers data. B) Scatter plot of PCA using ISSR and SCoT markers.

weight, shoot dry weight, root fresh weight and root dry weight were decreased by salinity stress. These findings concur with those made on several crops by Sadak and Abd Elhamid (2013).

Putrescine application or gamma radiation treatment of plants were successful in accelerating development in saline environments. This is in line with the findings of Zhao and Qin (2004), who discovered that exogenous application of putrescine improved root development in barley seedlings under salt stress. Furthermore, Khosroshahi and Ashari (2008) demonstrated that strawberry, apricot, peach, and sweet cherry fruits' soluble solids content, weight loss, and titratable acidity were all improved by the foliar application of polyamines, such as putrescine. Many physiological processes and plant response to biotic and abiotic stresses are regulated by substances like putrescine in plants (Alcázar et al. 2010). As they activate or inhibit vegetative growth, blooming, fruiting (Harez and Abbas 2015).

The efficiency of metabolic processes in plants can be increased by polyamine compounds, including putrescine. Additionally, the physiological functions of plants are improved because of the roots' increased ability to absorb nutrients from the soil. These present favorable results of putrescine can be explained by these two factors (Youssef et al. 2007). Moreover, putrescine can accelerate growth, cell division and elongation (El-Bassiouny and Mostafa 2008) by boosting the levels of endogenous growth regulators including auxins, cytokinin, and gibberellins while lowering the efficiency and quantity of growth inhibitors like abscisic acid (ABA).

A form of electromagnetic wave called gamma irradiation has a good effect on molecular penetration and can ionize materials by igniting their electrons (UNSC 2000). Ionized cells might be distinguished by the disruption of host DNA that resulted in noticeable changes in hereditary features. Because DNA has the capacity to repair itself after damage due to the mechanism of proofreading, the perturbation of DNA might be transitory rather than permanent, which would have less severe effects (Ali et al. 2016). Low levels of gamma radiation may have physiological effects on plant growth due to an interaction between gamma rays and cell-based compounds that results in the production of free radicals. According to, the effect of low doses can be summed up by the acceleration of cell division, cell expansion, enzyme activity, tolerance against biotic or abiotic stress, and the increase in plant yield. The

resulted free radicals can alter the primary components of cells (El-Beltagi et al. 2011). According to Aly et al. (2019), utilizing gamma rays increased all growth metrics in eggplant. Low doses of gamma radiation increase plant height which may be related to the ability of radiation to stimulate cell division and other crucial processes that improve nucleic acid synthesis and activation of RNA or protein synthesis (Asare et al. 2017).

Regarding the impact of salinity on the chemical components of the *V. faba* plant, rising salinity level resulted in a decrease in total soluble sugar. Salinity may inhibit photosynthesis activity and/or increase consumption of carbohydrates, according to the reduction in leaf photosynthetic pigments (Hassanein et al., 2009). Additionally, one of the alterations brought by salt and drought stress in plants is proline buildup, which is frequently thought to be implicated in stress resistance processes. The stabilization of proteins and membranes against the denaturation impact of excessive concentrations of salts and other damaging solutes may play a role in maintaining the structure of macromolecules and other organelles (Munns, 2002). With rising salinity levels, salt stress caused declines in total nitrogen and increment in proline and free amino acids (Abdelhamid et al. 2013). These results are very similar to those found by Taie et al. (2013). The decrease in protein synthesis and/or the rise in its breakdown can be linked to the reduction in total nitrogen.

Strong osmo-protectants known as compatible osmolytes (a group of tiny molecules that includes polyamines, glycinebetaine and TSS) help to mitigate the negative consequences of osmotic stress. Putrescine foliar spraying treatment resulted in a significant increase in the percentage of total carbohydrates and nitrogen. This increase in growth may be attributable to this substance's ability to stimulate physiological processes that were improved vegetative growth (El-Bassiouny and Mostafa, 2008). Additionally, putrescine increased the levels of proline, soluble sugars, and amino acids in wheat plants (Hussein et al. 2023). Moreover, putrescine's protective action on wheat's Rubisco protein may be the cause of variance in protein expression in plants developing under water deficiency stress (Hassan et al., 2020). Also, radiation offers protection from the effects of salt stress.

