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Sophisticated Genetic Studies on Onion through Using Gamma Rays

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ABSTRACT

Morphological, molecular and cytogenetically studies were revealed on fresh and old seeds of the onion cultivar (Giza 20) where it is considering the most famous genotype at the national research program of onion crop because it has growth, vitality and quality traits unrivaled and extensive agricultural spreading, especially in northern Egypt. The present investigation included fourteen traits of germination, viability, growth and quality in addition; cytogenetic and molecular identification using protein banding patterns electrophoresis (SDS-PAGE) for two classes of dry onion seeds (fresh and old lots). Both types of dry onion seeds were irradiated before planting with five doses of gamma rays; (10, 20, 40, 80, 100 RD), respectively then the agriculture process was done for the irradiated seeds of both onion lots beside the control. All measurements were calculated for the six doses of gamma rays (five doses and the control), while simple correlation coeffients was done for the optimum dose (10 RD) only. After holding all function measurements can reveal that the doses (10 , 20 , 80 RD) were the better and detected highly influential of germination , growth and quality traits of onion seeds and contributed to increase the level of genetic improvement compared to the control dose. From cytological investigation, only fresh lots have positive effects from radiation doses, where mitotic division have significant increase after (10 and 80 RD) doses and chromosomal aberrations increased but still in save limits , whilst mitotic indies as well as chromosomal aberrations of old lots have negative effects mainly after (20 and 40 RD) doses. Results of protein analysis revealed observation changes with the naked eye in protein profile between old and fresh seeds, where disappearance of some bands in old lots after gamma irradiation as well as in (80 and 100 RD) compared to fresh seeds. We can rank the three previous optimum doses according to the importance of genetic improvement and the percentages of positive induced mutagenesis from high to low as follow; (10 then 80 then 20 RD) in addition to prove that fresh seeds have been outperforming than old seeds not only in idealism three doses, but also in the rest of doses (40, 100 RD). This measure is a necessary and effective in the detection of commercial fraud cases resulting from mixing both lots of onion seeds. Radiation using gamma rays has positive benefits to fresh lots of onion seeds specially the optimum dose (10 RD).

Key words: Onion, Gamma irradiation, Mutagenesis, SDS-PAGE, Cytogenetic investigation, mitotic index and chromosomal aberrations.

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INTRODUCTION

Onion crop has been grown and used for thousands years BC not only as a food required to make a distinctive flavor, but also because it possesses a wide range of medical and medicinal properties, as well as the presence of multiple species such as white, red, yellow and green onion and each type has different taxonomic characteristics. Onion crop is considering one of the most vital and important food for humans and Egypt accounts 70% of the African production and its production is concentrating in the north of Egypt as a vegetable crop with high nutritional value.

If we reviewed the medical benefits of onions, we find that many uses of it such as nourishes the facial skin, enhancing the growth of hair follicles, scaling the peel as well as its vital and effective in strengthening the vision of the eye, a source of vitamins B6, anti-bacteria and fungi, treatment of asthma and tuberculosis, pneumonia, insomnia and also harmful to urine. Therefore, improvement the growth and germination of this crop were one of the priorities of onion research department in Egypt through traditional breeding programs such as hybridization among lines with highly criteria , then simple selection in the first generation and continues this stage several times to reach to genotypes of onion highly genetic stability which were related to resistance for environmental stresses such as salinity and water deficit conditions and enhancing seedling germination and quality traits , or through modern programs of improvement and development, such as biotechnology and gene transfer within molecular biology to achieve the same goals. Improvement using mutation is considering one of the most important programs for the improvement aims for the previous traits in onion seeds , through exposure the seeds or plants for different doses of cosmic rays such as gamma irradiation , which will have the fruitful and vital significant changes in the composition of DNA through increasing or decreasing the number of chromosomes and this will be a positive or negative change according for the desired traits to reach to safe doses on human health and surrounding organisms after eating the radioactive food. This will be achieved through the greatest effect on improving the strength and efficiency of seedling growth in onion seeds before planting. After all that has been listed, we can summarize the most important papers that were launched to improve onion and other crops through inducing mutagenesis using gamma rays.

Mitotic index is the most cellular factor that allows us to evaluate the regularity of cellular division **[1].** However, any positive or negative changes in mitotic division are explanation indicators in monitoring ecological pressure **[2].**The inhibition of mitotic action was may be due to the blocking of G1, suppressing cells from DNA synthesis **[3]** or the hindering of G2, blocking cells from entering mitosis **[4].** The Allium test can be obtained by seed germination **[5].** *Allium cepa* test has been considered an efficient test to show the existence of mutagenicity **[6]**, due to its kinetic characteristics of proliferation and chromosomes appropriate for this form of study **[7].** The chromosome abnormality test on root meristematic cells, used since 1938, **[8].** Many scientific studies have been detected the effect of radiation on onion plants, **[1] , [9-15].**

The aim of the current investigation is trying to know the effect of different doses of gamma rays on germination , growth and quality characteristics of onion crop including fresh and old seeds beside assessment molecular markers including protein banding patterns electrophoresis (SDS-PAGE) and cytogenetic parameters on the grounds that all these estimates are selecting on the basis rank to determine the appropriate and optimum dose of gamma rays which increasing the quality and germination seeds percentages and all the rest of morphological taxonomic traits.

MATERIALS AND METHODS

Laboratory and pot experiments were carried out at (Seed Technology Research Unit, Mansoura, Dakahlia Governorate, Seed Technology Research Department, Field Crops Research Institute, Agricultural Research Center, Genetic and Cytology Department, Genetic Engineering and Biotechnology Division, National Research Centre, Cairo, Dokki, Giza, Egypt) during 2016 season. Onion dry seeds included (fresh and old lots) of (Giza 20) were obtained from Central Administration for Seed Production (*CASP*) and irradiating before planting with five different doses of gamma rays (; i.e. 10, 20, 40, 80 and 100 RD, beside the control dose) induced by CO66 in Atomic Energy Commission to evaluate the effects of different gamma doses on high and low vigor of onion seeds, quality , vitality , germination % , seedling parameters, field emergence, cytological and molecular parameters, respectively.

Treatments and Experiments Design:-

Lab and pots experiments under greenhouse conditions were taken place; Lab. experiment performed with sterile Petri dishes of 15 cm diameter on filter paper beds in growth chambers. 30 seeds of each dose beside the control treatment (six treatments) were sown in single Petri dish for each dose on filter paper beds. Pots experiment was performed under greenhouse with plastic pots of 15 cm diameter and filled with 1 kg of air dried loamy soil which placed in oven for 1 day at 75°C. Onion dry seeds (fresh and old lots for the six treatments) were sown at the rate of 30 seeds / pot. Seeds were sown at the depth of 3 cm and replicated three times for the six treatments, irrigation was applied whenever required. Data regarding seedling emergence were recorded up to the 14 days of sowing and the plants were harvested after 15 days of germination. The treatments were arranged in Factorial Design in Randomize Complete Block Design (RCBD) with three replicates.

