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Effects of γ-radiation on chickpea (*Cicer arietinum*) varieties and their tolerance to salinity stress

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Effects of γ-radiation on chickpea (*Cicer arietinum*) varieties and their tolerance to salinity stress

Abstract: Chickpea (*Cicer arietinum* L.) is a bisexual and self-pollinated legume. It improves the soil fertility through its natural ability to fix atmospheric nitrogen with its symbiotic bacteria. Salinity is one of the most important abiotic stress factors affecting plant growth. γ-radiation is a very effective tool for inducing mutations in many plants. This study evaluated the γ-radiation effect on germination, cell division and plant growth of first-generation plants. Seeds of seven chickpea varieties were irradiated with γ-radiation doses ranging between 50 Gy and 600 Gy. Non-significant differences in germination percentage were recorded for seeds exposed to 50 Gy, 100 Gy, and 200 Gy of γ-radiation in comparison to the corresponding controls except ILC 484. The mitotic index (MI) of root cells increased at the low doses of 50 Gy, 100 Gy and 200 Gy comparing and reduced at the higher doses in all chickpea varieties to the control. All doses of γ-radiation induced a variable range of chromosomal abnormalities; the most common were bridges, laggard chromosomes, stickiness at metaphase, chromosome breaks, micronuclei and binucleate cells. The 300 Gy to 600 Gy doses induced degradation of nuclear membranes. The salinity treatments at 25 mM NaCl and 60 mM NaCl reduced seedling's growth of all cultivars. The dose of 100 Gy alleviated the impact of salinity at a concentration of 25 mM NaCl for all varieties, except FLIP 84-188 and FLIP 97-263. The 60 mM NaCl treatment significantly reduced early growth of all cultivars and its effect was not alleviated by the γ-radiation.

Key words: chickpea; γ-radiation; germination; mitotic index; chromosomal aberrations

Učinki γ-sevanja na sorte čičerke (*Cicer arietinum* L.) in njihova toleranca na slanostni stres

Izvleček: Čičerka (*Cicer arietinum* L.) je obojespolna samoprašna stročnica. Zaradi sposobnosti vezave atmosferskega dušika s simbiontskimi bakterijami izboljšuje rodovitnost tal. Slanost je eden izmed najpomembnejših abiotskih stresnih dejavnikov, ki vpliva na rast rastlin. V raziskavi je bil ovrednoten vpliv γ-sevanja na kalitev, celične delitve in rast rastlin F1 generacije čičerke. Semena sedmih sort čičerke so bila obsevana z γ-žarki v jakosti od 50 Gy do 600 Gy. V primerjavi s kontrolo so bile zabeležene neznačilne razlike v odstotku kalitve pri semenih, ki so bila izpostavljena 50 Gy, 100 Gy in 200 Gy γ-sevanja, razen pri sorti ILC 484. V primerjavi s kontrolo se je pri vseh sortah povečal mitotski indeks (MI) celic rastnega vršička korenine, ki so bile obsevane z majhnimi dozami 50 Gy, 100 Gy in 200 Gy ter zmanjšal pri obsevanju z večjimi dozami. Vse uporabljene doze γ-sevanja so vzpodbudile različen obseg kromosomskih aberacij. Najbolj pogoste so bile mostički, zaostali kromosomi in zlepljeni kromosomi v metafazah ter zlomljeni kromosomi, mikronukleusi in dvojedrene celice po delitvi. Doze sevanja z jakostjo od 300 Gy do 600 Gy so vzpodbudile razpad jedrnih membran. Slanostna obravnavanja s 25 mM NaCl in 60 mM NaCl so zmanjšala rast sejank vseh sort. Doza obsevanja s 100 Gy je zmajšala učinek slanostnega stresa 25 mM NaCl pri večini sort, razen pri sortah FLIP 84-188 in FLIP 97-263. Obravnavanje z dozo 60 mM NaCl je značilno zmanjšalo zgodnjo rast pri vseh sortah in negativnega učinka ni bilo mogoče zmanjašati z γ-obsevanjem.

Ključne besede: čičerka; γ-sevanje; kalitev; mitotski indeks; kromosomske aberacije

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1 INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an annual herbaceous self-pollinated legume (Ladizinsky & Adler, 1976). Its seeds are a significant source of proteins, carbohydrates, vitamins, minerals and unsaturated fatty acids (Jimenez-Lopez et al., 2020; Jukanti, Gaur, Gowda, & Chibbar, 2012). It is also important for sustainable agriculture since fixing atmospheric nitrogen via symbiotic bacteria provides rotational value to subsequent crops and improve the growth and yield of chickpea (Marques et al., 2020). The domesticated chickpea is divided into two major distinct chickpea types. One is the "microsperma" or 'desi' with small and dark colored seeds with reticulated surface and anthocyanin pigmented aerial parts and pink or purple flowers (Moreno & Cubero, 1978; Van der Maesen, 1972). The other is "macrosperma" or 'kabuli' with large seeds with beige seed coat and green aerial parts that lack anthocyanin pigmentation and with white flowers (Upadhyaya et al., 2008).

Soil salinity is considered to be one of the most common abiotic stresses controlling agricultural production around the world by threatening crop yield, and agricultural sustainability (Munns & Gilliham, 2015). It will become progressively more severe over time due to climatic changes, unsuitable irrigation and excessive fertilization (Sun et al., 2018). Salinity affects crops in two ways; by osmotic stress caused by high concentrations of salts in the soil, which make it harder for roots to absorb water, and by ion stress caused by an increased levels of soluble salts within the plant cells caused by exchangeable sodium (Na^+) during salinity stress (Munns et al., 2020). The impacts of salt stress on plants vary greatly depending on the type and dose of salt used, environmental factors, plant species, cultivars within a species, and plant development stages (Tabur, Avci, & Özmen, 2021). The osmotic stress induces formation of harmful free radicals, including reactive oxygen species (ROS) which causes oxidative damages and induces negative effects on the cell functional integrity (Gaafar, Hamouda, & Badr, 2016; Sharma, Jha, Dubey, & PessarakliM, 2012). Tolerance to salinity may consequently include variations in responses to these factors (Munns & Tester, 2008).

Gamma rays have been used frequently in mutation breeding of grain legumes (Abdelfattah Badr, El-Shazly, & Halawa, 2014; Chopra, 2005; El-Azab, Ahmed Soliman, Soliman, & Badr, 2018; Soliman, Elkelish, Souad, Alhaithloul, & Farooq, 2020). Many mutant crop varieties resistant to diseases, cold, salt and with desired qualities have been developed using γ-radiation (Chopra, 2005; Gnanamurthy, Mariyammal, Dhanavel, & Bharathi, 2012; Tshilenge-Lukanda, Kalonji-Mbuyi, Nkongolo, & Kizungu, 2013). Low frequency of γ-radiation may be beneficial, while the treatments with high doses can be harmful to germination, growth rate, vigor, pollen and ovule fertility (Singh, 2005). The γ-radiation has been used for mutation induction in chickpea (Amri-Tiliouine et al., 2018; Joshi-Saha, Reddy, Petwal, & Dwivedi, 2015; Wani, 2009). Assessment of LD50, lethality, injury, mitotic, and meiotic aberration frequency is required for determining sublethal doses for successful mutation breeding experiments (Bhat & Wani, 2017). At high doses, γ-radiation interact with several metabolites and cell components and can induce many cytogenetic mutations such as chromosomal rearrangements: chromatid and chromosome bridges, single and double fragments, micronuclei, and delayed chromosomes segregation (Abdelfattah Badr et al., 2014; El-Azab et al., 2018; Nazarenko & Izhboldin, 2017). Kamble and Patil (2014) reported the rate of cell division (as mitotic index) and induced qualitative and quantitative chromosomal aberration comprising chromosomes, clumping, polyploidy, ring formation, stickiness, chromatin bridges, laggards, multipolarity at anaphase in chickpea.