One of the key defensive responses of plant cells to gamma irradiation stress is the development of defense systems (Jan et al., 2012). The increase in the content of soluble protein is one of the plant's defenses against gamma irradiation damages, Hanafy and Ageeb (2018) discovered that leaves produced from irradiated seedlings with lower doses of gamma rays have higher total protein and proline levels. Additionally, Afrin et al. (2019) discovered that onion bulbs with low gamma ray doses had the highest nitrogen content.

According to Jităreanu et al. (2013), the mitotic index is the significant biological parameter that indicates the frequency of cell division and meristem growth. The current findings showed that the negative influence of NaCl on mitotic division of tested *V. faba* meristematic, which was directly related to the salt concentration. This mitodepressive effect on MI was accompanied by an increase in chromosomal abnormalities and the induction of micronucleus rate (Souguir et al. 2022). Mitotic depression may be due to decrease in cyclin dependent kinases (cdcs) activity (Zhao et al. 2014), accumulation of cells at G_1 phase inhibiting DNA synthesis or arresting the cell in G_2, hindering the cell to enter M phase (Mahfouz and Rayan 2017). Foliar application of putrescine and irradiation with gamma radiation mitigated the harmful effect of NaCl causing significant increase in MI frequencies with low mitotic abnormalities percentage as demonstrated before. Previously treatment with putrescine showed enhancement in MI after 6h that supports that polyamine is essential constituents of the cell and implicates in cell growth and proliferation (Gömürgen et al. 2005). El-Azab et al. (2018) proved that exposure to low levels of gamma radiation may stimulate ROS at a very low rate, which can speed up the passage of the cell cycle from G0 to G1, activating plant cell cycle machinery.

The frequencies of the various division stages changed because of treatment with NaCl. Following salt stress, prophase frequency increased considerably, whereas gamma radiation exposure significantly increased cell accumulation during metaphase. Putrescine caused non-significant changes in frequencies of the different phases. These variations in the frequencies of mitosis phases show that NaCl and gamma radiation affects the relative length of each phase of division as compared with the control. The prophase accumulation proves the toxic strength of a treatment caused by delaying in breakdown of nuclear membrane or delaying in chromosome compression because of blocking dividing cells at Chfr point that inhibits prophase/metaphase transition (Sobieh et al. 2014). While the accumulation of dividing cells in metaphase may be due to spindle apparatus disruption leading to prevention of metaphase/anaphase transition (Sobieh and Fahmy 2021). Chromosome stickiness was the most frequent form of abnormality seen with NaCl treatments. Chromosome stickiness indicates that NaCl has harmful effects that are permanent and cause cell death (Gömürgen et al. 2005).

Stickiness may be due to changes in nucleosomes formation and/or absent of specific non-histone proteins implied in chromosome organization essential for chromosome segregation and chromatid separation (Potapova and Gorbsky 2017). Chromosome bridge/or multibridge are developed due to strong adhesion between chromatin fibers which stick sister chromatids at metaphase and hold them together, this strong connection may prevent the correct separation of joined chromatids during mitosis (Bordin et al. 2023). The formation of spindle disturbance and multipolarity by gamma radiation may be resulted from inhibition of spindle fiber formation followed by the random distribution of the chromosomes in the cytoplasm (Singh and Roy, 2017). Moreover, the presence of ring chromosome could be result from loss of telomeric part of chromosome (Khanna and Sharma 2013). The induction of laggards indicates the clastogenic effect of any treatment causing loss of some genetic material (Sobieh et al. 2016). Laggards usually form micronuclei (El-Azab et al. 2018). As well, micronuclei can be formed when lagging free chromosomes cannot reach to the cell poles in the correct time to be included in the major nucleus (Utsunomiya et al., 2002). Micronuclei often serve as a marker of chromosomal instability or reflect sensitivity due to single gene polymorphisms as suggested by Luzhna et al. (2013). Direct relationship for MN induction and salt ions shows the mitodepressive effects of higher salt concentrations. It is also an indicator of cell division sensitivity to ion level, which may interfere with the cell cycle regulatory machines. Cl^- ions can induce variation of genome arrangements and mutations (Boyko et al. 2010) The highly positive relationship between gamma radiation and induction of MN could be due to that gamma radiation can produce chromosomal breaks in two chromosomes that tend to reunite forming a MN (Pampalona et al. 2016).