Note: - All traits studied were measurements for the two types of onion lots (fresh and old seeds).

Each dose for the six treatments (five doses beside the control) was sown in individual Petri dish three times.

Studied Traits:-

Fourteen traits were calculated for fresh and old onion seeds under the control and five doses of gamma rays after planting namely.; germination % , fresh weight , seedling dry weight , shoot length , root length , seedling length , germination energy % , SVI1 , SVI2 , germination rate , speed of germination , mean germination time , EC and field emergence , respectively.

Note :- (SVI) is means seedling vigor index and both types were calculated by **[16, 17],** field emergence was conducted according to the method by **[18],** Electrical conductivity by **[19 , 20]** and modified methods by **[21 , 22]** , respectively.

Statistical Analysis:-

All data were statistically analyzed as a factorial experiment design as the technique of analysis of variance (ANOVA) and described by **[23]**.

Improvement resulting from the irradiation:-

It was assessment by (the value of each trait for each dose of gamma rays) – (the value of each trait under the control dose) / (the value of each trait under the control dose) X 100.

Simple Correlation Coeffients:-

It was calculated for fresh and old onion seeds and described for all traits studied under the optimum dose of gamma rays (10 RD) only by **[24 , 25].**

Cytogenetic Characterization:-

The root tips were used for cytogenetic investigation after both fresh and old seeds were geminated, when roots were grown and reached about 1–1.5 cm in length, roots were fixed in ethanol : acetic acid (3:1) for 24 h, hydrolyzed in 1 M HCl for 12 min then stained with aceto-orcien for 24 h. Root tips were cut off in a drop of 45% acetic acid, macerated and squashed **[26].** Three replicates were performed for each treatment and scoring about 3000 cells which were done from at least 5 roots of each replicate. Mitotic Index (MI), frequencies of mitotic phases (prophase, metaphase, anaphase and telophase), RDR (relative division rate) calculated by the formula **[27].**

RDR % = (% of dividing cells in treated sample - % of dividing cells in control sample / (100 - % of dividing cells in control bulbs) X 100

Mitotic abnormalities were used as endpoints for determination of cytogenetic effects. The MI was calculated as the ratio between the number of mitotic cells and the total number of scored cells and expressed as percentage. The frequency of mitotic abnormalities was expressed as a percentage in relation to the number of cells in mitosis.

Qualitative characterization of protein using gel electrophoresis:

Storage protein in root tips of the two treated seeds of *Allium cepa* plants were analyzed using discontinuous polyacrylamide gel electrophoresis in the presence of sodium dodecylsulphate (i.e.) SDS-PAGE according to **[28],** Protein analyzer program was used for data analysis.

Statistical Analysis:-

All data were statistically analyzed as a factorial experiment design as the technique of analysis of variance (ANOVA) and described by **[23].**

Some Abbreviations:-

G%: Germination percentage , F.W: Fresh weight , S.D.W: Seedling dry weight , S.L: Shoot length , R.L: Root length , S. Leng : Seedling length , G.E %: Germination energy percentage , SVI1: Seedling vigor index one , SVI2: Seedling vigor index two , G.R: Germination rate , M.G.T: Mean germination time , S. of. G: Speed of germination, EC: Electrical conductivity, F.E: Field emergence, RD: Rad.

RESULTS AND DISCUSSION

Mean Performance:-

The obtained data from all traits studied in onion seeds (fresh and old lots) for all doses of gamma irradiation beside the control are shown in table (1). Firstly we observed that all mean values for the fourteen traits of fresh onion seeds were better than the mean values of old seeds. The highest mean values were detected in the traits ;(germination % , fresh weight , seedling dry weight , seedling length , germination energy % , germination rate , root length , shoot length , mean germination time , speed of germination , SVI2 and field emergence for the doses (10 , 20 , 80 RD) and the dose of (10 RD) only for SVI1which revealed highly values of genetic improvements under gamma irradiation for the class one of lots (fresh seeds) while the same traits not recorded any positive results or genetic improvements under the other doses (40 , 100 RD) compared with the control dose of gamma rays, in addition these results were less advantage in old seeds , respectively.

The values of EC trait were low under exposure for the three doses of gamma irradiation (10 , 20 , 80 RD) in both classes of lots and this was clearly evident in fresh lots more clearly than old lots indicating a high storage efficiency in fresh lots, and then the reason is probably due to the fresh seeds prevented transmission the food ingredients of seeds from the inside to the outer shell of the grain (pill cover) and reduced the migration of proteins , carbohydrates and transition to the outside of cover as well as prevent decomposition and maintain of it for a long time during the storage period.

It was similar in excellence the average data obtained in most traits studied under exposed to doses of (10, 20 and 80 RD) and given the highest effect for improving seeds and quality traits in onion lots compared with the control in fresh and old types, but of course it was highly in fresh lots more than the other one, while the (40,100 RD) doses did not achieve any progress or the impact of genetic improvement mentioned in the previous types of lots. These two doses had opposite effect and whenever increasing the dose of irradiation over all (80 RD) whenever decreasing all traits studied beside increasing the values of EC trait in seeds, So we can say that the best doses which causes full genetic improvement in most traits studied were (10, 20 and 80 RD) doses, respectively, **[29, 30] and [14, 15].**

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Table (1):- The Average data for all traits studied of onion seeds (Fresh and Old Lots) under the control and five doses of gamma rays.

Seed Treatments	Germination percentage		Fresh weight (mg)		Seedling dry weight (mg)		Shoot length (cm)		Root length (cm)		Seedling length(cm)		Germination Energy (%)	
A. Seed lots	F	O	F	O	F	O	F	O	F	O	F	O	F	O
Low vigor	65.0	60.0	0.155	0.145	0.016	0.012	7.50	7.0	7.0	4.88	14.0	13.50	64.0	33.0
High vigor	93.0	75.0	0.840	0.630	0.030	0.023	17.50	11.8	15.3	8.3	25.70	19.0	92.0	67.0
F. test	**	**	**	**	**	**	**	**	**	**	**	**	**	**
B. Gamma rays	F	O	F	O	F	O	F	O	F	O	F	O	F	O
Mean of Control	65.0	63.0	0.650	0.630	0.024	0.023	11.9	11.8	8.4	8.30	19.20	19.0	64.0	62.0
10 RD	93.0	61.0	0.690	0.590	0.027	0.014	13.0	7.4	15.3	6.60	23.95	18.35	92.0	42.0
20 RD	75.0	69.0	0.700	0.220	0.026	0.015	10.70	7.3	11.4	5.70	22.45	14.05	72.0	64.0
40 RD	69.0	65.0	0.155	0.145	0.017	0.013	9.30	7.2	8.0	5.0	16.0	13.90	67.0	40.0
80 RD	88.0	75.0	0.840	0.192	0.030	0.017	17.50	8.0	13.4	8.0	25.70	17.70	87.0	67.0
100 RD	69.0	60.0	0.188	0.177	0.016	0.012	7.5	7.0	7.0	4.88	14.0	13.50	70.0	33.0
Mean of Radiation	78.80	66.0	0.514	0.265	0.023	0.014	11.60	7.38	11.02	6.03	20.42	15.50	77.60	49.20
F. test	**	**	**	**	**	**	**	**	**	**	**	**	**	**
LSD at 0.05	1.12	1.65	0.033	0.024	0.013	0.005	3.34	3.65	1.88	2.06	1.06	1.15	1.33	1.65

F: Fresh lots, O: Old lots, **: Highly Significant.