Shah, Mirza, Haq, and Atta (2008) tested the effect of γ-radiation doses ranging from 100 Gy to 1200 Gy in the first generation (M1) of four chickpea genotypes. The germination percentage (GP) reduced gradually with increasing γ-radiation doses from 400 Gy 1200 Gy. Brahmi et al. (2014) reported that the 150 Gy dose was determined as the optimum causing 50 % reduction in seed survival of local chickpea variety, but higher, more than 250 Gy doses caused a slow decline in germination rate; reaching values lower than 10 % for treatments of over 650 Gy. The shoot lengths of nine *Cicer* species, including three kabuli and four desi types as well as two annual wild species were inhibited with a 200 Gy of γ-radiation and growth curves gradually decreased at the 300 Gy and 400 Gy doses (Toker, Uzun, Canci, & Ceylan, 2005). Melki, Mhamdi, and Achouri (2011) investigated the impact of low doses of γ-radiation from radioactive cobalt on chickpea growth, protein content in leaves and grains harvested from irradiated seeds. The dose of 20 Gy γ-radiation enhanced plant growth by 146.35 % compared to plants grown from non-irradiated seeds. Sohrabi, Heidari, and Esmailpoor (2008) evaluated the effect of NaCl salinity at different levels (0, 3, 6 and 9 dS m^{-1}) on chickpea and reported reduction of plant growth, pod number, flowers, seed mass and seed number.

The objective of this study is to evaluate the potential of induced mutation with γ-radiation in chickpea varieties to alleviate the effects of salinity stress treat-

ments on germination, seedling's growth and cell division and chromosomes in the M1 chickpea.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

Seven varieties of chickpea (FLIP 81-71, FLIP 84-188, FLIP 97-263, ILC 72, ILC 464, ILC 484 and ILC 2555) were obtained from International Center for Agricultural Research in Dry Areas (ICARDA), currently hosted by the Agricultural Research Center (Giza, Egypt), and used in this study.

2.2 EXPERIMENTAL SET-UP

Air-dried seeds of the chickpea varieties were irradiated with 50, 100, 200, 300, 400, 500 and 600 Gy of γ-radiation (dose rate 1.249 kGy h^{-1}). The irradiation was done at the Atomic Energy Center, Nasr City, Cairo, Egypt using irradiation device GSR D1 (Germany). The 50 % lethal irradiation dose (LD50) was determined by calculating the germination and survival percentage. Germination percentage (GP) of irradiated seeds for all doses and their controls was determined on the 7^{th} day of germination.

2.3 CYTOLOGICAL ANALYSIS

The chickpea seeds were germinated in Petri dishes and germinating roots (7 days) were fixed in freshly prepared Carnoy's fixative for 24 h and kept at 4 °C until used. The fixed roots were washed briefly with distilled water, hydrolyzed with 1N HCl for 8-10 min at 60 °C or for 20-25 min at room temperature. The hydrolyzed roots were washed briefly again with distilled water and stained with the basic fuchsin stain (Germany) for 15 min at 23 °C. The stained root tips were cut off and squashed in a drop of 45 % acetic acid, using coverslip. The slides were examined using the 40 × magnification of the light microscope (KRÜSS, Germany) and five slides were examined for each treatment. Photomicrographs of abnormal and control cells were taken with digital camera (Fujifilm FinePix JV100 12 MP Digital Camera, China).

The following data were measured and calculated for each treatment using the following equations

MI (%) = (Number of cells in mitosis / Number of all examined cells) × 100.

Abnormality type (%) = (Number of cells showing the specific abnormality type / Total number cells showing all abnormalities) × 100.

Total abnormalities (%) = (Total number of cells showing all abnormalities / Total number of all examined cells) × 100.

2.4 MORPHOLOGICAL ANALYSIS

For studying the effects of NaCl salinity and of γ-radiation doses and their combination, the treated and control seeds were sown in 30 cm wide plastic boxes containing 30 kg soil (EC = 0.6 ds cm^{-1}) with five replicates during the early winter season of 2018 -2019. Three treatments were applied, first: control plants were not treated by neither γ-radiation nor NaCl; second, the plants were treated with two concentrations of NaCl (25mM and 60mM NaCl); third, the plants were treated with γ-radiation doses (50, 100 and 200 Gy) and NaCl (25 mM NaCl or 60 mM NaCl) as combination treatments.

Seven vegetative growth parameters were measured after eight weeks from sowing: shoot length (cm), root length (cm), number of leaves per plant, shoot fresh biomass (g), root fresh biomass (g), shoot dry biomass (g) and root dry biomass (g).

2.5 STATISTICAL ANALYSIS

The data were statistically analyzed using the ANOVA by IBM SPSS Statistics 25 software. The significant difference between the treatments comparing to the control in the same variety was recorded at an alpha level of 0.05 according to the Least Significant Difference (LSD) test.

3 RESULTS

The seed GP calculations revealed no significant variations among varieties under normal condition. The highest GP of 100 % was recorded for 'ILC 484' and the lowest GP of (86.7 % (was recorded for ‹ FLIP 84-188'. In general, non-significant differences were recorded for seeds exposed to 50 Gy, 100 Gy, and 200 Gy doses of γ-radiation in comparison with the corresponding controls except 'ILC 484'. The GP values for the studied varieties slightly decreased at 300 Gy and decreased significantly at 400 Gy, 500 Gy and 600 Gy of γ-radiation (Figure 2). The maximum inhibitory effect on germination was recorded at 500 Gy for 'FLIP 81-71', 'ILC 72' (50 %)) and 'ILC 2555' (53.3 %) but the lowest value of

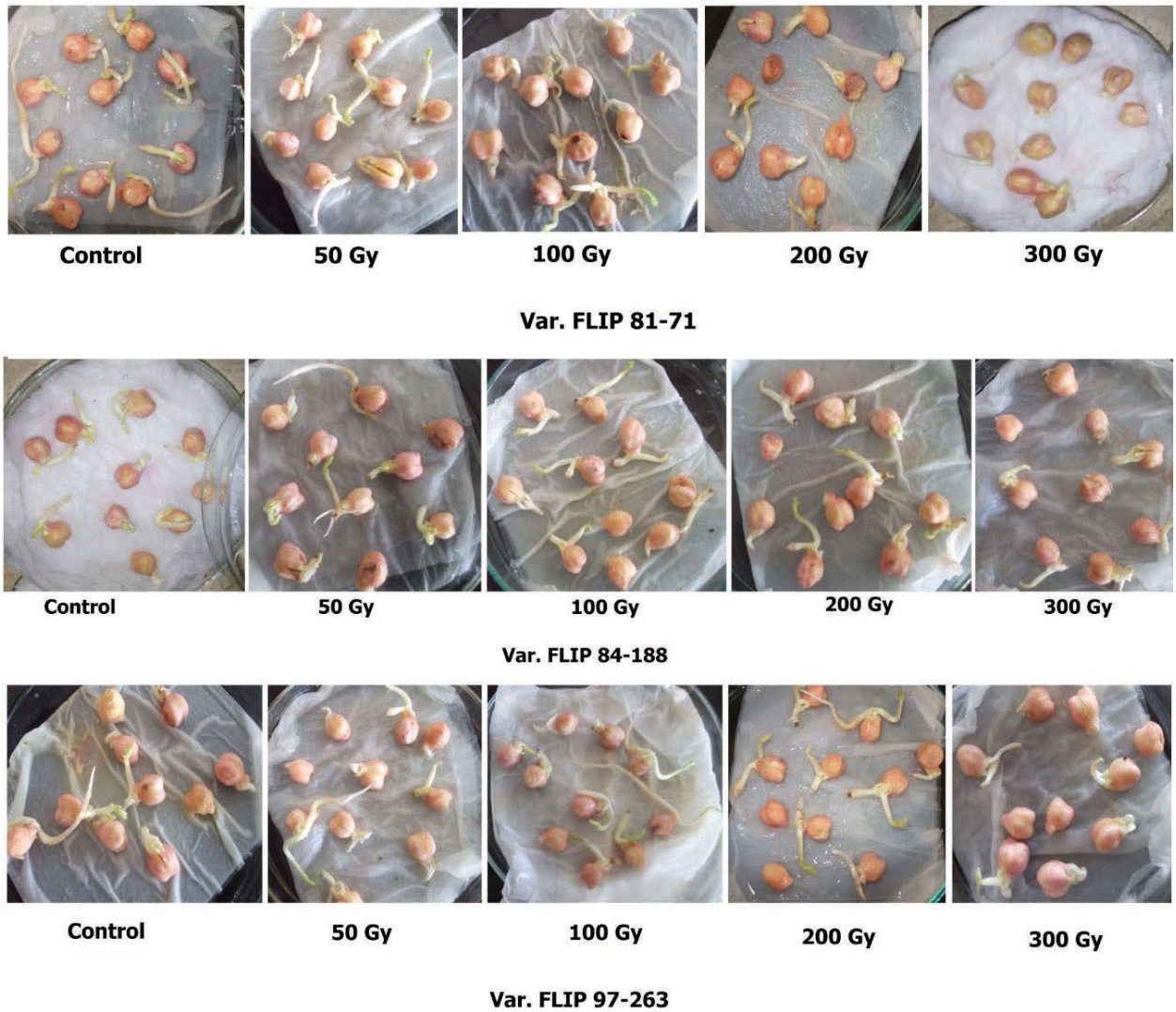

Figure 1: Photos illustrating the germinating seeds of the three chickpea varieties FLIP 81-71. FLIP 84-188, and FLIP 97-263 under control conditions and after exposure to 50, 100, 200, and 300Gy of γ-radiation

GP for the other four varieties was recorded at the maximum dose of 600 Gy of γ-radiation.