Many checkpoints control the cell cycle of eukaryotic cells. Before entering mitosis, cell cycle checkpoints measure the size of the cell, ensure accurate chromosome replication, and ensure chromosome integrity. The metaphase checkpoint then starts the correct segregation during mitosis through the mitotic spindle. The reversible phosphorylation of the regulatory protein cyclins is necessary for the cell cycle checkpoint. the activity Cdcs family mediates this phosphorylation (Fouad and Hafez 2018). Therefore, Cdcs are regulatory proteins that regulate transcription and restrict cell division in response to undesirable conditions (Ding et al. 2020). Cdcs are divided into eight types based on the putative cyclin-binding domains. One of them, CDCA, is encoded in *V. Faba* by the *cdc2* gene (Binarova et al. 1998). It has previously been demonstrated that CDCAs are involved in both G1/S and G2/M transitions (Hemerly et al. 1995). To promote MI in *Arabidopsis, cdc2* expression is upregulated prior to cell division (Hemerly et al. 1993). The reduction of salt's influence on the cdcs activities and consequently mitotic division was verified by the current data, where there was a considerable downregulation in cdc2 expression level after salt stress. Reduction in Cdcs and cyclin activity under salt stress was previously reported by Qi and Zhang (2020). In general, the downregulation of *cdc* genes expression is a stress response (Kitsios and Doonan 2011) leading to cell cycle arrest, prolonged S-phase progression, or delayed entry into mitosis (De Veylder *et al*. 2007). Previously, treatment of *V. faba* root tips with bohemine or roscovitine (inhibitors of *cdc2-k* gene) led to characteristic abnormalities in mitosis leading to prolonged prophase with intact nuclear envelope (Binarova et al. 1998).

On the contrary, the alleviation effect of both putrescine and gamma radiation caused highly significant upregulation in mitotic division rate alongside with *cdc2* expression level. The low concentration of NaCl with both putrescine and gamma radiation caused upregulation of the expression level for 2 and 4-folds respectively. This upregulation of *cdc2* expression level accompanied with increase in mitotic division is like the results of Fouad and Hafez (2018), who found increase in mitotic division associated with increase in *cdc2* expression level in *Allim cepa*.

Two fingerprint markers ISSR and SCoT were used to evaluate the genetic diversity among the eight treatment groups of *V. faba* and control. Present data show that ISSR and SCoT molecular markers have produced different patterns of DNA polymorphism and discriminated molecular variations among treated and control *V. faba*. This reveals the incidence of modifications at the molecular level among *V. faba* plants by different treatment used. Afiah et al. (2016) proved that ISSR was beneficial in realizing the genetic variation among *V. faba* genotypes and *V. faba* genotypes under salt stress respectively. The SCoT molecular marker technique is effective in assessing genetic variations between control and stress treated *V. faba* (Essa et al. 2023) and between control and mutants *V. faba* caused by gamma irradiation. These variations caused changes at the molecular and phenotypic levels. In *Vigna unguiculata* the appearance and the disappearance of amplicons in the mutant genotypes compared to control proved the impact of gamma irradiation on phenotypic and genotypic traits (Vanmathi et al. 2021). Moreover, gamma irradiation had the capabilities of induction genetic variation in the genotypes of Cowpea varieties, as assessed by RAPD and ISSR markers (Badr et al. 2014). In the same concern ISSR fingerprints differentiate the amaranth mutant from Ficha cultivar and K-433 hybrid and

showed that the genetic diversity produced may be part of the complex response to the gamma-radiance (Žiarovská et al 2013). Besides, modifications in control and gamma radiated treatments might be produced by inter microsatellite length polymorphism (Aly et al. 2019).

