Evaluation of genetic stability using SCoT markers and SDS-PAGE with gamma radiation on callus of (Atropa belladonna L.) and antioxidant activity.

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ABSTRACT

Due to the improvement of the mutant germplasm and its use in the creation of new mutant organisms, these mutations have been instrumental in meeting global food and nutritional protection challenges. Mutagenic therapies for plant breeding and for drug alkaloids development projects have contributed to a wide range of genetic variability. The primary commercial source of drug alkaloids such as atropine is *Atropa belladonna*. This paper focuses on the effect of gamma rays (γ-rays) on callus of *Atropa belladonna* at the molecular level and changes of biochemical metabolisms in callus. *Atropa belladonna* callus were irradiated with 0.0, 30, 60, 90, 120 and 150 Gy gamma rays after 6 weeks on induction media. Highly significant differences between effect of gamma rays (γ-rays) on callus of *Atropa belladonna* at 2,4-D 2 mg/L and Kin 0.5 mg/L respectively were recorded on callus fresh weight and callus dry weight. 120 Gy had higher callus fresh weight, dry weight, Dry matter content (%) than the control. The difference in ion levels were measured in control and callus irradiated. The results clearly decrease in ion levels with increase of gamma dose. On the other hand, the deferent of antioxidant activity were cleared between control and gamma ray's callus. The hist antioxidant activity dose 150 Gy which recorded 30.20 comparing with control 20.10. Polyacrylamide gel electrophoresis SDS-protein PAGE profiles were used. In irradiated plants, there were several new protein bands that could be used as markers for each dose. Using DNA-Start codon targeted (SCoT) polymorphism assay. There were two negative molecular markers which were found only in control as compared to irradiated callus, with molecular sizes 940 and 400 bp.

Keywords: *Atropa belladonna* L., γ-rays, protein SDS-PAGE, SCoT, Callus.

INTRODUCTION

Atropa belladonna L. is a perennial herbaceous plant and the most important commercial source of pharmaceutical tropane alkaloids in the Solanaceae family, commonly known as belladonna or deadly nightshades. Genus Atropa is medicinally important as it has a lot of benefit. Many bioactive compounds have so far been isolated from Atropa. This emphasizes on the need for the review of literature for reporting the additional information on the medicinal importance of other species of genus Atropa Maqbool et al. 2014.

γ-rays are energy intensive radiations that causes a range of seed cell random damage and nucleotide damage Zheng and Li 2017. They can also help to develop free and reactive oxygen radicals that indirectly disrupt the physiological and biological characteristics of the cells and remove the plant Kovacs and Keresztes 2002 and Wi *et al.*, 2007. New technologies such as tissue culture, gene transformation and technology for mutation breeding are potential solutions for improving the efficiency of modern agro systems (Ramakrishna and Shasthree 2016). The dose response of tissue cultured shoot tips to gamma irradiation has been identified by Shasthree *et al.*, (2009). The use of *in vitro* culture and radiation mutations has been an important way to introduce genetic diversity and rapidly propagate selected mutants. (Suprasanna *et al.*, 2012).

In taxonomic and genetic analysis, molecular markers are very useful. Start codon targeted (SCoT) polymorphism of the various DNA marker systems gaining prominence over other dominant DNA for its dominance for greater polymorphism and improved marker solvability, marker systems like RAPD and ISSR Gorji *et al.*, 2011. The SCoT primers are based on the initiation codon sequences of genes in preserved regions flanked by Ibrahim *et al.*, 2016, Mulpuri *et al.*, 2013. Rajput and Agrawal 2020 using SCoT and sequence related amplified polymorphism (SRAP) markers genetic fidelity of *ex vitro* acclimatized *Atropaacuminata Royle* ex Lind l. plants showed 96 percent similarity between mother plant and micro propagated plants. In comparison with the mother plant these plants

demonstrated better antioxidant DPPH activity.

SDS-PAGE (SDS-PAGE) is a biochemical technique that is used to analyze the effect of several treatments on the expression of genes. In studies of abiotic and biotic stresses, protein profiling is important **(**Mahmoud *et al.,* 2018**).** This study is the first to isolate protein from callus *Atropa belladonna* L. and there are no previous studies.