Cytological analysis of root meristematic cells, for all chickpea varieties, following seeds exposure to γ-radiation doses from 300 Gy to 600 Gy showed degradation of most nuclear membranes. Consequently, the cytological analyses were only made on the roots exposed to the γ-radiation doses of 50 Gy, 100 Gy and 200 Gy and the control (Figure 3). The MI and the chromosomal abnormalities of the treatments are presented in Table 1. The MI showed significant variation between the different doses of gamma irradiation compared to the control in 'FLIP 81-71', 'ILC 464' and 'ILC 484'. The lowest MI value (3.4) was scored in 'FLIP 97-263' at 50 Gy and 200 Gy while the highest MI value (7.3) was recorded in 'ILC 72' at 200 Gy. The results showed an increase in the MI with the increasing doses from 50 Gy to 200 Gy in all the studied chickpea varieties.

All the applied doses of γ-radiation induced a variable range of mitotic chromosomal abnormalities; bridges, laggard chromosomes, sticky metaphase, chromosome breaks, micronuclei and binucleated cells (Table 1). Five of the studied chickpea varieties (FLIP 84-188, FLIP97-263, ILC 464, ILC 484 and ILC 2555) showed significant difference in bridge percentage between the treatments. The appearance of laggard chromosome was more frequent in all treatments of all varieties except the 50 Gy of 'ILC 2555'. The only significant difference of laggard chromosome percentage was recorded in 'ILC 2555'. The highest value of laggard

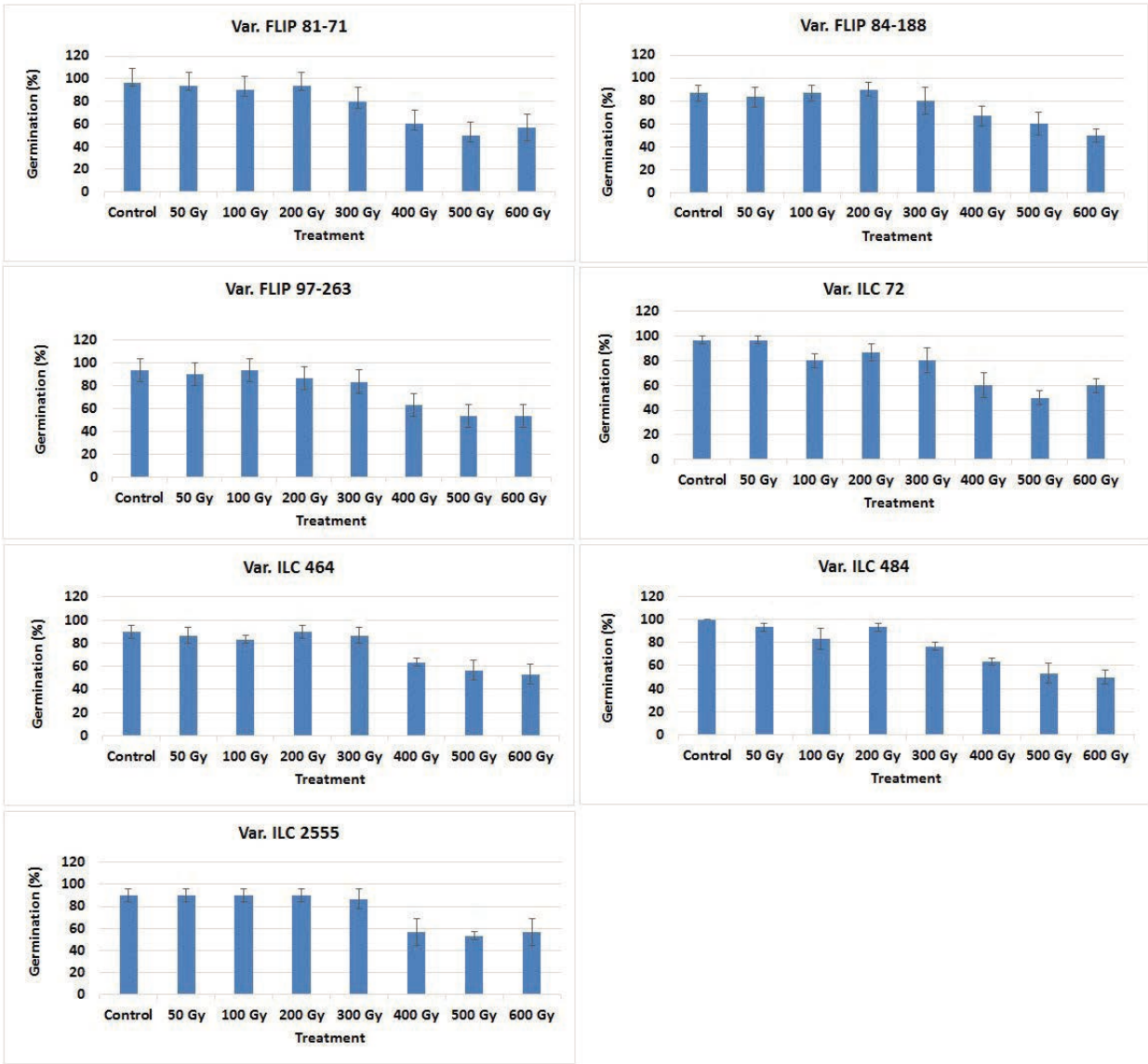

Figure 2: Germination of seven chickpea varieties under control conditions and after exposure to γ-radiation. The mean values ± standard errors are presented (n = 5)

chromosomes (74.5 %) was induced by 200 Gy in ILC 72, while the highest value of sticky metaphase (81.5 %) was induced by 200 Gy in ILC 464. In three varieties, ILC 72, ILC 484 and ILC 2555, a highly significant difference (p = 0.010, 0.000 and 0.001, respectively) in breaks was recorded. While 'FLIP 81-71' and 'ILC 464' showed significant difference (0.010, 0.002, respectively) in the presence of micronuclei, the significant difference of binucleated cells was recorded in 'FLIP 84-188' and 'FLIP 97-263'. The maximum values of the percentage of micronuclei and binucleated cells were 45.0 % and 27.3 %, respectively, induced by 50 Gy γ-radiation in 'ILC 464'. The total abnormalities increased with increased γ-radiation doses. The highest value of the total abnormalities induced by γ-radiation was 5.4%, which was recorded at 200 Gy dose in 'ILC 72' while the minimum value (2.0 %) was recorded at the 50 Gy dose in 'FLIP 81-71' and 'FLIP 97-263'. Highly significant difference appeared at the total abnormalities between all chickpea varieties (Table 1).

The shoots of all chickpea varieties grew above ground after 12 days of sowing. The seedlings treated with 600 Gy and 500 Gy died after three weeks of sowing while seedlings exposed to 400 Gy and 300 Gy died after five weeks of sowing.