A comparison of the level of polymorphism and discriminating efficacy of ISSR and SCoT showed that each of the two techniques can detect genetic variation among control and treated *V. faba*. But the results revealed that the ISSR markers were more efficient than SCoT for differentiating the genetic variation. ISSR markers effectively assessed the genetic diversity among the *V. faba* and produced a wide range of PIC and medium RP value, which indicates the presence of specific alleles in some genotypes, which can assist the differentiation of these genotypes from the others (Serry et al. 2019). PIC and Rp are reported to be better informative factors than MI to describe the discriminative power of a primer to distinguish various genotypes (Shingote et al. 2019(. Although ISSR was more efficient, both two markers produced high level of polymorphism. Therefore, a combination of ISSR (spanning selected repetitive sequences) and SCoT (targeting the start codon sequences of the DNA) can be considered as best markers for more expressive and dependable investigation of genetic variability as clarified by cluster analysis using UPGMA dendrogram and PCA scatter plot. They revealed high genetic variability of 10Gy+100mM NaCl. So, it can be concluded that gamma radiation was an effective means for initiation mutation in *V. faba*. These changes could be successfully identified by ISSR and SCoT analysis. Therefore, the recent progress in mutation breeding studies in relation with new technologies is quite critical to influence and improve plant breeding programs to overcome the climate changes.

CONCLUSION

Salinity is a severe problem, which affect plant growth and productivity. Plants have developed highly advanced stress tolerance mechanisms to adapt stresses. The application of putrescine or irradiated plants with gamma radiation were effective in enhancing growth, cell division under saline conditions and alleviating the harmful effect of salinity.

ACKNOWLEDGEMENTS

The authors are grateful to express their gratitude to the Egyptian Atomic Energy Authority (EAEA) Cairo-Egypt for supporting this research.

REFERENCES

Abdelhamid MT, Sadak MSH, Schmidhalter URS, EL-Saady M. 2013. Interactive effects of salinity stress and nicotinamide on physiological and biochemical parameters on faba bean plant. Acta biol. Colomb. 18(3):499-510.

Afiah SAI,.Abd El- Gawad ZA , Mohamed TH R , Al-Agwany H H.2016. Salt and Water Deficit Tolerance in some *Vicia faba* L. Genotypes in Relation to Pigments ISSR – PCR Markers and Stress Tolerance Indices. Egypt. J. Bot. 56(1):121-144.

Afrin MS, Kabir MA, Alam MS .2019. Effect of gamma radiation on the growth, yield and quality of four onion accessions. J Agric Vet Sci12(8):68-78.

Albrifcany M S, Hajer S, Askander A. 2022. Genetic diversity among some parental varieties of *Vicia faba* L. using ISSR and SCoT molecular marker techniques. J. of Agricult Chemist Biotechnol, Mansoura Univ. 13(12):129-137.

Alcázar R, Altabella T, Marco F, Bortolotti C, Reymond M, Koncz C, Carrasco P, Tiburcio A F, 2010. Polyamines: Molecules with regulatory functions in plant abiotic stress tolerance. Planta 231:1237-1249.

Ali H, Ghori Z, Sheikh S, Gul A .2016. Effects of gamma radiation on crop production. In: Crop production and global environmental issues. Springer, 27-78.

Aly AA, Eliwa NE, Abd El-Megid MH. 2019. Stimulating effect of gamma radiation on some active compounds in eggplant fruits. EJRSA 32(1):61-73.

Aly AA, Eliwa NE, Maraei RW.2019. Physiological and molecular studies on ISSR in two wheat cultivars after exposing to gamma radiation. Science Asia 45(5):436-445.

AOAC. Association of Official Agricultural Chemist. 1984. Official methods of analysis of the association of official analytical chemist. 14 ed. Washington. D.C. USA.

Asare AT, Mensah F, Acheampong S, Asare-Bediako E, Armah J .2017. Effects of gamma irradiation on agromorphological characteristics of okra (Abel- moschus esculentus L. Moench.). Adv Agric 3:1-7.

Badr A, El-Shazly HH, Halawa MM .2014. Cytological effects of gamma radiation and its impact on growth and yield of M1 and M2 plants of cowpea cultivars. Cytologia. 79(2):195-206.

Bates L S, Waldern R P, Teare I D.1973.Rapid determination of free proline for water stress studies determination of free proline for water stress studies. Plant and Soil. 3:205.