In this study, irradiation with different doses of gamma on callus *Atropa belladonna* L. was performed measuring the effect of radiation on callus, and resulting active substances as antioxidants, as well as studies on the molecular level of protein and genetic material with SCoT markers.

MATERIALS AND METHODS

Embryogenic callus culture Establishment

Embryogenic calli of (*Atropa belladonna* L.) were established from young leaf discs on callus maintenance medium comprised of (Murashige and Skoog 1962) supplemented with 2,4-D 2mg/L+Kin 0.5mg/L. Cultures were maintained initially in the dark for 4–6wk. at25±1°C, andthereafter, cultures were incubated in a culture room under 16-h light (100μmol m 2s1photon flux density) and 8-h dark photoperiod at 26±1°Cwith 70–80% relative humidity.

Irradiation

Atropa belladonna callus were treated with 6 doses of gamma radiation (0.0, 30, 60, 90, 120 and 150 Gy γ-rays) at the National Center for Radiation Science and Technology (NCR), Cairo, Egypt

Radio sensitivity and gamma studies.

Six grams of embryogenic calli *Atropa belladonna*, placed in 9.5-cm-diameter Petri plate, were exposed to 0.0, 30, 60, 90, 120 and 150 Gy gamma rays using Gamma Cell 220 (60 Co source) at dose rate of 9.6 Gy min^{-1} at the National Center for Radiation Science and Technology (NCR), Cairo, Egypt. Following irradiation, 1,500 mg calli were placed on a newly prepared Callus medium for maintenance. Sensitivity of the irradiated calli was recorded in terms of relative growth rate (RGR) after 15, 30 and 45 days of treatment as follows: RGR= (final fresh weight−initial fresh weight)/initial fresh weight. The experiments were performed with three replicates.

Tissue water content determination.

Immediately after removal from the medium, fresh weight (FW) of the calli was determined and tissue paper was blocked to remove excess water. After drying the calli in the hot air oven for 48 hours at 60°C, dry weight (DW) was recorded. The percentage of fabric water (TWC) of the calli was determined as follows: TWC (%) = [(FW−DW)/FW] ×100.The dry matter content was estimated according to the following equation: Callus dry matter (%) = Callus dry weight x100 / Callus fresh weight.

Elements Analysis

The oven-dried material (80°C) was used to determine the concentrations of sodium (Na^{+}), potassium (K^{+}), Nitrogen (N^{-}), calcium (Ca^{+2}) and magnesium (Mg^{+2}).100mg of the dry material was extracted in 50 ml of deionized water with continuous shaking and used for the determination of ions concentration on a microprocessor-based Ion Analyzer (Elico, India) using ion specific electrode (Na^{+}, K^{+}, N^{-}, Ca^{+2}, Mg^{+2}) ratio was calculated according to (Faithfull, 2002).

Antioxidation

The free radical scavenging activity of the samples were measured by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) test according to the method of Braca *et al.,* 2002 with some modifications. Dried callus of 10 mg was added to 3ml of a 0.004% methanol solution of DPPH. The mixture was vigorously shaken and left in the dark for 30 minutes, then absorption by UV-2401PC visible spectrophotometers was measured at 517 nm against white (Shimadzu, Kyoto, Japan). Lower absorption of the reaction mixture showed higher free radical activity of scavenging, which was analyses on the graph of the composite concentration inhibition percentage. Triple and averaged experiments were performed. The ability to scavenge the DPPH with the following equation was calculated: Scavenging ability (%) = [(A – B)/ A] × 100

A= O.D of blank, B= O.D of sample

Molecular genetic studies

SDS-protein PAGE electrophoresis.

SDS- PAGE (Laemmli, 1970) technique was carried out with some modifications.

DNA Extraction

Total DNA has been extracted using the Biospin plant DNA extraction kit from 1 g of

calls (Bio Basic Inc. Kit Leading Supplier and Manufactures of Life Science Products and services, Canada). Quality of DNA has been checked with electrophoresis of 1.0 percent agarose gel.

SCoT Technique.