The effect of the 60 mM NaCl treatment was sig-

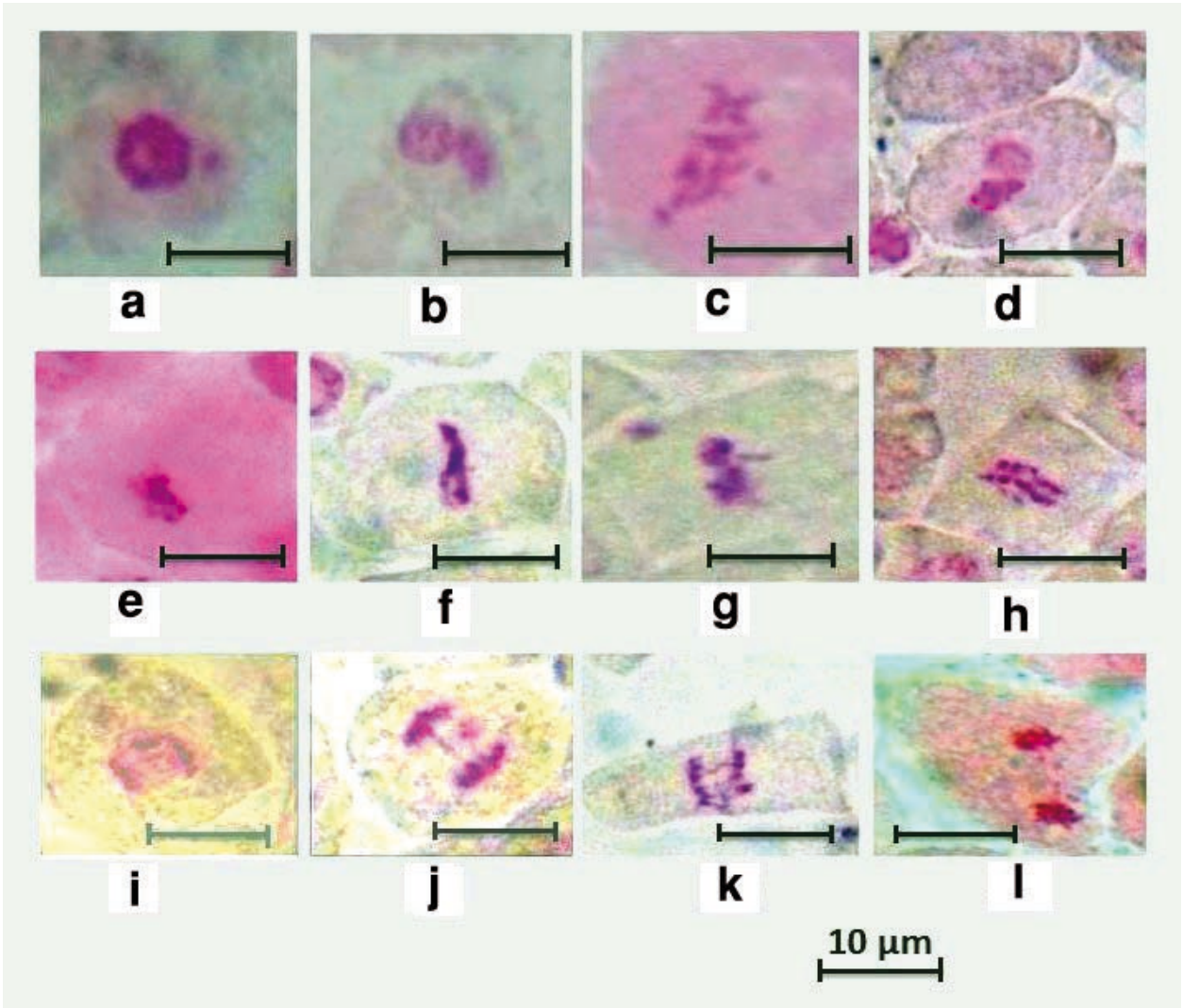

Figure 3: Photographs illustrating types of chromosomal abnormalities induced in the root meristems of seedlings of seven chickpea varieties exposed to three doses of γ-radiation (50 Gy,100 Gy and 200 Gy): a) micronucleus induced by 50 Gy; b) binucleated cell induced by 50 Gy; c) severe stickiness and disturbance at metaphase induced by 100 Gy; d) binucleated cell with sticky metaphase induced by 100 Gy;, e, f) stickiness at metaphase induced by 100 Gy; g) micronucleus, lag-chromosome and sticky metaphase induced by 200 Gy; h) multi-bridges with vagrant chromosome induced 50Gy; i) anaphase bridge induced by 200 Gy; j) anaphase bridge and lagging chromosomes induced by 50 Gy; k) anaphase multi-bridges induced by 50 Gy; l) telophase bridge break induced by 200 Gy.

nificantly higher than the 25 mM NaCl treatment in all studied varieties. In 'FLIP 81-71', measurements under the two salt treatments showed significant reductions in shoot and root traits. Combining low doses of γ-radiation with the low concentration of NaCl treatments alleviated the effect of salinity treatments particularly the shoot length, which scored the highest length in plants exposed to combination of 100 Gy of γ-radiation and 25 mM NaCl compared to the control value (Table 2). The data of 'ILC 72' illustrated that the combination of 100 Gy of *γ*-radiation and 25 mM NaCl treatment showed significant increase in shoot length (24.0 ± 0.58 cm) as compared with 25 mM salt treatment alone. On the other hand, the *γ*-radiation doses (50, 100 and 200 Gy) combined with 60 mM NaCl treatments showed non-significant increase in shoot length comparing with 60 mM salt treatment alone. The combination of γ-radiation doses of 50 Gy, 100 Gy and 200 Gy with the 25 mM NaCl, significantly increased root length (approx. 11 cm) compared to the control plants and plants exposed to 25 mM NaCl only of 'ILC 464'. The data of 'ILC 2555' showed root length reductions

Table 1: Cytological analysis of chickpea root meristem showing mitotic index (MI), specific and total chromosomal abnormalities after γ-radiation

Variety	Treatment	Total cells examined	MI (%)	LSD	Different abnormalities (%)												Total abnormalities (%)		
					Bridge	LSD	Lag. Chrom.	LSD	Sticky metaphase	LSD	Break	LSD	Micro nuclues	LSD	Binuclei	LSD	*Total*	One-way ANOVA		
																		LSD	F-value	p-value
FLIP 81-71	Control	2355	3.4 ± 0.8	-	25.0 ± 12.6	-	31.7 ± 15.9	-	43.3 ± 28.5	-	0.0 ± 0.0	-	0.0 ± 0.0	-	0.0 ± 0.0	-	1.5 ± 0.1	-	10.5	0.004
	50 Gy	2963	3.6 ± 0.3	ns	18.8 ± 10.5	ns	17.4 ± 9.0	ns	28.2 ± 17.4	ns	3.7 ± 3.7	ns	20.8 ± 7.0	*	11.1 ± 11.1	ns	2.0 ± 0.2	ns		
	100 Gy	3655	6.0 ± 0.8	*	24.1 ± 3.5	ns	36.2 ± 5.1	ns	35.7 ± 2.5	ns	0.7 ± 0.7	ns	2.0 ± 1.1	ns	1.3 ± 1.3	ns	2.4 ± 0.3	*		
	200 Gy	3967	5.5 ± 0.3	*	2.9 ± 2.9	ns	31.8 ± 14.1	ns	62.9 ± 19.4	ns	0.8 ± 0.8	ns	1.2 ± 1.2	ns	0.4 ± 0.4	ns	3.3 ± 0.3	*		
	One-way ANOVA		0.034		0.297		0.704		0.636		0.566		0.010		0.483					
FLIP 84-188	Control	5385	5.0 ± 1.3	-	43.3 ± 5.1	-	43.3 ± 5.1	-	13.3 ± 10.2	-	0.0 ± 0.0	-	0.0 ± 0.0	-	0.0 ± 0.0	-	0.7 ± 0.1	-	30.7	0.000
	50 Gy	3050	5.2 ± 0.3	ns	25.9 ± 2.0	*	49.9 ± 1.3	ns	7.3 ± 3.7	ns	0.0 ± 0.0[a]	ns	10.7 ± 4.3	ns	6.2 ± 1.7	*	2.1 ± 0.4	*		
	100 Gy	2900	5.3 ± 0.6	ns	7.8 ± 3.9	*	30.9 ± 15.5	ns	46.7 ± 26.7	ns	1.8 ± 0.9	ns	12.8 ± 6.4	*	0.0 ± 0.0	ns	2.9 ± 0.3	*		
	200 Gy	4100	6.8 ± 0.8	ns	10.9 ± 0.8	*	53.5 ± 3.5	ns	28.5 ± 2.3	ns	5.0 ± 2.5	*	1.0 ± 1.0	ns	1.0 ± 1.0	ns	4.8 ± 0.3	*		
	One-way ANOVA		0.484		0.000		0.308		0.292		0.087		0.106		0.007					
FLIP 97-263	Control	6155	3.6 ± 0.5	-	18.8 ± 10.5	-	69.9 ± 10.5	-	6.4 ± 3.2	-	5.0 ± 5.0	-	0.0 ± 0.0	-	0.0 ± 0.0	-	0.6 ± 0.1	-	7.3	0.011
	50 Gy	3566	3.4 ± 0.4	ns	29.2 ± 3.8	ns	46.9 ± 7.6	ns	6.0 ± 3.0	ns	0.0 ± 0.0	ns	10.6 ± 5.3	ns	7.4 ± 2.2	*	2.0 ± 0.2	*		
	100 Gy	3688	3.7 ± 0.4	ns	0.0 ± 0.0	*	56.0 ± 5.4	ns	30.9 ± 1.2	*	0.0 ± 0.0	ns	13.2 ± 6.6	ns	0.0 ± 0.0	ns	2.3 ± 0.5	*		
	200 Gy	2210	3.4 ± 0.1	ns	14.9 ± 1.2	ns	62.0 ± 6.0	ns	9.5 ± 1.5	ns	0.0 ± 0.0	ns	0.0 ± 0.0	ns	9.5 ± 1.5	*	2.5 ± 0.4	*		
	One-way ANOVA		0.923		0.037		0.262		0.000		0.441		0.117		0.001					