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Table (1): Continued.

Seed Treatments	SVI1		SVI2		Germination rate		Speed of Germination		Mean Germination Time		EC		Field Emergence	
A. Seed lots	F	O	F	O	F	O	F	O	F	O	F	O	F	O
Low vigor	965.5	898.4	1.150	0.830	0.63	0.60	17.0	14.0	5.60	4.99	0.03	0.10	58.50	50.0
High vigor	2231.7	1329.2	2.520	1.620	0.78	0.73	26.0	21.0	6.99	6.75	0.19	0.19	90.50	70.0
F. test	**	**	**	**	**	**	**	**	**	**	**	**	**	**
B. Gamma rays	F	O	F	O	F	O	F	O	F	O	F	O	F	O
Mean of Control	1246.9	1244.8	1.650	1.620	0.67	0.65	19.0	18.5	6.85	6.75	0.12	0.12	60.50	57.5
10 RD	2231.7	1132.4	2.520	0.855	0.78	0.68	26.0	17.0	6.90	5.50	0.09	0.10	90.50	67.50
20 RD	1307.1	971.10	1.950	1.035	0.72	0.70	21.0	19.0	6.88	5.55	0.03	0.11	84.0	64.50
40 RD	1102.3	898.4	1.310	0.975	0.65	0.62	17.0	15.0	6.80	5.15	0.19	0.19	58.50	50.0
80 RD	1517.0	1329.2	2.025	1.275	0.77	0.73	25.50	21.0	6.99	5.55	0.08	0.10	88.0	70.0
100 RD	965.5	931.0	1.150	0.830	0.63	0.60	19.50	14.0	5.60	4.99	0.10	0.185	59.50	55.0
Mean of Radiation	1424.72	1052.42	1.791	0.994	0.71	0.66	21.80	17.20	6.63	5.35	0.098	0.137	76.10	61.40
F. test	**	**	**	**	**	**	**	**	**	**	**	**	**	**
LSD at 0.05	3.15	3.77	1.06	0.22	0.45	0.47	3.67	4.18	4.66	4.56	0.03	0.07	1.54	1.89

F: Fresh lots, O: Old lots, **: Highly Significant.

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Table (2):- The percentages (%) of Positive and Negative Values of Induced Mutagenesis (M1 Generation) in Onion Seeds through Using gamma rays.

Seed Treatments	Germination percentage		Fresh weight (mg)		Seedling dry weight (mg)		Shoot length (cm)		Root length (cm)		Seedling length(cm)		Germination Energy (%)	
Types of Seeds	Fresh	Old	Fresh	Old	Fresh	Old	Fresh	Old	Fresh	Old	Fresh	Old	Fresh	Old
10 RD	43.07	-3.17	6.15	-6.34	12.50	-39.13	9.24	-37.28	82.14	-20.48	24.73	-3.42	43.75	-32.25
20 RD	15.38	9.52	7.69	-65.07	8.33	-34.78	-10.08	-38.13	35.71	-31.32	16.92	-26.05	12.50	3.22
40 RD	6.15	3.17	-76.15	-76.98	-29.16	-43.47	-21.84	-38.98	-4.76	-39.75	-16.66	-26.84	4.68	-35.48
80 RD	35.38	19.04	29.23	-69.52	25.0	-26.08	47.05	-32.20	59.52	-3.61	33.85	-6.84	35.93	8.06
100 RD	6.15	-4.76	-71.07	-71.90	-33.33	-47.82	-36.97	-40.67	-16.66	-41.20	-27.08	-28.94	9.37	-46.77
Mean of Improvement	21.22	4.76	-20.83	-57.96	-3.33	-38.25	-12.60	-37.45	31.19	-27.27	6.35	-18.41	21.24	-20.64

Table (2):-Continued.

Seed Treatments	SVI1		SVI2		Germination rate		Speed of Germination		Mean Germination Time		EC		Field Emergence	
Types of Seeds	Fresh	Old	Fresh	Old	Fresh	Old	Fresh	Old	Fresh	Old	Fresh	Old	Fresh	Old
10 RD	78.97	-9.02	52.72	-47.22	16.41	4.61	36.84	-8.10	0.72	-18.51	-25.0	-16.66	49.58	17.39
20 RD	4.82	-21.98	18.18	-36.11	7.46	7.69	10.52	2.70	0.43	-17.77	-75.0	-8.33	38.84	12.17
40 RD	-11.59	-27.82	-20.60	-39.81	-2.98	-4.61	-10.52	-18.91	-0.72	-23.70	58.33	58.33	-3.30	-13.04
80 RD	21.66	6.78	22.72	-21.29	14.92	12.30	34.21	13.51	2.04	-17.77	-33.33	-16.66	45.45	21.73
100 RD	-22.56	-25.20	-30.30	-48.76	-5.97	-7.69	2.63	-24.32	-18.24	-26.07	-16.66	54.16	-1.65	-4.34
Mean of Improvement	14.26	-15.44	8.54	-38.63	5.96	2.46	14.73	-7.02	3.15	-20.76	-18.33	14.16	25.78	6.78

Positive and Negative Percentages of Induced Mutagenesis (M1 Generation):-

After reviewing all the average data for studied traits under five doses of gamma radiation compared with the control for the two types of onion seeds (fresh and old) , **(Table, 2)** can be summarized the following results.

The first dose of gamma rays (10 RD) revealed eleven positive induced mutagenesis in the traits (Germination percentage , fresh weight , Seedling dry weight , root length , shoot length , seedling length , germination % , SVI1 , SVI2 , mean of germination time and Speed of germination) for fresh seeds only and the three traits .,(germination rate , EC and field emergence) for the two types of onion seeds compared with the control , where this dose achieved genetic improvement superiority over the standard dose in the previous percentages. The second dose (20 RD) detected six positive induced mutagenesis compared with the control dose in the traits., (Germination percentage , Germination Energy (%) , Germination rate , Speed of germination , EC , field emergence) for fresh and old seeds , seven positive induced mutagenesis for the traits (fresh weight , seedling dry weight , root length , seedling length , SVI1 , SVI2 and mean germination time) for fresh seeds only , while no positive induced mutagenesis was generated for shoot length trait under this dose for the two types of onion lots , So this dose of irradiation exhibited high genetic improvement for enhancing the germination % , seedling growth and quality traits for onion seeds.

On the same track, the third dose (40 RD) recorded two positive induced mutagenesis only compared with the control. The first one was obtained for the fresh and old seeds in germination % trait, while the second positive induced mutagenesis was revealed for new seeds only in germination Energy (%) trait, which indicates that this dose were not effective for the genetic improvement in onion seeds through studding all traits and left a bad impact in germination %, seedling growth and quality traits, So we are not recommended for using it for onion seeds in any other study.