SCoT assay performed with random decamer primers obtained from DNA amplification was done using 7 SCoT primers (Table 1). Polymerase chain reaction (PCR) was carried out in a volume of 25 µL containing reaction buffer with $MgCl_2$, primer, (dNTPs), Taq polymerase and genomic DNA. The PCR mixture was subjected to 40 cycles in PCR with variable denaturation and annealing temperature. The products of amplification were stored at 4°C till further usage. Amplified products along with external size standard were stained with ethidium bromide and separated in a horizontal gel electrophoresis unit using 1.5 % agarose gel Ibrahim *et al.,* 2016.

Statistical analysis.

The data were statistically analyzed on complete randomized design system according to Snedecor and Cochran (1980). Means were compared by using least significant difference (LSD) at 5% levels of probability.

RESULTS

Effect of gamma irradiation on callus of *Atropa belladonna.*

The Effect of γ-rays on embryogenic calli and determination of optimal dose, of *Atropa belladonna* were established on MS medium supplemented with 2,4-D 2 mg/L + Kin 0.5 mg/L and fresh proliferating yellow is white callus were used for assessing γ-rays radiosensitivity (Fig.1 and Table 2). Dose radiation has influenced growth significantly and a decrease in RGR has been observed when the dosage increases). (Fig. 1). Patterns of calli growth were expressed as fresh weight of calli (mg), dry weight of calli (mg), dry matter of calli (%). Under the effect of (120 Gy) for 15, 30 and 45 days, callus fresh weight (mg) and callus dry weight (mg) and dry matter (2.65, 0.426 and 16.07 %) were highly significant, respectively compared with the control (2.04, 0.166 and 8.13 %). The irradiated calli turned entirely brown at higher doses (150 Gy). 120 Gy were therefore considered the best dose for Atropa belladonna embryo genic calli. The fresh and dry weight of leaves stems and roots of roselle plant were significantly increased because of γ-rays compared with control El Sherif *et al.,* (2011).

Ion levels under gamma irradiation stress in callus of *Atropa belladonna.*

Another important characteristic in gamma stress is the difference in ion levels; hence the contents of (K^+, Na^+, Ca^{2+}, N^- and Mg^{2+} Content) have been observed under various gamma treatments. The data presented in Tables (3) illustrate the effect of different doses of γ-rays (0.0, 30, 60, 90, 120 and 150 Gy) levels on elements % (K^+, Na^+, Ca^{2+}, N^- and Mg^{2+} Content) in callus of *Atropa belladonna*. γ-rays significantly ($p < 0.05$) decreased the K^+ content of callus for all doses of gamma (Table 3). The mean relative decrease in K^+ content of callus of all *Atropa belladonna* callus under doses of gamma (0.0, 30, 60, 90, 120 and 150 Gy were 3.300±0.39, 3.897±0.41, 2.190±0.39, 2.350±0.05, 0.707±0.50 and 1.790±0.69%, respectively. On the other hand, callus Na+ content decreased significantly in gamma ($p < 0.05$) for all gamma doses (Table 3). In Na^+, the mean relative decreases were 3.717±0.35, 5.19±0.44, 3.453±0.05, 3.193±0.36, 0.080±0.51 and 1.085±0.26 percent of all *Atropa belladonna* callus at gamma (0,0, 30, 60, 90/1, 120, and 150Gy), respectively. The same results were obtained for N^-content and the quality of Callus N^- decreased significantly in gamma ($p<005$) (Table 3). The mean relative reductions of all gamma *Atropa belladonna* callus (0.0, 30, 60, 90, 120, and 150Gy) in N- were 3.457±.032, 3.213±.054, 0.920±0.05, 0.640±.084, 1.010±0.05 and 3.040±0.02, respectively. The consistency of Callus Ca^{2+} in gamma ($p<005$) was substantially less in terms of Ca^{2+} material (Table 3). The mean relative reductions in Ca^{2+} were 1.174±0.01, 0.646±0.05, 0.083±0.45, 0.063±0.07, 0.545±0.10or 0.819±0.21for all the Gamma *Atropa belladonna* callus (0.0, 30, 60, 90, 120, and 150 Gy) for Ca^{2+}. Gamma component ($p<005$) for Mg^{2+} was significantly lower (Table 3). The average relative Mg^{2+} reductions for all Gamma *Atropa belladonna* callus (0,0,0, 30, 60, 90, 120 and 150 Gy) were 0.685±0.11, 0.410±0.05, 0.456±0.07, 0.318±0.06, 0.325±0.07and 0.506±0.09.