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ILC 72	Control	5890	4.0 ± 0.9	-	16.7 ± 16.7	-	66.7 ± 16.7	-	0.0 ± 0.0	-	0.0 ± 0.0	-	0.0 ± 0.0	-	16.7 ± 16.7	-	0.4 ± 0.1	-	36.9	0
	50 Gy	3000	5.0 ± 1.2	ns	17.7 ± 9.2	ns	53.3 ± 3.3	ns	11.8 ± 8.3	ns	0.0 ± 0.0	ns	13.3 ± 13.3	ns	3.8 ± 3.8	ns	3.2 ± 0.4	*		
	100 Gy	2800	6.7 ± 0.5	ns	19.8 ± 1.1	ns	47.2 ± 2.8	ns	13.0 ± 1.5	ns	7.6 ± 2.7	*	11.0 ± 3.1	ns	0.0 ± 0.0	ns	3.4 ± 0.3	*		
	200 Gy	4600	7.3 ± 1.5	ns	7.1 ± 0.8	ns	74.5 ± 1.9	ns	10.1 ± 1.7	ns	4.4 ± 0.2	ns	4.0 ± 0.1	ns	0.0 ± 0.0	ns	5.4 ± 0.5	*		
	One-way ANOVA		0.184		0.789		0.185		0.206		0.01		0.523		0.503					
ILC 464	Control	3441	3.7 ± 0.4	-	26.1 ± 3.9	-	21.1 ± 10.6	-	52.8 ± 12.1	-	0.0 ± 0.0	-	0.0 ± 0.0	-	0.0 ± 0.0	-	0.3 ± 0.2	-	42.4	0
	50 Gy	3065	4.5 ± 0.3	ns	0.0 ± 0.0	*	18.5 ± 18.5	ns	9.2 ± 4.9	*	0.0 ± 0.0	ns	45.0 ± 11.7	*	27.3 ± 11.7	*	2.2 ± 0.3	*		
	100 Gy	3350	7.0 ± 0.3	*	17.7 ± 1.5	*	52.9 ± 10.6	ns	30.3 ± 8.5	ns	1.0 ±1.0	ns	3.2 ± 3.2	ns	10.5 ± 9.1	ns	4.4 ± 0.3	*		
	200 Gy	3910	4.9 ± 0.3[b]	*	0.0 ± 0.0	*	18.5 ± 1.0	ns	81.5 ± 1.0	*	0.0 ± 0.0	ns	0.0 ± 0.0	ns	0.0 0.0	ns	2.2 ± 0.2	*		
	One-way ANOVA		0.001		0.000		0.195		0.001		0.441		0.002		0.094					
ILC 484	Control	3529	3.9 ± 0.3	-	38.1 ± 14.3	-	38.9 ± 20.0	-	14.7 ± 9.8	-	0.0 ± 0.0	-	8.3 ± 8.3	-	0.0 ± 0.0	-	0.5 ± 0.1	-	20.8	0
	50 Gy	4200	6.4 ± 0.3	*	2.8 ± 2.8	*	40.4 ± 29.9	ns	5.7 ± 3.0	ns	0.0 ± 0.0	ns	25.6 ± 14.4	ns	25.6 ± 17.9	ns	2.8 ± 0.4	*		
	100 Gy	2800	5.4 ± 0.4	*	1.5 ± 1.5	*	55.7 ± 1.6	ns	13.0 ± 2.1	ns	11.7 ± 1.0	*	1.2 ± 1.2	ns	16.9 ± 3.5	ns	2.9 ± 0.4	*		
	200 Gy	3100	5.4 ± 0.2	*	20.3 ± 0.6	ns	40.8 ± 0.6	ns	14.5 ± 0.1	ns	10.6 ± 0.5	*	5.0 ± 0.2	ns	8.9 ± 0.4	ns	5.1 ± 0.6	*		
	One-way ANOVA		0.004		0.024		0.899		0.602		0.000		0.252		0.301					
ILC 2555	Control	6345	4.5 ± 0.2	-	38.8 ± 3.6	-	61.2 ± 3.6	-	0.0 ± 0.0	-	0.0 ± 0.0	-	0.0 ± 0.0	-	0.0 ± 0.0	-	0.5 ± 0.1	-	16.8	0.001
	50 Gy	3540	4.6 ± 0.3	ns	20.8 ± 11.0	ns	0.0 ± 0.0	*	26.7 ± 18.7	ns	0.0 ± 0.0	ns	41.7 ± 21.3	*	10.8 ± 5.8	*	2.6 ± 0.3	*		
	100 Gy	3050	4.5 ± 0.0	ns	9.6 ± 0.8	*	51.2 ± 1.2	ns	39.2 ± 1.8	*	0.0 ± 0.0	ns	0.0 ± 0.0	ns	0.0 ± 0.0	ns	2.8 ± 0.2	*		
	200 Gy	4150	4.7 ± 0.3	ns	9.4 ± 4.8	*	36.5 ± 9.6	*	41.7 ± 4.4	*	7.5 ± 1.8	*	0.0 ± 0.0	ns	4.9 ± 2.4	ns	3.9 ± 0.5	*		
	One-way ANOVA		0.825		0.033		0.00		0.054		0.001		0.057		0.12					

Mean values ± SE (n = 5), and p-values (ANOVA) are presented. ns = non-significant difference, * denotes significant difference against the control in the same variety at $p < 0.05$ according to LSD test. Abbreviations: MI, Mitotic Index, Lag. Chrom., Laggard chromosome, Sticky metaphase, Micronucleu

Table 2: Morphological measurements of chickpea plants following seed exposure to NaCl treatments alone and in combination with *γ*-radiation