Binarova P, Dolez J, Draber P, Heberle-Bors E, Strnad M, szló Bögre L. 1998. Treatment of Vicia faba root

tip cells with specific inhibitors to cyclin-dependent kinases leads to abnormal spindle Formation. The Plant J 16(6):697-707.

Bordin ER, Santos F, Mannes Y, Martins de Freitas A, Ramsdorf WA.2023. Single and mixture cytogenetic effects evaluation of atrazine and glyphosate herbicides at environmentally relevant concentrations on Allium cepa root meristem cells. Ecotoxicol. Environ. Contam.18(1):38-50. https://doi.org/10.5132/eec.2023.01.06

Boyko A, Golubov A, Bilichak A, Kovalchuk I.2010. Chlorine Ions but Not Sodium Ions Alter Genome Stability of Arabidopsis Thaliana. Plant Cell Physiol. 51:1066-1078.

Caplin N, Willey N.2018. Ionizing Radiation, Higher Plants, and Radioprotection: From Acute High Doses to Chronic Low Doses.Front. Plant Sci. 9: 847.

Castro-Guerrero NA, Cui Y, Mendoza-Cozatl D. 2016. Purification of translating ribosomes and associated mRNAs from soybean (Glycine max). Curr. Protoc. Plant Biol 1:1-12. https://doi.org/10.1002/cppb.20011.

Chesnokov Yu V, Artemyeva AM. 2015. Evaluation of the measure of polymorphism information of genetic diversity Agricult. Biol. 50(5):571-578.

Collard B C, Mackill D J.2009. Start codon targeted (SCoT) polymorphism: A simple, novel DNA marker technique for generating gene-targeted markers in plants. Plant Mol. Biol. Rep. 27: 86-93.

De Veylder L, Beeckman T, Inze D. 2007. The ins and outs of the plant cell cycle. Nat. Rev. mol. cell. Biol. 8:655-665.

Demiral T, Türkan ˙I. .2005. Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. Environ. Exp. Bot. 53:247-257.

Ding L, Cao J, Lin W, Chen H, Xiong X, Ao H, Yu M, Lin J, Cui Q. 2020.The Roles of Cyclin-Dependent Kinases in Cell-Cycle Progression and Therapeutic Strategies in Human Breast Cancer. Int J Mol Sci. 21:1960.

El-Azab EM, Soliman M, Soliman E, Badr A. 2018. Cytogenetic Impact of Gamma Irradiation and Its Effects on Growth and Yield of Three Soybean Cultivars. Egypt. J. Bot. 58(3):411-422.

El-Bassiouny H M S, Mostafa H A. 2008. Physiological responses of wheat plant to foliar treatments with arginine or putrescine. Austr. J. of Basic Appl Sci. 2(4):1390-1403.

El-Beltagi HS, Ahmed OK, El-Desouky W .2011. Effect of low doses γ-irradiation on oxidative stress and secondary metabolites production of rosemary (*Rosmarinus officicnalis L.*) callus culture. Radiat Phys Chem 80:968-976.

Essa SM, Wafa H A, Mahgoub E.-S.I., Hassanin A A, Al-Khayri J M, Jalal A S, El-Moneim D A, ALshamrani S M, Safhi F A, Eldomiaty. A S.2023. Assessment of eight faba bean (*Vicia faba* L.) cultivars for drought stress tolerance through molecular, morphological, and physiochemical parameters. Sustainab. 15:3291. https://doi.org/10.3390/su15043291

FAO Statistics. 2021. Faosta, Url: http://faostat3.fao.org/download/Q/QC/E

FAOSTAT. 2022. Food and Agriculture Organization of the United Nations. Retrieved from http://www.fao.org/faostat

FAOSTAT 2018. Food and Agriculture Organization of the United Nations. Retrieved from http://www.fao.org/faostat/en/#data/QC

Fouad AS, Hafez RM. 2018. The effects of silver ions and silver nanoparticles on cell division and expression of cdc2 gene in *Allium cepa* root tips. Biol. Plant. 62(1):166-172. https://doi.org/10.1007/s10535-017-0751-6166

Gömürgen A N, Mutlu , Bozcuk S.2005. Effects of Polyamines (Putrescine, Spermidine and Spermine) on Root Tip Mitosis and Chromosomes in *Allium cepa* L Cytologia 70(2):217-224.

