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Improved Performance of Egyptian Clover Seed Using Gamma Ray.

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ABISTRACT

The present study was conducted to utilize the gamma irradiation to alleviate the deterioration of Egyptian clover seed quality caused by artificial aging. Egyptian clover seeds (c.v. Serw1) were artificially aged at 41C° and 100% relative humidity for 3 and 5 days and then they were irradiated with gamma ray doses (0, 50, 100, 150 and 200 Gy) of radioactive cobalt (60 CO) gamma rays on seed viability as measured by different laboratory tests including Standard germination (SG), and Electrical Conductivity (EC), Tetrazolium test (TZ), evaluation of seedling vigor indicators, and in addition to filed emergence (FE) under field conditions. The results revealed that artificial ageing increased electrical conductivity of seed leaching; but it decreased the number of viable seeds colored by tetrazolium Formozan (TZ). The germination percentage of aged seeds at standard conditions was also reduced and consequently seedling vigor traits, field emergence, relative field emergence showed the same trend. Exposing aged clover seed to Gamma rays (150 Gy) compared with other treatments improved germination traits, field emergence, relative field emergence. However, at higher dose of 200 Gy stress was evident and significant decreases in all parameters were observed when the seeds were aged for 5 days. Generally, the harmful effects of artificial aging (or poor storage conditions) on clover (c.v. Serw 1) seed performance under laboratory and filed conditions can alleviated by gamma irradiation dose of (150 Gy). Further investigation is needed including high doses of gamma radiation more than 200 Gy and seeds of various crop species stored naturally at controlled and open air storage, in order to confirm the results and find out the right recommendation.

Keywords: Egyptian clover , gamma ray, germination and seedling vigor.

INTRODUCTION

Egyptian clover (*Trifiolium alexandrium, L.*) is a traditional forage crops that is grown annually in Egypt in winter and spring. After several cutting for animal feeding and hay use, few field are left for an extra month to harvest seed (Abdel-Galil *et al.* 2014). In Egypt some growers attend their needs of clover seed from owns reminder seed from previous season (carry over seed). During storage or accelerated aging conditions, biochemical changes include loss of enzymes activity, cell membrane injury reduced production of ATP and respiration activity, accumulation of chromosome aberrations and toxicants and increasing free acid (Copeland and McDoland, 1995). These biochemical changes led to decreasing seed germination, field emergence, seed and seedling vigor and finally crop productivity. It should be regarded that the storage conditions of 40 to 45 C° and high relative humidity (75-100%) is considered a practical technique for predicting the relative storability 0f seed lots (Delouche *et.al,*1973). They mentioned that seed tends to lose its viability due to ageing process, even under ideal storage conditions; then yield per unit area will decrease. It's therefore essential to minimize the harmful effects of artificial aging or prolonging storage period and improve seed quality before sowing.

Pre-sowing seed treatments such as gamma radiation may improve in seed performance under field conditions (Hafsa Ali *et al.,* 2016).Gamma rays have been proved economical and effective as compared to other ionizing radiations such as electric field, magnetic field, laser radiation, microwave radiation because of its easy availability and the power of penetration which helps in its wider application for the improvement of various plant species (Moussa, 2006). Gamma radiation used to enhance seed germination (Bhargava and Khalatkar 1987).Also, it has influence on plant growth and development by inducting genetical, cytological, biochemical, physiological and morphogenetic changes in cells and tissues depending on the levels of irradiation (Rabie *et al.,* 1996 and Akshatha *et al.,* 2013), also have yield and quality (Munishamanna et al., 1998; Latha and Nair, 1999 and Kim *et al.,* 2004). It is very important to determine the right dose where low doses improve seed germination meanwhile high doses have decreased germination and normal seedlings (Mahto *et al.,*1989; and Yassein and Amina, Aly, 2014). Also,they reported that increasing, dose of gamma rays up to 100 Gy, gradually increased the germination percentage and then decreased gradually with increasing the gamma rays dose. The stimulatory effects of gamma rays on germination may be attributed to the activation of RNA synthesis, increased enzymatic activation (Akshatha *et al.*,2013), growth of roots and leaves (Thapa, 1999) and increase yield of some plants such as sunflower (Abo-Hegazi *et al.,* 1988). Taking into consideration the previous efforts, the aim of this study was to utilize gamma irradiation to alleviate the deterioration of Egyptian clover seed quality caused by artificial aging.