Redical-scavening activities of the different callus γ-rays' doses from *Atropa belladonna*.

Different dried of callus gamma rays doses from *Atropa belladonna* were used as antioxidant, and the results are show in Fig. (2). The redical-scavening activities for different dried of callus gamma rays doses were examined comparing with 1,1-Diphenyl-2-Picrylhdydrazyl (DPPH) solution for 30 min.

The results indicate that the highest radical-scavening activity was given by dose 30 Gy (40.70) followed by 120 Gy (39.00), 90 Gy (31.10), dose 150 Gy (30.20) and 60 Gy (29.90); respectively. The control had the lowest effect of radical-scavening activity; 21.10.

SDS-Protein electrophoresis

The total protein revealed by SDS-page were used to detect the genetic differences between the irradiated calluses. These results showed a mixture of 46 polypeptide bands ranging in size from 9.154 to154.238 kDa (Table 4), 11 bands of which were polymorphic and 17 bands of which were unique with different molecular weight (Table 4 and Fig.3). In gamma ray doses, the largest number of bands 12 were found in dose 120 and 150 Gy with 100% polymorphic value. Dose 60 Gy gave the minimum number of bands 4. However, data in (table 4) show high number of new protein bands can be used as markers between irradiated plants every dose. However, there were unique bands in each dose which varied in number (5, 2, 3, 1 and 1) for (30, 60, 100, 120 and 150 Gy), respectively and sizes (108.157, 53.985, 48.624, 42.031 and 23.643 KDa) for 30 Gy, (82.341 and 53.384 KDa) for 60 Gy, (76.128, 54.390 and 24.727 KDa) for 90 Gy, (151.384 KDa) for 120 Gy, and (9.154 KDa) for 150 Gy, respectively.

Overall, SDS-PAGE analysis for callus proteins have shown two types of bands: polymorphic (through certain bands of dose and not others) and special (band appeared in only one dose). These bands varied in terms of quantitative and qualitative weight, concentration, relative mobility, and fractionation. These bands can also be used for the characterization of every germplasm as biochemical markers. Various bands of protein were compared, and some doses vanished while, others emerged. The resulting patterns showed heterogeneity between callus of different gamma ray doses (Fig. 3).

Table 5 show similarity matrices for callus of *Atropa belladonna* based on SDS-PAGE protein analysis. The highest similarity between 30, 150 and 120 Gy was recorded (4.123). The lowest similarity between 120 Gy and 150 Gy (1.414).

The clustering of control and gamma radiation callus *Atropa belladonna* which are produced by UPGMA cluster analysis based on Nei and Li coefficients (Fig. 4). The research is divided into two groups with distance of 3.7. The first group is composed of only 150 Gy and 120 Gy with distance of 1.5. The second group is divided into three subgroups at distance 3.5. The first subgroup consists of 90 Gy and 60 Gy with distance of 2.6, while the other groups consist of 30 Gy only in one group with distance 3.5 and the control only in one group with distance of 3.

SCoT polymorphism

For polymorphic screening seven primers of SCoT were used. The analysis of genotypes with seven SCoT primers provided a total of 66 amplicons with a range between 178.2 to 1351bp. There were 66 bands, ranging from 6 (Primer SCoT-2 and SCoT-11) to 14. (Primer SCoT-10). In addition, the seven primary molecular markers are classified as positive molecular markers (41 molecular markers), which are compared with controls of several molecular dimensions in irradiated callus (Table 6 and Fig.5). On the contrary, two negative molecular markers with molecular sizes 940 and 400 bp were found only in control in comparison with irradiated callus. In addition, in both control and irradiated callus 25 common molecular markers were identified (Table 6 and Fig.5).