Gupta K, Dey A, Gupta B. 2013. Plant polyamines in abiotic stress responses. Acta Physiol Plant. 35(7):2015-2036.

Hammer, Q, Harper D A, Ryan PD, 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontol. Electron. 4(1):9.

Hanafy RS, Ageeb S. 2018. Physiological and molecular studies on the effect of gamma radiation in fenugreek (*Trigonella foenum* L.) plants. J Genet Eng Biotechnol. 16(2):683-692.

Harez RO, Abbas AJ. 2015. Effect of spraying with organic fertilizer (humic acid) and the Brassinosteroid growth regulator on knots, shedding and some traits of vegetative, flowering and fruiting growth of local orange *Citrus sinensis* L Diyala J Agric Sci. 7(2):78-89.

Hassan N, Ebeed H, Aljaarany A. 2020. Exogenous application of spermine and putrescine mitigate adversities of drought stress in wheat by protecting membranes and chloroplast ultra-structure. Physiol. Mol. Biol. Plants 26:2339245.

Hassanein RA, Bassony FM, Barakat DM, Khalil RR. 2009. Physiological effects of nicotinamide and ascorbic acid on Zea mays plant grown under salinity stress. 1- Changes in growth, some relevant metabolic activities and oxidative defense systems. Res J Agric Biol Sci. 5(1):72-80.

Hemerly A, De Almeida Engler J, Bergounioux C. Montagu M, Engler G, Inzé D, Ferreira P.1995. Dominant

negative mutants of the CDC2 kinase uncouple cell division from iterative plant development. EMBO J. 14:3925-3936.

Hemerly AS, Ferreira P, Engler J, Van Montagu M, Engler G, lnze D.1993. *cdc2a* expression in Arabidopsis is linked with competence for cell division. Plant Cell 5:1711-1723.

Hirt H, Heberle-Bors E. 1994. Cell cycle regulation in higher plants. Sem. Dev. Biol. 5:147-154.

Hoagland DR, Arnon DI. 1950. The water-culture method for growing plants without soil. Circular. Calif. Agric. Exp. Stn. 347:32.

Hussein HA, Alshammari SO, Abd El-Sadek ME, Kenawy SKM. Badawy AA. 2023. The Promotive Effect of Putrescine on Growth, Biochemical Constituents, and Yield of Wheat (Triticum aestivum L.) Plants under Water Stress. Agriculture 13(3):587. https://doi.org/10.3390/agriculture13030587

Isayenkov S, Maathuis FJM. 2019. Plant salinity stress: many unanswered questions remain. Front. Plant. Sci. 10:80. https://doi.org/10.3389/fpls.2019.00080.

Jan S, Parween T, Siddiqi TO. 2012. Effect of gamma radiation on morphological, biochemical, and physiological aspects of plants. Environ. Rev. 20:17e39.

Jităreanu A, Pădureanu S, Tătăringă G, Tuchiluş C, Stănescu U.2013. Evaluation of phytotoxic and mutagenic effects of some cinnamic acid derivatives using the Triticum test. Turk J Biol. 37:748-756.

Khanna N, Sharma S. 2013., "Allium cepa root chromosomal aberration assay: a review," Indian J. Pharm. Biol. Res. 1:105-119.

Khosroshahi MRZ, Ashari EM. 2008. Effect of Putrescine application on post-harvest life and physiology of strawberry, apricot, peach and sweet cherry fruits. J. Agric. Sci. Technol. 45:219-230.

Kitsios G, Doonan JH. 2011. Cyclin dependent protein kinases and stress responses in plants. Plant Signal. Behav. 6:204-209.

Kuznetsov VV, Shevyakova NI. 2007. Polyamines and stress tolerance of plants. Plant Stress. 1:50-71.

Livak KJ, Schmittgen TD. 2001.Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods. 25(4):402-408.

Lowery O H, Rosebrough N J, Farr A L, Ronodall R J.1951. Protein measurement with Folin reagent, J. Biol. Chem. 193(1):265-275.