MATERIALS AND METHODS

This experiment was carried out at Seed Technology Research Unit, Mansoura, Seed Technology Research Department and Experimental farm of Tag El-Eiz Station, Agricultural Research Center (ARC), Egypt, during 2016 year. Egyptian clover seed (cv. Serw1) was obtained from Forage Crops Research Department, ARC, Giza. Seed samples were subjected to aging treatment which includes high temperature of 41C° for 3 and 5 days and high relative humidity of 100%. The relative humidity was regulated in a closed container by use water as outlined by (Delouche and Baskins, 1973). The seeds were dried at room temperature until its moisture content was equal its original level (14%).

Egyptian clover seed of artificially aged and non-aged were irradiated with gamma ray doses (0, 50, 100, 150 and 200 Gy) at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The gamma radiation was derived from a Cobalt-60 (60Co) source. Samples were stored in plastic bags in a refrigerator under the temperature 4-6 °C. Laboratory experiment was arranged in a completely randomized design with four replicates. Then the seed samples were subjected to the following tests:

Standard germination test: Three replicates of 100 seeds from each treatment were placed in on two sheets of filter paper in 14 cm Petri-dishes. Then, they were put a growth chamber at 20 Co for 7 days. The Normal and abnormal seedlings were counted and expressed as germination percentage according to the international rules of ISTA (1999). Other calculations were also made including shoot and root length of 10 normal seedlings (cm) which were measured at the end of the germination test. The average of seedling dry weight of 10 normal seedlings were made after drying the seedlings in hot-air oven at 85 ^{0}C for12 hours according to Krishnasamy and Seshu (1990).

The abnormal seedlings and their types (without primary root or shoot); Germination Rate (GR) according to the equation outlined by (Bartlett ,1937):

$$GR=a+(a+b)+(a+b+c)+\ldots\ldots\ldots..(a+b+c+\ldots.+m) \div n((a+b+c+\ldots.+m)$$

Where (a,b,c and m) = number of seeds emerged at the first count, second and final account and n is the number of counts.

Also, Mean germination time (MGT) was calculated based on the equation outlined by Ellis and Roberts (1981).

$$MGT = \Sigma Dn \div \Sigma n$$

Where (n) is the number of seeds, which were germinated on day, D is number of days counted from the beginning of germination.

The Electrical Conductivity Test (EC): It was conducted to evaluate the electrical leakage from the seed according to the procedures outlined by Matthews and Alison (1987). Three replicates 100 seeds were weighted to decimal places. Then, the seeds were put in beakers containing 250 ml of distilled water. The beakers were placed in an incubator at 30 Co for 24 Hours. The conductivity of seed steep water was measured immediately after the removal of the samples from the incubator, using mullet –cell conductivity meter (CMD 830 WPA) and sample. The results were reported as (mhos/g seeds). EC= Reading of replicate / weight of seeds.

The Tetrazolium (TZ) test was utilized for assessing seed viability by following the producers out lined by International Rules for Seed Testing (ISTA, 1999). The seeds are soaked in water at 30 C^o for about 16 hour's, after which the embryos were excised, together with a thin layers and endosperm. The embryos were then submerged in tetrazolium chloride solution at 30 C^o for about 24 hours, followed by rinsing in water and removal of the endosperm layer. The embryos of the seed were evaluated according to its staining pattern as viable and non- viable with a Binocular Microscope.

Field emergence: Three replications of 100 seeds from each treatment were sown in a complete randomize block design at Tag El-Eiz Agricultural Research station. All cultural practices for growing Egyptian clover crop were done as recommended. Seedling emergence was recorded at time intervals until constant (after 22 days from seed sowing). Relative Field emergence (RFE denotes to the percentage of viable seeds produced plants in the field) was calculated based on the following equation:

$$RFE = \frac{FE}{G}$$

Where: RFE = Relative field emergence; FE = Mean Seedling Field Emergence of all seed; G = Mean standard germination of all seed.