Table 7 shows Jaccard coefficient similarity matrices for gamma radiation callus of *Atropa belladonna* based on targeted start codon (SCoT) analysis. Table 7 shows the highest similarity between gamma radiation 60 Gy and gamma radiation 90 Gy was recorded (93). The lowest similarity was on the other hand, between 120 Gy and 150 Gy and control (80).

The clustering of control and gamma radiation callus *Atropa belladonna* which are produced by UPGMA cluster analysis based on Nei and Li coefficients (Fig. 6). Research is divided into two groups with distance 4. The first is composed of only 150 Gy and 120 Gy. At distance 3.5, two subgroups are divided into the second group. The first subgroup consists of 90 Gy and 60 Gy, while the other group consists of 30 Gy and control.

DISCUSSION

Generally, mutagenic treatments for the recovery of somaclonal variants are not applied to cell cultures. But a frequency increase in somaclonal variants was usually observed in this research when mutagenic treatments were used. Mutagenesis was reportedly needed to recover the specific variant isolated in some cases. The main physical mutagen for inducing genetic variation is gamma irradiation. The combination of induction and *in vitro* technology is a more efficient solution for

improving modern farm ecosystem productivity (Rudulier et al. 1984). The starting point of any breeding program is genetic variation. For variants, the combination of irradiation of explants and *in vitro* regeneration is most efficient. Novak (1987) described the dose response of tissue cultured to gamma. In this study effects on calluses, morphogenesis, chemicals, ion contents and genetics have been examined using *in vitro* mutagenesis. In various explant materials, the effects of gamma rays on tissue culture were shown (Shasthree et al. 2009; Degani and Pickholz 1973). Callus growth was favored by lower irradiation doses than by higher doses. These results have been supported by (Shasthree et al. (2009). EL-Shaer *et al.*, 2016 study the effect of gammas 5 and 10 Kr and of some growth regulators (BA, 2,4-D and NAA) these investigations were performed using the technique of tissue culture. Radioactive callus to produce *Moringa oleifera* secondary plant components were studied.

The first step of this program was the assessment of radio-sensitivity of *Atropa belladonna* to establish the appropriate mutagenesis dose. Tissue response to a given dose of radiation indicates this to select exact mutagenesis dose. Taras et al.1999 and Bajaj 1970 on callus cultures of *Phaseolus vulgaris*.

Depending on the gamma dose Gamma rays (γ-rays) can affect living organism cells. The gamma rays have triggered free radical modifications in plant cells. Gamma rays are energy intensive, membranes damaging ion radiation. A method of successful detection of such damage is ion leakage controlled by relative conductivity in *Arabidopsis* irradiated with gamma rays Zheng and Li 2017.

It has been pointed out that plant stress reactions frequently lead to changes in the metabolism of proteins. Several proteins in a variety of stress environments are synthesized and stored in plant tissues. proteins shown by SDS-PAGE can be used for a variety of purposes such as genetic diversity, biosystematics analysis, polygenetic link recognition and evolutionary interrelationships of organisms from different natural habitats Khater*et al*., 2016.This polymorphism may be a genetic marker since it can be their variability is typically highly inherited and highly polymorphic. Protein polymorphisms resulting from insertions or deletion between mutated protein band sites are also codominant. Found in accord with Bhat and Kudesia 2011protein profile of five species (*Solanum melongena*, *S. xanthocarpum*, *Datura alba*, *Lycopersiconesculentum* and *Capsicum annum*) of family Solanaceae, SDS-PAGE has been widely used in recent years for resolution of genetic diversity Karihaloo *et al*.,2004.

In addition, new (unique) bands typically occur due to various changes in structural DNA (*e.g*., splits, transpositions, deletions) resulting in modifications in amino acids and hence the protein shaped Mondini *et al*., 2009.