Variety	Treatment	Shoot length (cm)	LSD	Root length (cm)	LSD	No. of leaves / plant	LSD	Fresh biomass (g) Shoot	LSD	Fresh biomass (g) Root	LSD	Dry biomass (g) Shoot	LSD	Dry biomass (g) Root	LSD
FLIP 81-71	Control	24.33 ± 0.92	-	8.77 ± 0.15	-	25.00 ± 3.22	-	1.33 ± 0.04	-	0.45 ± 0.03	-	0.31 ± 0.02	-	0.07 ± 0.01	-
	25 mM NaCl salt	19.33 ± 1.86	*	8.07 ± 0.87	ns	20.33 ± 3.84	ns	0.9 7 ± 0.11	*	0.49 ± 0.09	ns	0.28 ± 0.003	ns	0.06 ± 0.006	ns
	60 mM NaCl salt	13.83 ± 1.59	*	4.67 ± 0.58	*	6.00 ± 0.58	*	0.53 ± 0.088	*	0.30 ± 0.058	ns	0.21 ± 0.009	*	0.03 ± 0.003	*
	50 Gy+25 mM NaCl	23.00 ± 1.53	ns	8.50 ± 0.35	ns	16.67 ± 0.88	*	1.43 ± 0.075	ns	0.55 ± 0.029	ns	0.33 ± 0.012	ns	0.08 ± 0.003	ns
	100 Gy+25 mM NaCl	25.00 ± 1.53	ns	7.03 ± 0.61	*	23.00 ± 1.16	ns	1.30 ± 0.058	ns	0.38 ± 0.103	ns	0.36 ± 0.073	ns	0.03 ± 0.003	*
	200 Gy+25 mM NaCl	20.33 ± 0.88	ns	7.70 ± 0.49	ns	23.33 ± 0.88	ns	1.10 ± 0.058	*	0.26 ± 0.006	*	0.31 ± 0.039	ns	0.03 ± 0.000	*
	50 Gy+60 mM NaCl	13.33 ± 2.40	*	6.10 ± 0.31	*	7.67 ± 1.20	*	0.78 ± 0.012	*	0.36 ± 0.024	ns	0.28 ± 0.007	ns	0.03 ± 0.002	*
	100 Gy+60 mM NaCl	17.33 ± 1.20	*	5.50 ± 0.68	*	14.00 ± 1.16	*	1.05 ± 0.053	*	0.24 ± 0.023	*	0.33 ± 0.012	ns	0.03 ± 0.000	*
	200 Gy+60 mM NaCl	17.67 ± 0.33	*	4.93 ± 0.30	*	12.00 ± 11.16	*	0.92 ± 0.009	*	0.25 ± 0.006	*	0.28 ± 0.038	ns	0.03 ± 0.000	*
FLIP 84-188	Control	21.17± 0.17	-	10.83 ± 1.09	-	22.00 ± 3.06	-	1.38 ± 0.034	-	0.50 ± 0.024	-	0.22 ± 0.019	-	0.05 ± 0.003	-
	25 mM NaCl salt	19.17 ± 2.40	ns	6.50 ± 1.53	*	20.00 ± 5.03	ns	0.62 ± 0.100	*	0.32 ± 0.107	*	0.18 ± 0.015	ns	0.03 ± 0.013	ns
	60 mM NaCl salt	20.17 ± 0.93	ns	8.67 ± 0.44	*	9.67 ± 1.20	*	1.04 ± 0.028	ns	0.32 ± 0.023	*	0.21 ± 0.018	ns	0.03 ± 0.000	*
	50 Gy+25 mM NaCl	9.83 ± 0.93	*	4.87 ± 0.32	*	17.33 ± 1.45	ns	0.52 ± 0.108	*	0.14 ± 0.009	*	0.16 ± 0.022	*	0.01 ± 0.001	*
	100 Gy+25 mM NaCl	15.00 ± 2.89	*	6.50 ± 0.58	*	19.67 ± 1.20	ns	0.97 ± 0.274	*	0.31 ± 0.022	*	0.16 ± 0.023	*	0.02 ± 0.005	*
	200 Gy+25 mM NaCl	11.00 ± 1.16	*	5.20 ± 0.25	*	11.67 ± 0.88	*	0.62 ± 0.079	*	0.24 ± 0.007	*	0.12 ± 0.006	*	0.02 ± 0.001	*
	50 Gy+60 mM NaCl	12.00 ± 1.16	*	4.83 ± 0.15	*	7.00 ± 1.16	*	0.67 ± 0.064	*	0.19 ± 0.026	*	0.19 ± 0.024	ns	0.02 ± 0.001	*
	100 Gy+60 mM NaCl	12.67 ± 0.88	*	6.43 ± 0.41	*	8.33 ± 0.33	*	0.72 ± 0.051	*	0.29 ± 0.015	*	0.13 ± 0.009	*	0.03 ± 0.001	*
	200 Gy+60 mM NaCl	16.33 ± 0.88	*	9.40 ± 0.21	ns	9.33 ± 0.67	*	0.62 ± 0.129	*	0.35 ± 0.018	*	0.23 ± 0.034	ns	0.04 ± 0.003	ns
FLIP 97-263	Control	26.83 ± 1.92	-	5.90 ± 0.86	-	21.67 ± 3.283	-	2.10 ± 0.048	-	0.42 ± 0.052	-	0.64 ± 0.018	-	0.07 ± 0.009	-
	25 mM NaCl salt	24.67 ± 0.93	ns	4.93 ± 0.98	ns	18.67 ± 2.728	ns	0.65 ± 0.036	*	0.21 ± 0.064	*	0.33 ± 0.024	*	0.04 ± 0.007	*
	60 mM NaCl salt	19.67 ± 2.60	*	3.90 ± 0.31	*	12.33 ± 0.882	*	0.75 ± 0.124	*	0.24 ± 0.045	*	0.30 ± 0.027	*	0.04 ± 0.007	*
	50 Gy+25 mM NaCl	18.83 ± 0.60	*	4.27 ± 0.50	*	18.33 ± 3.756	ns	1.02 ± 0.089	*	0.30 ± 0.072	ns	0.45 ± 0.057	*	0.05 ± 0.003	ns
	100 Gy+25 mM NaCl	18.33 ± 1.67	*	8.07 ± 0.12	*	14.67 ± 1.764	*	1.24 ± 0.121	*	0.44 ± 0.035	ns	0.51± 0.035	*	0.07 ± 0.009	ns
	200 Gy+25 mM NaCl	16.67 ± 2.40	*	3.87 ± 0.34	*	13.33 ± 1.764	*	1.05 ± 0.187	*	0.2833 ± 0.022	*	0.48 ± 0.055	*	0.05 ± 0.002	ns
	50 Gy+60 mM NaCl	16.00 ± 1.53	*	3.60 ± 0.23	*	18.67 ± 0.882	ns	0.76 ± 0.067	*	0.26 ± 0.021	*	0.33 ± 0.013	*	0.05 ± 0.001	ns
	100 Gy+60 mM NaCl	12.33 ± 1.45	*	7.97 ± 0.24	*	13.00 ± 1.155	*	0.66 ± 0.023	*	0.43 ± 0.032	ns	0.33 ± 0.021	*	0.06 ± 0.006	ns
	200 Gy+60 mM NaCl	14.00 ± 1.53	*	5.17 ± 0.524	ns	7.33 ± 1.202	*	0.88 ± 0.209	*	0.3867 ± 0.039	ns	0.40 ± 0.073	*	0.06 ± 0.007	ns