Seven positive induced mutagenesis were generated for both types of onion seeds in the traits., (germination % , germination Energy (%) , SVI1 , germination rate , speed of germination , EC , field emergence) through using the fourth dose of gamma rays (80 RD) , while the rest traits studied were exhibited the same results for fresh seeds only under irradiation for the same dose , table (2). Therefore, this dose beside the two doses (10 and 20 RD) are considering the strongest and the best three doses of radiation used to improve onion seeds and increasing the efficient of germination, growth and quality traits in addition, the safety of them for human and animal, So we are recommending for using them to improve and increase all germination and growth traits not only for onion seeds but also for the other crops. .

With respect to the fifth dose of gamma rays (100 RD),This dose is considering the weakest , worst and least influential dose in terms of the degree of genetic improvement. It was generated four positive induced mutagenesis compared to the control for the traits. (Germination % , germination energy % , speed of germination and EC) for fresh seeds only , while the other traits studied under treated with this dose were revealed negative percentages of induced mutagenesis as measured with the control dose for the both two classes of onion seeds or were not recorded any results and genetic improvement mentioned , respectively. This is the biggest proof of a genetic improvement in the form of mutation due to exposure dry seeds to different doses of gamma rays and the improvement rate varies from dose to another dose as we mentioned earlier.

From the summary previous results we note that the highest percentages of genetic improvement and positive induced mutagenesis were generated in the doses (10 , 20 , 80) for all traits studied and arranged from the highest to the lowest as follows; (10 RD), then (80 RD), then (20 RD) , respectively. In the end, we can clear that the reason for the genetic improvement in some traits studied of irradiated onion seeds with gamma rays at the doses of (10 , 20 and 80 RD) that may be due to a clear change in the composition of DNA (mutation) and may also lead to a modify in the gene action responsible for sensitive of highly doses of gamma rays to resistance for these highly doses , Thus, this modification may causes some shift in gene expression and leads to manufacture a group of proteins that play this role for resistance highly levels not only for irradiation , but also for salinity , water stress , toxicity of heavy metals and raising the efficiency of seed germination and quality of treatment seeds under these conditions , **[31] and [14].**

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Simple Correlation Coeffients Among all Traits Studied for the optimum dose of gamma rays (10 Rd) for fresh seeds:-

Note the presence of little and moderate effect in the relationship between all traits studied when exposed to the first dose of radiation (10 RD), so this dose had given a genetic improvement in a moderate form and will be briefing it in table (3) and fig (1). Germination % , fresh weight , seedling dry weight and shoot length traits were significant and highly significant positively correlated with the other traits , in addition root length , seedling length and germination energy % traits were recorded the same results with the other traits in table (3). On the same context ; SVI1 , SVI2 , germination rate and mean germination time traits were revealed significant and highly significant positively correlated with germination % , fresh weight , seedling dry weight , shoot length , root length , seedling length and germination energy % traits beside the same results were revealed between field emergence and the rest of traits , while significant and highly significant negatively of correlation coeffients were detected among EC and the other traits studied , respectively. This dose is considering the ideal treatment and which has achieved remarkable progress in genetic improvement associated with the characteristics of germination , seedling growth of onion seeds process, also extinguish a marked increasing in the quality attributes, which has the greatest impact on creating useful and constructive proteins in (DNA) and this due to a major role to bring the development and modification of the genetic reaction associated with enhancing the previous traits , These results were similar with the results obtained by **[32-34].**

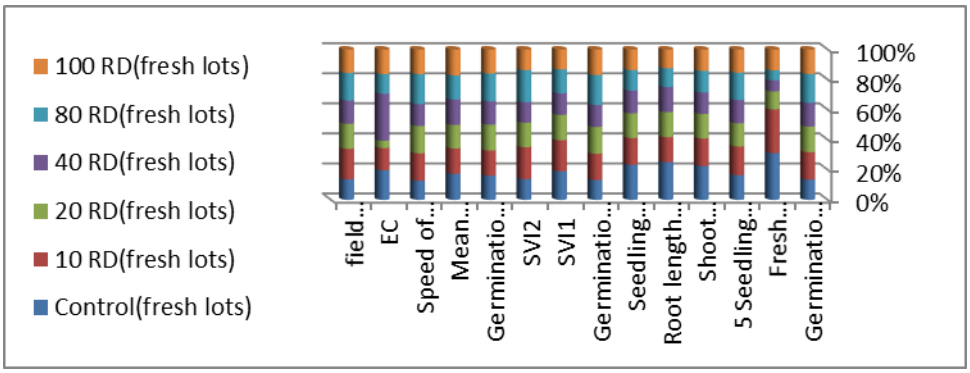

Fig (1):- The improvement resulted for all traits studied through using the five doses of gamma rays compared to the control in fresh lots.

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Table (3):- Simple correlation coefficients among all Traits Studied under the optimum Dose of Gamma rays (10 RD) for onion fresh seeds.

Studied Traits	G %	F.W	S.D.W	S.L	R.L	S. Leng	G.E %	SVI1	SVI2	G.R	M.G.T	S.OF.G	EC	F.E
G %	1.0													
F.W	0.88**	1.0												
S.D.W	0.87**	0.72**	1.0											
S.L	0.70**	0.80**	0.60**	1.0										
R.L	0.67**	0.84**	0.76**	0.58*	1.0									
S. Leng	0.78**	0.69**	0.80**	0.70**	0.74**	1.0								
G. E (%)	0.90**	0.92**	0.77**	0.80**	0.81**	0.56*	1.0							
SVI1	0.89**	0.50*	0.58*	0.65**	0.80**	0.90**	0.55*	1.0						
SVI2	0.55*	0.57*	0.88**	0.77**	0.66**	0.86**	0.77**	0.67**	1.0					
G.R	0.77**	0.65**	0.79**	0.68**	0.73**	0.85**	0.70**	0.83**	0.77**	1.0				
M.G.T	0.80**	0.84**	0.73**	0.55*	0.70**	0.62**	0.50*	0.90**	0.88**	0.68**	1.0			
S.OF.G	0.85**	0.70**	0.88**	0.73**	0.79**	0.58**	0.64**	0.89**	0.63**	0.81**	0.85**	1.0		
EC	-0.57*	-0.66**	-0.78**	-0.88**	-0.54**	-0.66**	-0.77**	-0.84**	-0.67**	-0.80**	-0.59*	-0.79**	1.0	
F.E	0.66**	0.84**	0.69**	0.75**	0.52*	0.55*	0.86**	0.77**	0.73**	-0.77**	0.57*	0.50*	-0.87**	1.0

***significant at 5%, **significant at 1%**

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Simple correlation coeffients among all traits studied using the optimum dose (10 RD) of gamma rays for old seeds:-

This dose is considering the most radiation dose that showed a marked advantage as it happened before in the fresh seeds, so they revealed significant and highly significant positively correlated coeffients between all traits studied among them except the correlation between EC and the rest of traits, where it recorded negatively correlated in table (4) and fig (2), respectively. we can say that this dose is safely on the quality , vitality of seeds , do not effect on human health , does not have any residual effect in the soil and that it is the first gateway to the process of genetic improvement for onion seeds through enhancing germination % and the related traits with it.