It is predicted that the SCoT markers are connected to functional genes and corresponding characteristics, so that the amplicons can be translated to gene target marker systems. Also these markers are multilocuses that are useful in achieving high genetic polymorphism. Xiong *et al*., 2011. These changes with gamma will correlate with the photoproduct levels of the Taq polymerase binding sites after radiation in the DNA template. Furthermore, the results obtained suggested that the SCoT marker can be used to effectively evaluate the variation between treated *Atropa belladonna*. These results is agree with previous results that obtained by (Xiong *et al*., 2011) in groundnut and (Luo *et al*., 2010) mango. *Atropa belladonna* had a higher resolution power of SCoT markers than potato (Gorji*et al*., 2011) and like resolving power of SCoT markers in Dendrobium.

Rajput and Agrawal 2020 *Atropa acuminata* Royle ex Lindl is a therapeutic herb critically endangered, well known for its immense therapy against countless illnesses. 96% Similitude between the mother plants and the micropropagated plants has been revealed by the genetic fidelity of *ex vitro* acclimated plants using SCoT and sequence-relate amplifying polymorphism markers (SRAP). In comparison to mother plants, these plants have shown higher activities with antioxidants (DPPH test).

CONCLUSION

Optimal mutagenic agents as gamma radiation played an important role in producing callus growth and improving secondary metabolites. The reduction in gamma irradiation increased the fresh weight of callus, while the greater gamma irradiation was lethal to the callus of *Atropa belladonna*. Moreover, the SDS-PAGE and SCoT-PCR approach can be used as an investigative technique for growth alterations caused by gamma rays. It should be noted that the 120 Gy and150 Gy dose produced the highest number of DNA fragments (48 bands), followed by the control dose given (47 bands).

Low doses of 30, 60 and 90 Gy showed beneficial genetic effects on callus for breeding purposes with gamma ray treatments and mutant plant variants could be easily identified using SCoT-PCR analysis. SCoT -10 gave 10 positive and total number of bands 14 so we recommend it for the future studies**.**

REFERENCES

Bajaj, Y.P.S. Saettler, A.W., Adams, M.W. 1970: Gamma irradiation studies on seeds, seedlings and callus tissue cultures of *Phaseolus Vulgaris* L. Radiation Botany.10, 2, 119-124.

Bhat, T.M., Kudesia, R 2011: Evaluation of Genetic Diversity in Five Different Species of Family Solanacea using Cytological Characters and Protein Profiling. Genetic Engineering and Biotechnology Journal. 20.

Braca, A., Sortino, C.P. 2002: Antioxidant activity of flavonoids from *Licanialicaniae flora*. J. Ethnopharmacol. 79:379-381.

Degani, N., Pickholz, D. 1973: Direct and indirect effect of gamma. J. Plant. 62.

EL-Shaer, H.F.A., Abd El-Hady, M.S., Nassar, M., Nabil, A. 2016: Molecular genetic studies on (*Mungbean oleifera* Lam) under effect of gamma radiation by tissue culture techniques. J. Biol. Chem. Environ. Sci. 11(4): 503 - 527.

El Sherif, F., Khattab, S., Ghoname, E., Salem, N., Radwan, K. 2011: Effect of Gamma Irradiation on Enhancement of Some Economic Traits and Molecular Changes in Hibiscus Sabdariffa Life Sci., J, 8(3).

Faithfull, N.T. 2002: Methods in Agricultural Analysis: A Practical Handbook. CABI, Publishing, 8(19): 1765-1780.

Gorji, A.M., Poczai, P., Polgar, Z., Taller, J. 2011: Efficiency of arbitrarily amplified dominant markers (SCoT, ISSR and RAPD) for diagnostic finger printing in tetraploid potato. Am. J. Potato Res. 88, 226–237.

Ibrahim, S.D., Adawy, S.S., Atia, M.A.M., Alsamman, A.M., Mokhtar, M.M. 2016: Genetic diversity, variety identification and gene detection in some Egyptian grape varieties by SSR and SCoT markers. Plant Omics 9(5): 311-318.

Karihaloo, J.L., Kaur, M., Singh, S. 2004: Seed protein diversity in *Solanum melongena* and its wild and weedy relatives. Genetic Resources and Crop Evolution, 49: 533-539.

Kovacs, E., Keresztes, A. 2002: Effect of gamma and UV-B/C radiation on plant cells. Micron;33: 199–210.

Laemmli, U.K. 1970: Cleavage of structural proteins during the assemble of the head of bacteriophage T4. Nature, 227: 680.