Ludovici G M, Oliveira de Souza S, Chierici A, Cascone M G, d'Errico F, Malizia A. 2020. Adaptation to Ionizing Radiation of Higher Plants: From Environmental Radioactivity to Chernobyl Disaster. J. Environ. Radioact. 222:106375.

Luzhna L, Kathiria P, Kovalchuk O. 2013. Micronuclei in genotoxicity assessment: From genetics to epigenetics and beyond. Front. Genet. 4:131.

Magyar Z, Mészaros T, Miskolczi P. et al. 1997. Cell cycle phase specificity of putative cyclin-dependent kinase variants in synchronised alfalfa cells. Plant Cell. 9:223-235.

Mahfouz H, Rayan W. 2017. Antimutagenics effects of Stigmasterol on two salt stressed *Lupinus termis* Cultivars Egypt J Genet Cytol. 46:253-272.

Matkovics B, Kecskemeti V, Varga S I, Novak Z, Kertesz Z. 1993. Antioxidant properties of di-and polyamines. Comp Biochem Physiol Comp Biochem. 104:475-479.

Munns R. 2002. Comparative physiology of salt and water stress. Plant Cell Environ. 25(2):230-250.

Nigg E. 1995. Cyclin-dependent kinases: key regulators of the eukaryotic cell cycle. Bioessays. 17: 471-479.

Pampalona J, Roscioli E, Silkworth WT, Bowden B, Genescà A, Tusell L, Cimini D. 2016. Chromosome bridges maintain kinetochore microtubule attachment throughout mitosis and rarely break during anaphase. PLoS ONE 11(1):e0147420.

Potapova T, Gorbsky GJ. 2017. The Consequences of chromosome segregation errors in mitosis and meiosis. Biology 6(1):12.

Qahtan AA, Al-Atar A, Abdel-Salam EM, El-Sheikh MA, Gaafar ARZ, Faisal M. 2021. Genetic diversity and structure analysis of a worldwide collection of faba bean (*Vicia faba*) genotypes using ISSR markers. Int. J. Agric. Biol. 25:683-691.

Qi F, Zhang F. 2020. Cell Cycle Regulation in the Plant Response to Stress. Front. Plant Sci. 10:1765.

Rosen H. 1957. A modified ninhydrin colormetric analysis for amino acid nitrogen. Archives Biochemist Biophys. 67(1):10-15.

Sadak MSh, Abd Elhamid EM. 2013. Physiological response of flax cultivars to the effect of salinity and salicylic acid. J Appl Sci Res. 9(6):3573-3581.

Sequera-Mutiozabal M, Antoniou C, Tiburcio AF, Alcázar R, Fotopoulos V. 2017. Polyamines: emerging hubs promoting drought and salt stress tolerance in plants. Curr Mol Biol Rep. 3:28-36.

Serry N, Ali KH, Hussein MSM. 2019. Phenotypic, Biochemical and Molecular Characterization of New Egyptian Mango Genotypes. Hortsci J Suez Canal Univ. 8(1):55-68.

Shingote PR, Amitha Mithra SV, Sharma P, Devanna NB, Arora K, Holkar SK, Khan S, Singh J, Kumar S, Sharma TR, Solanke AU. 2019. LTR retrotransposons and highly informative ISSRs in combination are potential markers for genetic fidelity testing of tissue culture-raised plants in sugarcane. Mol Breed 39(2):25.

Singh D, Roy BK. 2017. Evaluation of malathion-induced cytogenetical effects and oxidative stress in plants using Allium test. Acta Physiol. Plant. 39(4):92.

Snedecor GW, Cochran WG. 1980. Statistical methods, Lowa State University Press/Ames. pp. 177-195.

Sobieh Sh S, Fahmy DM. 2021. Assessment of antimitotic and programmed cell death stimulation potentials of *Galium sinaicum* (Delile ex Decne) Boiss. at the cellular level. Caryol 74(2):21-35. https://doi.org/10.36253/ caryologia-1057

Sobieh Sh S, Tawab SA, Fahmy DM. 2014. Deregulation of mitosis progression and cytotoxic effect triggered in *Allium cepa* L. Roots by *Rubus sancatus* Schreber Extract. Life Sci J 11:1047-1058.