From the previous results it could be concluded that the dose of gamma rays (10 RD) was detected highly genetic improvement and exhibited highly significant and positively correlated for all traits studied of old lots in onion cultivar (Giza 20) ,Table (4) and Fig **(2)** , respectively, **[32 , 34].**

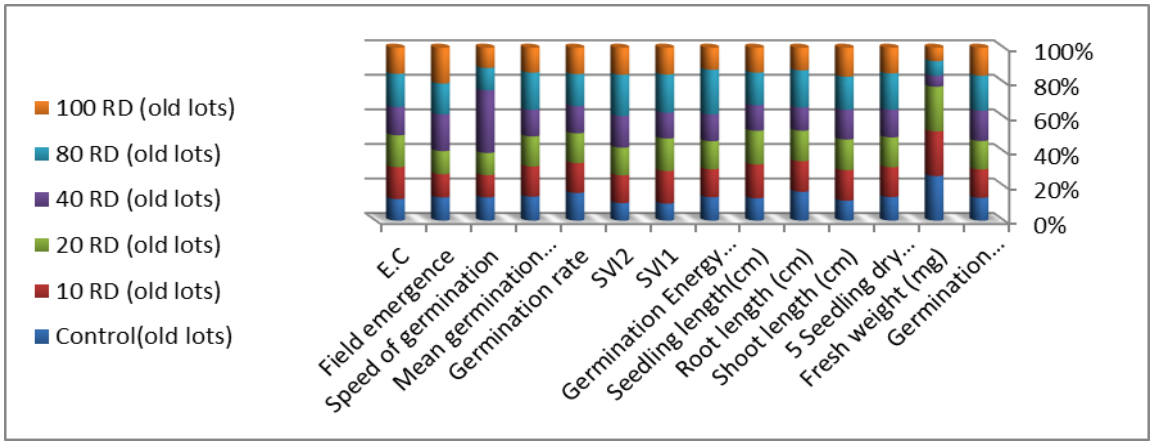

Fig (2):- The improvement resulted for all traits studied through using the five doses of gamma rays compared to the control in onion old seeds.

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Table (4):- Simple correlation coefficients among all Traits Studied under the optimum Dose of Gamma rays (10 RD) for onion old seeds.

Studied Traits	G %	F.W	S.D.W	S.L	R.L	S. Leng	G.E (%)	SVI1	SVI2	G.R	M.G.T	S.OF.G	EC	FE
G %	1.0													
F.W	0.67**	1.0												
S.D.W	0.83**	0.54*	1.0											
S.L	0.88**	0.58*	0.66**	1.0										
R.L	0.80**	0.61**	0.67**	0.70**	1.0									
S. Leng	0.60**	0.55*	0.83**	0.81**	0.77**	1.0								
G. E (%)	0.67**	0.77**	0.60**	0.60**	0.74**	0.80**	1.0							
SVI1	0.55*	0.83**	0.67**	0.70**	0.80**	0.58*	0.84**	1.0						
SVI2	0.70**	0.66**	0.80	0.55*	0.84**	0.75**	0.78**	0.55*	1.0					
G.R	0.62**	0.73**	0.59*	0.63**	0.60**	0.71**	0.63**	0.80**	0.63**	1.0				
M.G.T	0.50*	0.54*	0.51*	0.77*	0.81**	0.71**	0.81**	0.80**	0.69**	0.50*	1.0			
S.OF.G	0.78**	0.82**	0.88**	0.60**	0.50*	0.89**	0.63**	0.75**	0.52*	0.70**	0.78**	1.0		
EC	-0.77**	-0.55*	-0.64**	-0.73**	-0.67**	-0.72**	-0.83**	-0.50*	-0.69**	-0.88**	-0.83**	-0.53*	1.0	
F.E	0.59*	0.88**	0.77**	0.72**	0.87**	0.83**	0.67**	0.75**	0.55*	0.60**	0.77**	0.72**	-0.84**	1.0

*significant at 5%, **significant at 1%

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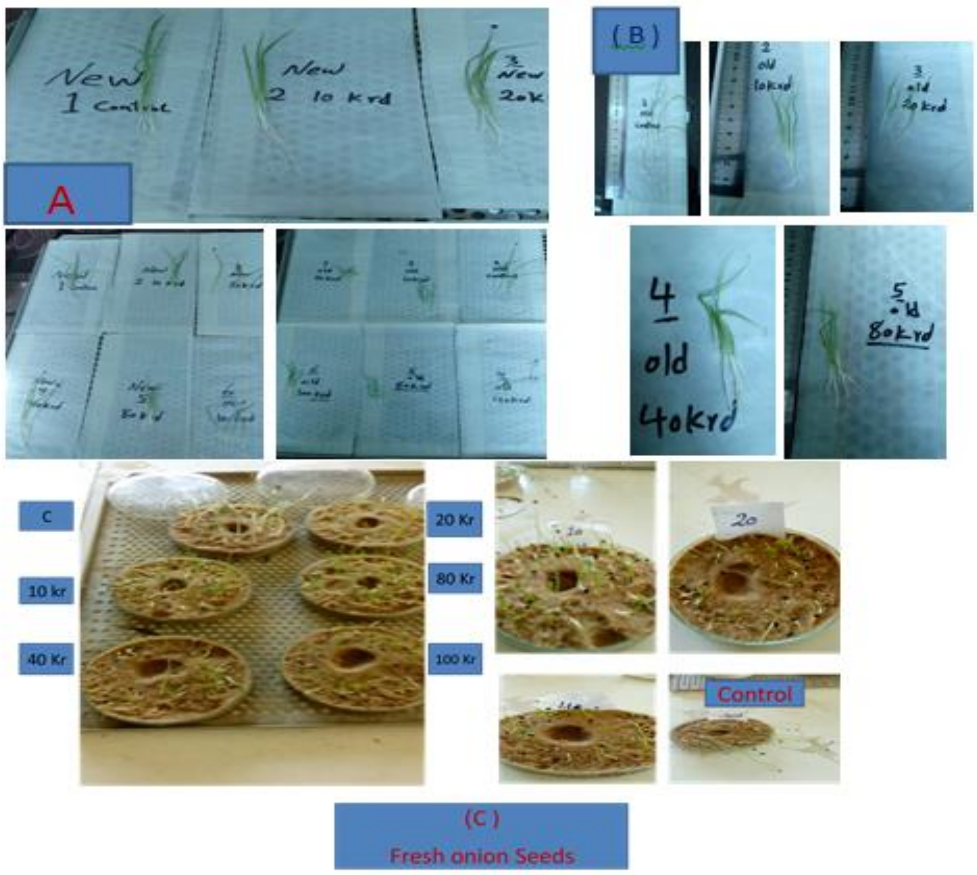

Fig (3):- Germination %, root and shoot Length for the two types of onion Seeds (Fresh and Old lots) through using the five doses of gamma rays beside the control.