All data were subjected to the statistical analysis according to the technique of analysis of variance (ANOVA) of completely randomized design for laboratory experiment meanwhile, field experiment were analyzed by the complete randomize block design , as described by Gomez and Gomez (1984), means were compared using least significant differences (LSD) at 5%.

RESULTS AND DISCUSSION

Table (1) showed the effect of artificial aging and gamma radiation treatments on viability of clover seed as expressed by germination percentage, electrical conductivity, and viable embryos staining by tetrazolium solution, field emergence percentage and relative field emergence. Artificial aging reduced the capability of seeds to germinate especially at longer time (5 days) of the treatment where seed germination was reduced from 89% (control) to 64%. According to the International Seed Testing Association (ISTA) the germination percentage of certified clover seed should not less than 85%. Beyaz *et al.* (2016) repotted that aging is a progressive process accompanied by accumulation of toxic metabolites, which progressively depress germination and growth of seedlings with increased age (Tales 2 and 3). It is obvious that important physiological and biochemical change are taken place in the embryo and endosperm during deterioration. The significance of these changes in relation to deterioration due to seed aging is not fully understood.

It is also obvious the values of EC of aged seed was increased by increasing the duration of aging treatment where more cell membrane damage are expected to take place and the weaker the membranes the lager the quantity of electrolytes leached from the seeds, and the greater the conductivity of the steep water. The EC value of controlled sample was (0.023 *mmhos/g seeds*), but it was increased to (0.034 and 0.039 *mmhos/g seeds*) after aging for 3 and 5 days, respectively, but the results of the EC test inversely proportional other testing results.

The results in Table 1, showed that aging treatment reduced the percentage of stained embryos decrease from 90 % to 82% and 66%. It's well-known that the TZ is used to indicate the activity of enzymes of the dehydrogenase group which are responsible for reduction procession in living tissue. In the present of active enzymes the seed or the embryos turned to red-colored, but in the absence of active enzymes dead tissues of the seed remain unstained.

The Correlation coefficient between field emergence and standard germination, tetrazolium test, relative field emergence as shown in Table (4) was a positive and significant, (R= 0.883, 0.930 and 520, respectively), but the correlation coefficient between field emergence and the results of the EC test was negative (R=-0.787) indicting inversely proportional relationship with field emergence .

With respect to the effect of gamma rays dose on the viability criteria (Table 1), there was a slightly positive effect only for gamma rays dose (150 gry) .The other gamma rays doses exerted various effect. However, gamma rays improved the performance of aged clover seeds especially when aging period was 5 days. This result revealed that more deteriorated seeds were more positively respondent to radiation treatment compared with less deteriorated ones, suggesting the use of gamma rays particularly the 150 dose to improve the performance of low viability as expressed by seed germination, embryos staining by tertazolium solution and EC of seed steep water or poor storability seeds as indicated by seed aging. The germination percentage of seeds aged for 5 days was 64% and became 70% as the seeds were subjected to gamma ray dose of 150 Gy. Identically, the percentage of staining embryos of seeds aged for 5 days was 66% and increased to 72%. The field emergence results of the seed sample were consistently with those previously mentioned.

Table 1. Effect of artificial aging and gamma ray dose on standard germination% (SG), electrical conductivity (EC), tetrazolium test (TZ), field emergence (FE) and relative field emergence (RFE).