Sobieh Sh S, Zeinab MHKh, Abeer AR, Naglaa ANY. 2016. In vitro and in vivo genotoxicity and molecular response of silver nanoparticles on different biological model systems. Caryol 69:147-161.

Souguir D, Abd-Alla HI, Hörmann G, Hachicha M. 2018. Chromosomal and nuclear alterations in the root tip cells of *Vicia faba* induced by sodium chloride. Water Environ Res. 90:164-171.

Souguir D, Berndtsson R, Mzahma S, Filali H, Hachicha M. 2022. Vicia micronucleus test application for saline irrigation water risk assessment. Plants 11:462. https://doi.org/10.3390/ plants11030462

Spencer LMM, Forster BP, Jankuloski L. 2018. Manual on mutation breeding, 3rd ed.; Food and Agriculture Organization of the United Nations (FAO): Rome, Italy.

Taie H, Abdelhamid MT, Dawood MG, Nassar RM. 2013. Presowing seed treatment with proline improves some physiological, biochemical and anatomical attributes of faba bean plants under sea water stress. J Appl Sci Res. 9(4):2853-2867.

UNSC .2000. Sources and effects of ionizing radiation: sources (Vol. 1). United Nations Publications.

Utsunomiya KS, Bione NCP, Pagliarini MS. 2002. How many different kinds of abnormalities could be found in unique endogamous maize plant? Cytologia, 67:169-176.

Vanmathi S, Arulbalachandran D, Soundarya V. 2021. Effects of gamma radiation on quantitative traits and genetic variation of three successive generations of cowpea (Vigna unguiculata (L.) Walp.) Plant Sci. Today, 2021 8(3):578-589. https://doi.org/10.14719/ pst.2021.8.3.1054

Velikova V, Yordanov I., Edreva A. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. Plant Sci. 151:59-66.

Venkatesan J, Ramu V, Sethuraman Th, Sivagnanam Ch, Doss G. 2021. Molecular marker for characterization of traditional and hybrid derivatives of *Eleusine coracana* (L.) using ISSR marker. J Genet. Eng. Biotechnol. 19:178.

Wild SA, Corey RB, Lyer JG, Voigt GK. 1985. Soil and plant analysis for tree culture. Oxford and IPH Publishing Co., New Delhi, India, pp. 529-546.

Wisniewski M, Nassuth A, Teulieres CH, Marque CH, Rowland J, Cao PHB. 2014. Genomics of cold hardiness in woody plants. Crit Rev Plant Sci. 33:92-124.

Xavier MN, Torres Novaes JA, Cavalcante Silva AC, Alves AVS, Araujo Santos MJ, Pantale˜ao SM, Scher R, d'Errico F, Oliveira de Souza S. 2023. Cytogenetic effects of β-particles in *Allium cepa* cells used as a biological indicator for radiation damages. J Environ Radioact. 259:107-109.

Xiong F, Zhong R, Han Z, Jiang J, He L, Zhuang W, Tang R. 2011. Start codon targeted polymorphism for evaluation of functional genetic variation and relationships in cultivated peanut (*Arachis hypogaea* L.) genotypes. Mol Biol Rep 38:3487-3494.

Youssef EAE. 2007. Increasing drought tolerance of gladiolus plants through application of sustainable agricultural development. Agadir, Morocco, pp. 299-326.

Zhang XZ. 1993. Crop physiology research method. China Agricultural Press, Beijing, pp. 145-146.

Zhao F, Qin P. 2004. Protective effect of exogenous polyamines on root tonoplast function against salt stress in barley seedlings. Plant Growth Regul. 42:97-103.

Zhao L, Wang P, Hou H, Zhang H, Wang Y, Yan S, Huang Y, Li H, Tan J, Hu A, Gao F, Zhang Q, Li Y, Zhou H, Zhang W, Li L. 2014. Transcriptional regulation of cell cycle genes in response to abiotic stresses correlates with dynamic changes in histone modifications in maize. PLoS One 9:e106070

Žiarovská J, Ražná K, Labajová, M. 2013. Using of inter microsatellite polymorphism to evaluate gamma-irradiated amaranth mutants. Emir J Food Agric 25(9):673-681.

Ziêtkiewicz E, Rafalski A, Labuda D.1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics. 20:176-183.