Cytogenetic Investigation:-

Results of cytological analysis were listed in table (5), where mitotic index (MI) increased after most doses in case of fresh seeds while decreased after (100 RD) dose only. This increase found to be highly significance after (10 and 80 RD) doses with significant increase in values of chromosomal aberrations (CA) but still in save limits. Phase index (PI) of mitotic division mainly have no drastic change after treatment for fresh seeds except in case of (100 RD) dose were recorded significant change compared to control. Relative division rate (RDR) recoded positive values except (100 RD) dose were found negative value. But in case of treated old seeds with gamma irradiation, MI decreased, PI changed and CA increased significantly after all doses while RDR were recorded negative values.

All chromosomal aberrations were shown in table (5) and figures (4 , 5), where the most appeared aberration was nuclear lesions (fig. 4b-c and fig. 5b, c & d) followed by stickiness mainly in metaphase (fig. 4 e, f and g), beside many abnormalities as bridges, ring chromosome, fragments, c-metaphase and split metaphase.

Nuclear lesions found to be as the most prominent abnormality in this study as they found in many irradiated cells in many studies which this abnormality recovered with undiscovered mechanism. Most abnormality discovered in our data listed in metaphase stage and the most apparent one were stickiness, *Allium cepa* cells always sensitive to external environmental effects as physical and/or chemical factors. Percentage of mitotic index and chromosomal aberrations were dose dependent.

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Table (5):- Cytological analysis of fresh and old treated *Allium cepa* germinated seeds after five doses of gamma irradiation beside the control.

Plant	Treatment	% Mitotic Index (MI)	RDR	% Phase Index (PI)				% Chromosomal Aberrations (CA)
				Prophase	Metaphase	Anaphase	Telophase	
Fresh	Control	15.2±0.83	-	20.2±0.74	33.9±1.21	27.5±1.15	18.4±1.21	0.93±1.23
	10	19.8±2.11**	+5.42	21.7±1.07	35.2±1.39	25.8±0.99	17.3±1.72	1.84±2.71*
	20	16.7±1.30	+1.77	20.6±1.12	34.1±1.09	26.1±1.87	19.2±1.9	1.26±1.56
	40	15.5±1.05	+0.35	20.9±0.96	34.7±1.12	28.0±1.44	16.4±2.02*	1.19±1.88
	80	18.6±1.98**	+4.0	22.3±1.51	35.8±1.45	27.9±0.86	14.0±2.19*	1.72±2.11*
	100	12.8±2.17*	-2.83	24.1±1.95*	37.8±1.74*	22.9±2.03*	15.2±2.37*	3.06±2.76**
Old	Control	13.8±0.76	-	21.6±1.38	35.2±1.33	25.9±1.87	17.3±1.69	1.53±1.23
	10	10.6±2.29*	-3.71	24.49±2.92*	39.11±2.18*	18.02±3.04**	18.38±1.93	8.42**
	20	9.7±2.15*	-4.76	25.7±2.52*	39.3±2.43*	18.4±2.27**	16.6±2.72	11.50±3.88**
	40	8.2±3.29**	-6.5	26.8±3.19*	40.3±2.83*	17.2±3.51**	15.7±2.51	15.45± 4.59**
	80	11.5±1.95	-2.67	24.04±2.17	38.51±2.07	22.3±2.07*	15.15±2.11	7.38± 3.51**
	100	12.2±2.01	-1.86	23.8±2.39	38.4±2.81	20.2±2.74**	17.6±1.43	8.84±3.94**

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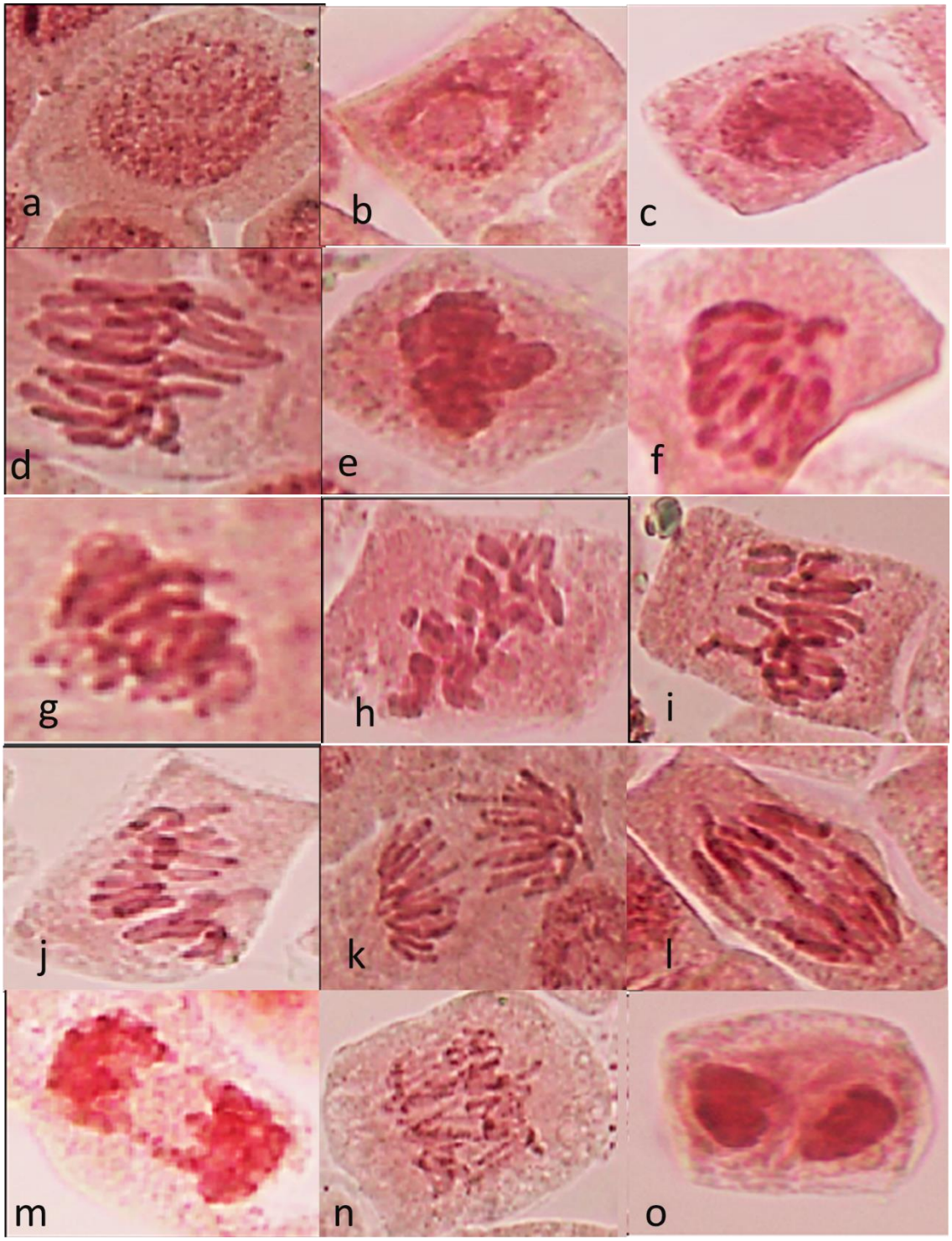