Treatments		SG %	EC *(mmhos/gm/seed)*	TZ%	FE%	RFE%
Control		89	0.021	90	87	98.0
Artificial aging	41Co for 3day	80	0.034	82	76	94.0
	41Cofor 5day	64	0.039	66	60	93.9
Gamma radiation dose	50 gry	88	0.022	89	83	94.3
	100 gry	89	0.021	90	88	99.0
	150 gry	92	0.019	93	89	96.7
	200 gry	88	0.020	88	86	97.7
Aging 41C for 3day	50 gry	80	0.026	81	76	95.1
	100 gry	81	0.024	81	78	96.5
	150 gry	83	0.023	84	81	97.7
	200 gry	80	0.024	81	77	96.3
Aging 41C for 5day	50 gry	65	0.032	66	60	92.3
	100 gry	66	0.029	67	64	97.0
	150 gry	70	0.028	72	66	94.5
	200 gry	68	0.030	69	65	95.7
LSD at 5%		3	0.003	2	9	2.2

Akshatha et al. (2013) reported that the stimulatory effects of gamma rays on seed viability as expressed by its ability to germination may be attributed to the activation of RNA synthesis, increased enzymatic activation. Also they mentioned that gamma rays influences on plant growth and development by inducting genetically, cytological, biochemical, physiological and morphogenetic changes in cells and tissues depending on the levels of irradiation , low doses improve seed germination meanwhile high doses have decreased germination and normal seedlings.

Table 2. Effect of artificial aging and gamma ray dose on germination rate (GR), mean germination time (MGT), shoot length (SL), root length (RL) and seedling dry weight (SDW) of onion seed.

Treatments		GR	MGT (day)	Seedling vigor SL (cm)	RL (cm)	SDW (gm)
Control		0.790	2.8	1.4	2.2	0.017
Artificial aging	41Co for3day	0.690	2.9	1.1	1.9	0.015
	41Co for 5day	0.470	3.2	0.7	1.6	0.012
Gamma radiation dose	50 gry	0.795	2.8	1.3	2.2	0.017
	100 gry	0.799	2.6	1.4	2.3	0.017
	150 gry	0.810	2.4	1.5	2.3	0.019
	200 gry	0.801	2.6	1.3	2.1	0.018
Aging 41C for 3day	50 gry	0.692	2.8	1.1	1.9	0.012
	100 gry	0.695	2.8	1.2	2.0	0.017
	150 gry	0.710	2.6	1.3	2.1	0.019
	200 gry	0.702	2.7	1.2	2.0	0.018
Aging 41C for 5day	50 gry	0.472	3.1	0.7	1.6	0.012
	100 gry	0.476	3.0	0.9	1.6	0.012
	150 gry	0.480	2.9	1.1	1.8	0.013
	200 gry	0.476	3.0	1.0	1.6	0.013
LSD at 5%		0.133	Ns	0.6	Ns	0.004

Table 3. Effect of artificial aging and gamma ray dose on abnormal seedling (ABS), un-germinated seed (UGS), stunted seedling (SS), seedling without shoot (SWS) and seedling without root (SWR).

Treatments		ABS%	Type of abnormal seedlings UGS%	SS%	SWS%	SWR%
Control		11	3	3	3	2
Artificial aging	41Co for 3day	20	8	6	3	3
	41Co for 5day	36	14	13	5	4
Gamma radiation dose	50 gry	12	4	3	3	2
	100 gry	11	4	3	2	2
	150 gry	8	3	2	2	1
	200 gry	12	3	3	3	3
Aging 41Co for 3day	50 gry	20	8	6	3	3
	100 gry	19	8	5	3	3
	150 gry	17	7	6	2	2
	200 gry	20	8	7	3	2
Aging 41Co for 5day	50 gry	35	13	13	5	4
	100 gry	34	12	13	5	4
	150 gry	30	11	11	4	4
	200 gry	32	11	12	5	4
LSD at 5%		2	2	2	2	2

Table 4. Correlation coefficient between field emergence and other viability seedling vigor tests.