Table 2 continued

ILC 72	Control	24.30 ± 2.00	-	3.67 ± 0.44	-	17.00 ± 1.00	-	0.68 ± 0.09	-	0.06 ± 0.003	-	0.30 ± 0.012[a]	-	0.011 ± 0.001	-
	25 mM NaCl salt	18.50 ± 2.08	*	4.50 ± 1.53	ns	15.33 ± 0.88	ns	0.77 ± 0.09	ns	0.17 ± 0.064	*	0.3± 0.015	ns	0.029 ± 0.011	*
	60 mM NaCl salt	14.33 ± 0.88	*	3.43 ± 0.35	ns	7.67 ± 0.88	*	0.74 ± 0.07	ns	0.10 ± 0.008	ns	0.32 ± 0.015	ns	0.017 ± 0.001	ns
	50 Gy+25 mM NaCl	18.33 ± 1.20	*	4.90 ± 0.21	ns	20.33 ± 1.45	ns	0.78 ± 0.04	ns	0.14 ± 0.019	*	0.30 ± 0.018	ns	0.023 ± 0.003	*
	100 Gy+25 mM NaCl	24.00 ± 0.58	ns	5.37 ± 0.59	ns	19.00 ± 3.61	ns	1.15 ± 0.11	*	0.07 ± 0.012	ns	0.46 ± 0.044	*	0.011 ± 0.002	ns
	200 Gy+25 mM NaCl	20.67 ± 1.20	ns	5.30 ± 0.47	ns	18.67 ± 0.88	ns	0.96 ± 0.09	*	0.10 ± 0.007	ns	0.39 ± 0.035	*	0.016 ± 0.001	ns
	50 Gy+60 mM NaCl	14.87 ± 0.59	*	4.07 ± 0.58	ns	12.00 ± 0.58	*	0.41± 0.01	ns	0.08 ± 0.009	ns	0.16 ± 0.006	*	0.014 ± 0.002	ns
	100 Gy+60 mM NaCl	13.57 ± 0.96	*	6.20 ± 0.46	*	10.33 ± 0.88	*	0.36 ± 0.03	ns	0.10 ± 0.009	ns	0.14 ± 0.015	*	0.017 ± 0.001	ns
	200 Gy+60 mM NaCl	13.43 ± 0.74	*	4.57 ± 0.52	ns	11.67 ± 0.33	*	0.38 ± 0.04	ns	0.09 ± 0.003	ns	0.15 ± 0.018	*	0.014 ± 0.001	ns
ILC 464	Control	28.73 ± 1.47	-	9.8 3 ± 0.73	-	22.67 ± 2.67	-	1.79 ± 0.243	-	0.43 ± 0.123	-	0.48 ± 0.066	-	0.06 ± 0.012	-
	25 mM NaCl salt	24.17 ± 0.44	*	9.00 ± 0.29	ns	16.33 ± 2.03	*	1.06 ± 0.045	*	0.31 ± 0.009	ns	0.29 ± 0.012	*	0.05 ± 0.001	ns
	60 mM NaCl salt	20.00 ± 1.16	*	6.33 ± 0.60	*	10.33 ± 1.45	*	0.98 ± 0.015	*	0.26 ± 0.015	*	0.26 ± 0.004	*	0.04 ± 0.003	*
	50 Gy+25 mM NaCl	26.00 ± 0.58	ns	11.17 ± 0.44	ns	18.00 ± 1.16	ns	1.35± 0.035	*	0.59 ± 0.009	ns	0.37 ± 0.009	*	0.09 ± 0.001	*
	100 Gy+25 mM NaCl	28.67 ± 0.88	ns	11.50 ± 0.76	ns	22.00 ± 0.56	ns	1.98 ± 0.079	ns	0.60 ± 0.013	*	0.53 ± 0.021	ns	0.09 ± 0.002	*
	200 Gy+25 mM NaCl	18.00 ± 1.16	*	10.97 ± 0.26	ns	19.6 7± 0.88	ns	1.03 ± 0.091	*	0.60 ± 0.003	*	0.28 ± 0.025	*	0.09 ± 0.000	*
	50 Gy+60 mM NaCl	17.50 ± 2.02	*	7.50 ± 1.26	*	15.00 ± 4.73	*	0.92 ± 0.092	*	0.36 ± 0.102	ns	0.25 ± 0.025	*	0.05 ± 0.013	ns
	100 Gy+60 mM NaCl	18.83 ± 0.73	*	6.50 ± 0.29	*	10.67 ± 0.88	*	0.95 ± 0.037	*	0.26 ± 0.009	ns	0.26 ± 0.010	*	0.04 ± 0.003	*
	200 Gy+60 mM NaCl	17.00 ± 1.16	*	6.83 ± 0.44	*	10.67 ± 0.88	*	0.91 ± 0.039	*	0.28 ± 0.009	ns	0.25 ± 0.011	*	0.05 ± 0.002	*
ILC 484	Control	19.67 ± 1.01	-	4.27 ± 0.56	-	21.00 ± 2.52	-	1.44 ± 0.23	-	0.26 ± 0.04	-	0.49 ± 0.08	-	0.067 ± 0.009	-
	25 mM NaCl salt	17.00 ± 0.58	ns	6.93 ± 0.12	*	33.00 ± 2.03	ns	1.79 ± 0.10	ns	0.42 ± 0.01	*	0.6 ± 0.03	ns	0.103 ± 0.003	*
	60 mM NaCl salt	14.00 ± 0.58	*	9.57 ± 0.54	*	8.33 ± 0.88	*	1.3 ± 0.12	ns	0.47 ± 0.05	*	0.35 ± 0.08	ns	0.028 ± 0.002	*
	50 Gy+25 mM NaCl	25.50 ± 1.32	*	6.47 ± 0.35	*	25.00 ± 1.73	ns	1.89 ± 0.16	ns	0.14 ± 0.01	*	0.64 ± 0.06	ns	0.022 ± 0.001	*
	100 Gy+25 mM NaCl	21.33 ± 1.20	ns	8.57 ± 0.35	*	24.33 ± 1.76	ns	1.53 ± 0.24	ns	0.26 ± 0.02	ns	0.45 ± 0.08	ns	0.034 ± 0.003	*
	200 Gy+25 mM NaCl	24.00 ± 1.15	*	8.50 ± 0.38	*	22.00 ± 1.15	ns	1.72 ± 0.05	ns	0.28 ± 0.02	ns	0.58 ± 0.02	ns	0.036 ± 0.003	*
	50 Gy+60 mM NaCl	15.33 ± 1.45	*	6.17 ± 0.67	*	13.00 ± 1.15	*	1.18 ± 0.04	ns	0.28 ± 0.02	ns	0.4 ± 0.02	ns	0.027 ± 0.002	*
	100 Gy+60 mM NaCl	14.33 ± 1.45	*	7.73 ± 0.24	*	10.67 ± 1.20	*	0.81 ± 0.36	ns	0.21 ± 0.01	ns	0.27 ± 0.12	ns	0.027 ± 0.001	*
	200 Gy+60 mM NaCl	16.00 ± 1.15	*	6.13 ± 0.49	*	12.67 ± 1.45	*	0.83 ± 0.37	ns	0.24 ± 0.02	ns	0.28 ± 0.13	ns	0.031 ± 0.003	*

Continued on the next page

ILC2555	Control	22.83 ± 0.73	-	10.17 ± 1.01	-	24.67 ± 0.88	-	1.26 ± 0.130	-	0.33 ± 0.110	-	0.24 ± 0.045	-	0.03 ± 0.006	-
	25 mM NaCl salt	23.83 ± 1.59	ns	8.67 ± 0.44	ns	20.33 ± 1.45	*	1.35 ± 0.231	ns	0.22 ± 0.092	ns	0.25 ± 0.066	ns	0.01 ± 0.003	*
	60 mM NaCl salt	21.17 ± 0.60	ns	7.33 ± 0.60	*	10.33 ± 0.88	*	0.97 ± 0.072	ns	0.25 ± 0.065	ns	0.19 ± 0.012	ns	0.03 ± 0.006	ns
	50 Gy+25 mM NaCl	23.17 ± 0.60	ns	10.73 ± 0.19	ns	25.67 ± 1.76	ns	1.04 ± 0.022	ns	0.37 ± 0.072	ns	0.24 ± 0.012	ns	0.04 ± 0.003	*
	100 Gy+25 mM NaCl	25.33± 2.19	ns	7.90 ± 0.70	*	24.67 ± 0.88	ns	1.48 ± 0.231	ns	0.49 ± 0.198	ns	0.34 ± 0.023	*	0.05 ± 0.006	*
	200 Gy+25 mM NaCl	18.50 ± 1.26	*	5.83 ± 0.52	*	11.67 ± 0.88	*	1.04 ± 0.134	ns	0.35 ± 0.045	ns	0.21 ± 0.021	ns	0.03 ± 0.003	ns
	50 Gy+60 mM NaCl	20.00 ± 2.08	ns	9.07 ± 0.23	ns	11.67 ± 0.88	*	1.24 ± 0.084	ns	0.26 ± 0.095	ns	0.26 ± 0.018	ns	0.03 ± 0.006	ns
	100 Gy+60 mM NaCl	20.00 ± 1.15	ns	6.60 ± 0.47	ns	10.67 ± 0.67	*	1.06 ± 0.054	ns	0.39 ± 0.042	ns	0.26 ± 0.009	ns	0.04 ± 0.003	ns
	200 Gy+60 mM NaCl	16.33 ± 1.20	*	3.97 ± 0.29	ns	10.00 ± 1.00	*	0.67 ± 0.021	*	0.28 ± 0.025	ns	0.18 ± 0.006	ns	0.03 ± 0.000	ns

Mean values ± SE are presented (n = 5). ns = non-significant difference, * denotes significant difference against the control in the same variety at $p < 0.05$ according to LSD test

under all treatments except combination of 50 Gy and 25 mM NaCl treatments. The number of leaves per plant increased by the treatments with the 25 mM NaCl and its contribution with all doses of γ-radiation in 'ILC 484', while the *γ*-radiation doses in combination with the 25 mM and 60 mM NaCl induced significant reduction in shoot length, number of leaves and shoot and root biomass comparing to the control and salt treatment in 'FLIP 97-263'. The combination of 100 Gy *γ*-radiation and 25 mM salt treatment induced significant increase in shoot fresh biomass comparing to the salt treatment only in 'FLIP 84-188' (Table 2).