Figure (4):- Types of chromosomal aberrations found in the two onion seed lots after treated with five gamma rays beside the control. a-normal interphase, b-c- nuclear lesions, d-normal metaphase, e-g-sticky metaphase, h-c-metaphase, i-forward chromosome, j-split metaphase, k-normal anaphase

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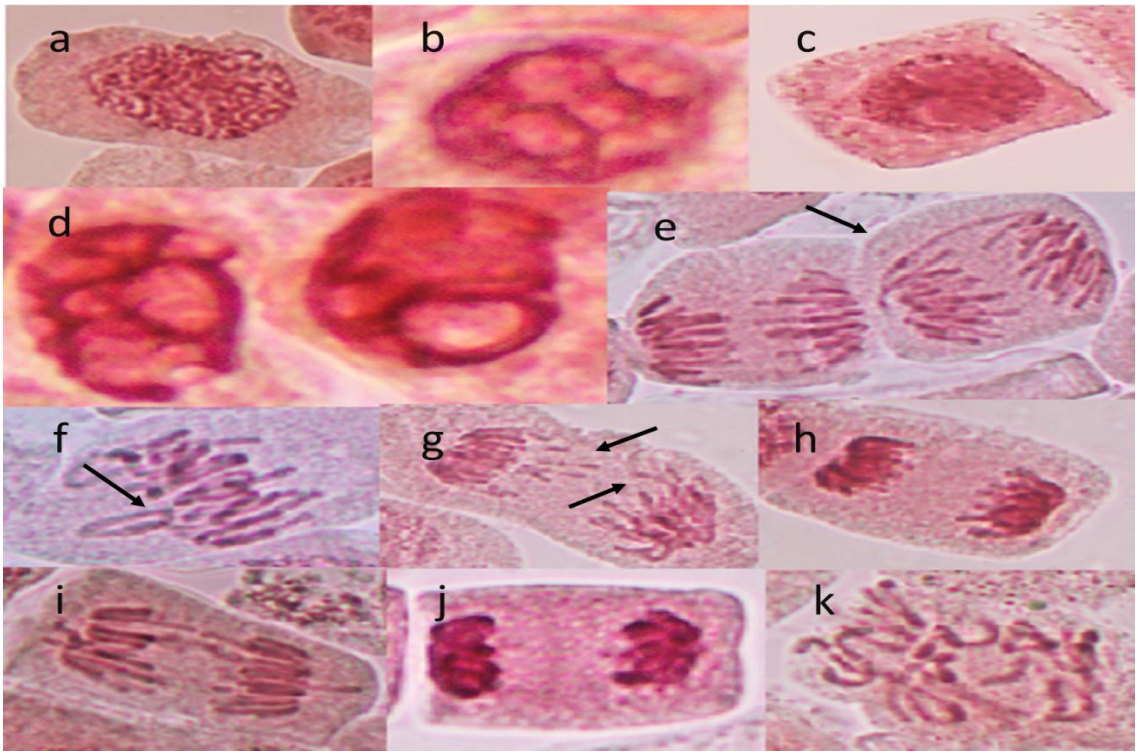

Figure (5):- Types of chromosomal aberrations found in the two onion seed lots after treated with five gamma rays beside the control. a-normal prophase, b-d-nuclear lesions, e-bridge, f-ring chromosome, g-fragments, h-early telophase, i-bridge, j-sticky anaphase and k-abnormal metaphase.

Protein electrophoresis:

SDS-PAGE results showed low number of bands may be revealed the low protein content of onions root tips and difficulties to show this gel image after many tries. The data presented in table (6) and figure (6) revealed the disappearance of some bands in old lot of onion seeds after all doses and control, all bands in old seeds found in different position with differ in molecular weight and relative migration. Five different band found in root tips of the treated and untreated germinated seeds, three of them found in control (37.6, 22.5 and 18.4 KDa), the band of molecular weight (18.4 KDa) disappear after (80 RD) dose where the band (22.5 KDa) disappear after (100 RD) dose in the case of fresh seeds. But in case of old seeds the mobility of protein band were different after all doses, new bands were appeared (19.7 KDa) and (12.6 KDa) while the upper two bands (37.6 and 22.5 KDa) disappear. These results suggest that the negative effect of seed storage and may be the way to distinguish between fresh and old seeds using these doses of gamma rays.

[35] Study the proteins property of three oil seeds (soybean, peanut and sesame) were investigated following Î³-irradiation (0.0, 0.5, 1.0, 2.0, 3.0, 5.0 and 7.5 KGy). The effect of Î³-radiation on total protein solubility, albumin, globulin and SDS-ME fractions were studied using SDS-polyacrylamide gel electrophoresis. The results showed that solubility of total protein were decreased and reached to the maximum decrease using irradiation dose of 7.5 KGy compared to control. The interesting phenomena are that albumin and globulin fractions decreased in its solubility while the SDS-ME fraction increased. These phenomena may be due to the effect of gamma radiation on the protein, which may dissociate this fraction to small subunits, and rearrangement to form a complex protein even high or small molecular weight proteins solubilized only in SDS-ME fraction. The changes in protein profile were depended even on radiation dose and on the nature of oil seeds; soybean, peanut and sesame.

[9] studied the effect of different doses of gamma rays i.e. 10 , 20 , 40 , 80 and 100 Kr on germination , viability , seedling growth and some morphological traits in onion seed and revealed that seed germination percentage was developed at lowest levels of gamma rays (10 and 20 k rad) and was very resistance for the highest doses, beside proved that seedling growth was reduced gradually with using doses up to 40 k rad.

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Using of gamma rays at doses (0.0 and 20Gy) performed from a cobalt source (60Co) with strength of 500 Ci and the dose rate of 0.54 Gy/min-1 for applying soybean dry seeds before growing and the results proved that the lowest dose of gamma rays was the reason for increasing biomass accumulation and grain yield for both conditions of irradiation and water deficit treatment and revealed that pre-treatment with the lowest dose of gamma rays (20 Gy) used for irradiating dry seeds of soybean before growing could be useful for increasing water stress tolerance and slash low yield due to low water during growing stages, consequently, it may be fruitful administration gadget to restore life and improve agriculture in arid reigns Known as highly severe dryness , **[11].** Investigation the seeds of cowpea plants under two treatments of salinity and irradiation using gamma rays through studding electrophoretic of proteins revealed 3forms of adjustments, the first one was vanished some protein bands, the second switch was related with induced selectively increased and synthesis of new set of protein. Some of these reactions were detected under irradiation and salinity conditions, whilst another's were manufactured by gamma rays or salinity stress, **[12].**

[13] revealed the different effects of gamma rays on shallot chromosomes (Allium ascalonicum Linn) during using the doses (0, 10, 20, 30, 40, 50, 60, 70 and 80 Gy) at the dose rate of 0.0078 Gy and perceived that root tip squashes were found by light microscope, one thousand cells per specimen, to expose chromosome anomaly , while no anomaly were showed in the cells of plants irradiated to 70 and 80 Gy.