Studied traits	Field emergence
Standard germination %	0.883^{**}
Tetrazolium test %	0.930^{**}
Relative field emergence %	0.520^{**}
Electrical conductivity	-0.787^{**}
Mean germination time	-0.264^{ns}
Germination rate	0.817^{**}
Abnormal seedlings	-0.923^{**}

Tables (2 and 3) showed that seedling characters were influenced by artificial aging and gamma radiation and the results have had similar trend as their effect on seed viability. This may explain by the statement reported be Akshatha *et al.* (2013), in which the positive effect of gamma ray of the right dose was extend to further stages followed seed germination. The Correlation coefficients between field emergence and seedling vigor as expressed by germination rate (GR) and abnormal seedling (%) were significant (R=0.817, 0.-.923), respectively .However, The Correlation coefficients between field emergence (FE) and the mean germination time (MGT) was insignificant (R=-0.264) and the reason for this result might be due to the fact that the value of MGT is calculated at time intervals form the time at which the seeds started to germinate until the end of the test.

The highest results of seedling parameters (root lengths, root lengths, seedling fresh weight, seedling dry matter content were obtained from 150 Gy treatment (Tables 2 and 3). In control and in the doses of 50 and 200 Gy. seedlings grown from seeds irradiated aged seed were observed to grow slower than that of irradiated with other doses. The lowest results recorded from 200 Gy gamma radiation could be attributed to inhibitory effect of higher gamma ray. These results were parallel to those of Xiuzher (1994) and Rabie *et al.,* (1996), who reported that seed irradiation with high doses of gamma rays, disturb protein synthesis water ex-change and enzyme activity, production of growth hormones. Furthermore, the results of Chandorkar and Clark (1986), and Stoeva *et al.* (2001) came to the same conclusion in which irradiation with high doses of gamma rays affected water exchange and enzyme activity.

From the previous results it was obvious that the inhibitory effect of gamma radiation on seed viability was observed in the doses over 150 Gy. The importance of this study is to provide primary results for future investigation including high doses of gamma radiation more than 200 Gy and seeds of various crop species stored naturally at controlled and open air storage.

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تحسين أداء تقاوي البرسيم المصري باستخدام أشعة جاما

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فى هذه الدراسة تم تعريض بذور البرسيم المصري صنف (سرو ١) للإجهاد الصناعي على درجة حرارة ٤١ °م ورطوبة نسبة ١٠٠% لمدة ٣ ، ٥ أيام ، ثم تم تعريضها لجرعات مختلفة من أشعة جاما (٠ ، ٥٠ ، ١٠٠، ١٥٠ و ٢٠٠ جراي) ، بهدف تجنب الضرر الذى لحق بالبذور نتيجة الاجهاد الصناعى .وتم تقييم حيوية التقاوى وقوة انبات البادرات بعدد من التجارب المعملية الموصى بها بالقواعد الدولية لفحص البذور . كما اجريت تجربة حقلية لتحديد نسب التكشف الحقلى للبذور المعاملة . وتوصلت الدراسة إلى عدد من النتائج أهمها أن تعريض البذور للإجهاد الصناعي قد أدى إلى زيادة نسبة البذور التالفة وبالتالى انخفضت نسبة الانبات القياسي والبادرات غير الطبيعية وزادت درجة التوصيل الكهربي لمنقوع البذور ، وانخفض عدد أجنة البذور الملونة باللون الأحمر (اختبار التترازليم). وكان معامل الارتباط معنويا بين معظم القياسات المعملية ونسبة التكشف الحقلى. كما أدت معاملة البذور بجرعة من اشعة جاما (١٥٠ جراي) إلى تقليل الضرر الذى لحق بالبذور جزئيا وتحسن أداء البذور وأعطت بادرات قوية تحت ظروف المعمل والحقل . وفى المقابل فان زيادة جرعة الاشعاع إلى (٢٠٠جراي) أدى إلى تأثير سلبى على القياسات المعملية والحقل مما يدل على أن مزيد من الضرر قد لحق بالبذور. وكان للتفاعل بين الإجهاد الصناعي ومعاملة أشعة جاما تأثير معنوي على معظم القياسات . وفى ضوء هذه النتائج ، فانه يقترح استخدام هذه الجرعة لتحسين أداء تقاوى البرسيم المصرى خاصة المخزنه منها. كما يقترح إجراء فحوصات جديدة تشمل جرعات أخرى وتقاوى محاصيل أخرى للوقوف على صحة النتائج من عدمه وإصدار التوصية المناسبة.