4 DISCUSSION

All varieties used in the current study, except 'ILC 484', germinated in the control range when irradiated low doses of γ-radiation (50 Gy, 100, Gy 200 and 300 Gy) , which in agreement with Shah et al. (2008), who reported that germination was not affected in the desi variety Pb2000 at γ-radiation doses of 100 Gy, 200 Gy and 300 Gy. High doses of γ-radiation (400 Gy, 500 Gy and 600 Gy) on the other hand decreased the GP significantly compared with the low doses and the control. The inhibition of germination, seedling growth, and other biological responses were frequently observed (Abdelfattah Badr et al., 2014; Kim, Lee, Back, Kim, & Lee, 2000; Toker et al., 2005). The reduction of GP at high doses of γ-radiation, has been reported in many plants including chickpea (Joshi-Saha et al., 2015; Melki & Sallami, 2008; Shah et al., 2008). Low doses of irradiation, like low levels of other abiotic stresses, may increase the anti-oxidative capacity of the cells by producing ROS that mediate the acceleration of cell cycle entry to G0/G1 leading to a positive effect on the plant cell cycle machinery (Feher, Ötvös, Pasternak, & Pettkó-Szandtner, 2008; Sharma et al., 2012). On the contrary, high doses of γ-radiation may result in cell cycle arrest at G2/M phase during somatic cell division and/or damage in the genome (El-Azab et al., 2018; Preuss & Britt, 2003). However, the cytogenetics during germination under abiotic stress is not well understood and requires attention.

The retarded germination of seeds exposed to high doses of NaCl stress and the slow growth of seedlings under these treatments may be associated with slow cell division at the early emergence of seminal root and shot. It is widely accepted that the first action of abiotic stress on germination is moisture deficit resulting in poor plant stand at the early seedling phase and hampers early crop establishment (Kaydan & Yagmur, 2008; Shao, Chu, Jaleel, & Zhao, 2008). Mitotic index was approved as an efficient short-term genetic bioassay via the United States Environmental Agency through the Gene-Tox Program in 1981 (Waters & Auletta, 1981) and was used as an indicator to characterize the cell activity and proliferation (Scofield, Jones, & Murray, 2014). Low doses of γ-radiation induced an increase in the proportion of dividing cells, whereas higher doses resulted in reduction in mitotic activity. A dose-dependent increase in mitotic indices was observed in cowpea following exposure to γ-radiation ranging from 10 to 300 Gy (Girija, Gnanamurthy, & Dhanavel, 2013). Similar findings were also found in cowpea cultivars (Abdelfattah Badr et al., 2014) and in soybean cultivars (El-Azab et al., 2018). In plant root tips, arrest in cell cycle progression is caused by check points that mediate the entry of cells into S-phase and mitosis (De Veylder, Joubès, & Inzé, 2003). The cell often spontaneously continues cycle progression, but this is often followed by genome instability allowing cell survival at the cost of tolerating mutation including chromosomal abnormalities (Hartig & Beck, 2006).

As explained in the results section, the cytological effects of γ-radiation on cell division in the root tip mitosis was made on plants following exposure to the low doses (50, 100 and 200 Gy). Higher γ-radiation doses from (300 to 600 Gy) caused degradation of most nuclear membranes in the root meristematic cells of all varieties. This result is in agreement with Arian and Maqbool (2011) who reported that doses of 150 to 300 Gy induced oxidative damages and inhibition of cell division in chickpea root tip cells. The γ-radiation also affected the cell division phases forming different abnormality types. The total abnormalities percent showed a highly significant difference at all the studied varieties. The total number of abnormal cells increased with the increase of γ-radiation doses of all the studied varieties. Similar result was reported by (Wani, 2009) in chickpea following γ-radiation and ethyl methane sulphonate and their combination treatments.

Chromosomal abnormalities induced by γ-radiation include stickiness of chromosomes (Dhanavel, Gnanamurthy, & Girija, 2012). The highest value of sticky metaphase was recorded in 'ILC 464' at 200 Gy. Chromosome stickiness might be formed due to changes in specific non-histone proteins, histone proteins and DNA breaks induced during chromosome condensation (Piskadlo, Tavares, & Oliveira, 2017). The appearance of free and the lagging chromosomes was more frequent in all the treatments in the studied chick pea varieties except at 50 Gy in 'ILC 2555'. The lagging chromosomes at ana-telophase might be formed due to the failure of spindle fibers to push the respective chromosomes to the poles because of exposure to

γ-irradiations. The ataxia telangiectasia and Rad3-related (ATR) plays an essential role in suppressing replication stress from DNA damage. A mitosis-specific and R loop–driven ATR pathway supports faithful chromosome segregation, preventing formation of lagging chromosomes (Kabeche, Nguyen, Buisson, & Zou, 2018). Chromosomal bridges are commonly attributed to dicentric chromosomes originating from chromosome exchange after chromosome double strand breaks (Cornforth & Goodwin, 1991). Chromosome breakage is usually considered to involve the DNA molecule responsible for the linear stability of the chromosome. This aberration is the result of unfinished repair of DNA (Grant, 1978). Micronuclei usually arise from lagging chromosomes and fragments, which fail to reach the pole region in time and are included in the daughter cells as micronuclei (A Badr, 1986; Kumar, 1998). The micronuclei were more frequency observed in cells exposed to γ-radiation at low dose of 50 Gy, in all the varieties. The number of micronuclei could illustrate the individual sensitivity level to mutagens (Koteles, 1996; Köteles, Bojtor, Szirmai, Berces, & Otos, 1993).

All plants exposed to 60 mM NaCl treatment died before reaching maturity. This result is in agreement with Khan, Siddique, Munir, and Colmer (2015) who stated that salinity severely inhibited plant growth, and led to some tissue death resulting in plant deaths. Haleem (2012) reported that high concentrations of NaCl treatments at 50, 100 and 200 mM caused depression in plant growth, total soluble protein content, photosynthetic pigments content, nucleic acids contents and all yield characteristics, and concluded that seed irradiation with γ-rays moderates the adverse effect of salinity stress compared to non-irradiated seeds. Khan et al. (2015) stated that the 60 mM NaCl treatment also reduced stem and root dry mass of all chickpea genotypes when compared to their controls. Even at low (20 mM and 25 mM) salt concentration, chickpea growth was reduced significantly (Sadiki & Rabih, 2001). Salinity of 3 dS m^{-1} in field soils was reported to be the threshold for reduced shoot growth and yield in chickpea (Katerji, Van Hoorn, Hamdy, Mastrorilli, & Oweis, 2005; Rao, Giller, Yeo, & Flowers, 2002).

5 CONCLUSION

The γ-radiation doses above 300 Gy induced degradation of nuclear membranes, whereas lower doses did not affect or slightly enhanced mitotic activities but induced different types of chromosomal abnormalities. The total number of abnormal cells increased with the increase of γ-radiation doses in all the studied varieties. Gamma-rays induced various types of qualitative and quantitative chromosomal aberration including chromosome bridges, laggard chromosomes, stickiness, chromosome breakage and micronuclei. The salinity treatments at 25 mM NaCl and 60 mM NaCl reduced seedling's growth of all cultivars estimated as root and shoot length and biomass production. The application of γ-rays can moderate the adverse effect of low levels of salinity stress compared to non-irradiated seeds. The γ-radiation dose of 100 Gy alleviated the impact of NaCl salinity in chickpea plants at a concentration of 25 mM NaCl for all varieties, except 'FLIP 84-188'and 'FLIP 97-263'. On the other hand, the 60 mM NaCl treatment significantly reduced early growth of all cultivars and its effect was not alleviated by the γ-radiation application.

6 STATEMENTS AND DECLARATIONS

Conflict of interest: The authors declare no competing interests.

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