[14] detected the role of gamma irradiation for improving plant growth, morphological, biochemical, and molecular characters in (Allium sativum) through using different doses ranging from 10 to 150 Gy and revealed that plant growth trait was related with doses of gamma rays in addition, pigments fractions and total components of carbohydrate were also decreased with highly doses of γ- radiation , respectively.

[15] Used Caesium-137 as gamma ray source through six different doses treatment i.e. 0, 5, 7, 9, 11, 13 and 15 Gray to assessment the radio sensitivity and study cryptogenic analysis of two entries of (*Zingiber Officinale)* Roscoe ; Bentong and Tanjung Sepat , respectively. The final results reported that the best doses for ginger genotypes (Bentong and Tanjung Sepat) were observed at 8.14 Gy and 9.38 Gy ,beside gamma rays recorded the lowest significant of the average permanence level of rhizomes and this reduction was more observed in Bentong genotype. From the previous results of cryptogenic analysis it could be concluded that the highest doses of gamma rays (5, 7, 9, and 11 Gy) recorded a major role in showing the highest rate of chromosomal deviation in cells.

[10] Study the effect of different doses of gamma rays on *A. cepa* bulbs, they chromosome behavior and antioxidant enzymes on 3rd and 30th day after gamma radiation. They found positive correlation between chromosomal aberrations and antioxidant enzymes related protection mechanism of cell has been established.

The mutagenic efficiency of gamma irradiation on *Allium cepa* was studied by **[36].** Five doses of gamma rays (viz. 5kR, 10kR, 15 kR, 20kR and 25kR) were used for irradiation the seeds. Chromosomal aberrations were summarized in this investigation as dicentric, tricentric, ring, minute, deletion, fragment, laggard, bridge and micronuclei in treated germinated root tip mitosis, percentage of abnormality were increased with increasing doses. The chromosomal abnormalities were dose dependent.

Table (6) :- Protein banding pattern for the two types of onion seeds after treatment with five doses of gamma rays (10, 20, 40, 80, 100 RD) beside the control).

M.W. OF Bands By KDa	RF	Fresh						Marker	Old					
		Cont.	10	20	40	80	100		100	80	40	20	10	Cont.
116	0.445	-	-	-	-	-	-	+	-	-	-	-	-	-
97.4	0.697	-	-	-	-	-	-	+	-	-	-	-	-	-
66.2	0.699	-	-	-	-	-	-	+	-	-	-	-	-	-
37.6	0.758	+	+	+	+	+	+	+	-	-	-	-	-	+
22.5	0.774	+	+	+	+	+	-	-	-	-	-	-	-	+
19.7	0.779	-	-	-	-	-	-	-	+	+	+	+	+	-
18.4	0.799	+	+	+	+	-	+	+	+	+	+	+	+	+
12.6	0.861	-	-	-	-	-	-	-	+	+	+	+	+	-

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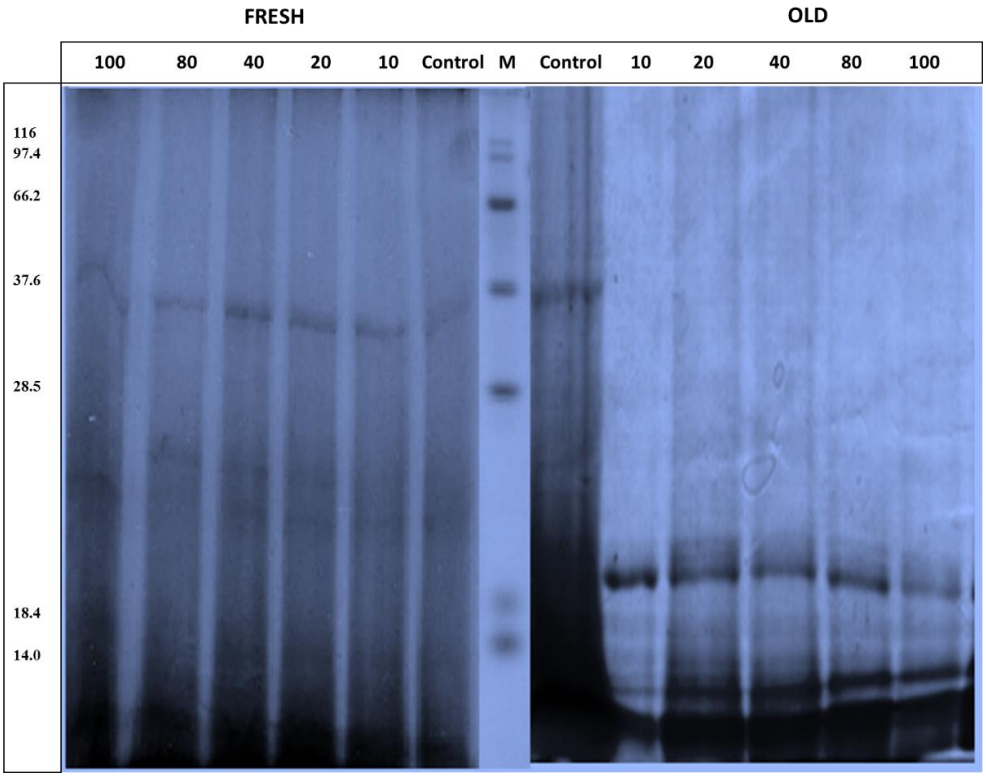

Figure (6):- Protein banding pattern for the two onion seed lots (fresh and old) after treatment with five doses of gamma rays (10, 20, 40, 80, 100 RD) beside the control).

CONCLUSION

This study was carried out on the two lots types (fresh and old seeds) for the onion cultivar (Giza 20) which performed from agriculture research center because it is considering highly agricultural widespread and has many genetic traits. Dry seeds were irradiated using five doses of gamma rays (10 , 20 , 40 , 80 , 100 RD) beside the control for each class of seeds (fresh and old). Fourteen agro-morphological traits were calculated for each type of seeds under the six doses in addition, cytogenetic and molecular characterization using protein banding patterns electrophoresis (SDS-PAGE). The final results proved that the doses (10, 20, 80 RD) were the optimum treatments and outperformed on the rest doses under studding measurements for all traits studied. The results of molecular and cytogenetic investigation were very important and fruitful on fresh and old seeds So, the dose of (10 RD) is considering the most safe dose and recommended for using it on a large scale in the genetic improvement programs of the onion crop for high percentages of germination, yield and quality traits. The Allium test was proven to be a very reliable indicator of radiation exposure, as the macroscopic parameter data have shown. The technique has the advantage of low cost, ease in handing and short test time.

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