Luo, C., He, X.H., Chen, H., Ou, S.J., Gao, M.P. 2010: Analysis of diversity and relationships among mango cultivars using Start Codon Targeted (SCoT) markers. Biochem. Syst. Ecol. 38, 1176–1184.

Mahmoud, F.E., Rashed, M.A., Khalil, K.M., Abou-Deif, M.H. 2018: Identification and characterization of heat shock protein in four Egyptian maize inbred lines (*Zea mays* L.). J. Agric. Sci., 26(2A), 1203-1211.

Maniatis, T., Frictsch, E.F., Sambrook, J. 1982: Molecular cloning. A laboratory manual. Cold Spring Habor Laboratory Publisher, New York.

Maqbool, F., Singh, S., Kaloo, Z., Jan, M. 2014: Medicinal importance of Genus AtropaRoyle - A review. International Journal of Advanced Research. 2, 2, 48-54.

Mondini, L., Noorani, A., Pagnotta, M.A. 2009: Assessing plant genetic diversity by molecular tools. Diversity. 1: 1 9-35.

Mulpuri, S., Muddanuru, T., Francis, G. 2013: Start codon targeted (SCoT) polymorphism in toxic and non-toxic accessions of *Jatropha curcas* L. and development of a codominant SCAR marker. Plant Sci. 207, 117–127.

Murashige, T., Skoog, F. 1962: A Revised Medium for Rapid Growth and BioAssays with Tobacco Tissue Cultures. Plant Physiology, 15, 473-497.

Novak, F.J., Micke, A. 1987: *In vitro* mutants technology for crop improvement in developing countries, Sabrao 19:82-85

Shasthree, T., Imran, M.A., Narsinga, R.N., Mallaiah, B. 2009: Effect of physical mutagen (Gamma rays) on differentiation and multiple shoot production from different explants of *Erithrinavariegate*L. BTAIJ 3(4):261-266

Snedecor, G.W., Cochran, W.G. 1980: Statistical methods.7th ed. Lowa State Unv. Press., Ames., Lowa, U.S.A.

Suprasanna, P., Jain, S.M., Ochatt, S.J., Kulkarni, V.M., Predieri, S. 2012: Applications of *in vitro* techniques in mutation breeding of vegetatively propagated crops. In: Shu QY, Forster BP, Nakagawa H (eds)Plant mutation breeding and biotechnology. CAB International, Wallingford. 369–383.

Rajput, Agrawal, 2020: Micropropagation of *Atropa acuminata Royle* ex Lindl. (a critically endangered medicinal herb) through root callus and evaluation of genetic fidelity, enzymatic and non-enzymatic antioxidant activity of regenerants. Acta Physiologiae Plantarum. 42(11).

Ramakrishna, D., Shasthree, T. 2016: Highly efficient somatic embryogenesis development from leaf cultures of *Citrulluscolocynthis* (L.) Schrad Article in Physiology and Molecular Biology of Plants. June.

Rudulier, D.L., Strom, A.R., Dandekar, A.M. 1984: Molecular biology of osmoregulation. Science. 224:1064-1068.

Taras, T.C., Szala, L., Krzymanski, J. 1999: An *in vitro* mutagenesis selection system for *Brassica napus* L. In: proceedings of 10th interna-tional Rapeseed Congress, Canberra, Australia.

Zheng, G., Li, W. 2017: Profiling membrane glycerolipids during γ-ray-induced membrane injury. BMC Plant Biology. 203.

Wi, S.G., Chung, B.Y., Kim, J.S., Kim, J.H., Baek, M.H., Lee, J.W., Kim, Y.S. 2007: Effects of gamma irradiation on morphological changes and biological responses in plants. Micron.;38:553–64.

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

Table 1: List of 7 SCoT primers used in finger printing in irradiated calls *Atropa belladonna*.

Primer name	Primer Sequence
SCoT -1	5'-CAACAATGGCTACCACCA-3'
SCoT -2	5'-CAACAATGGCTACCACCC-3'
SCoT -6	5'-CACCATGGCTACCACCA-3'
SCoT -8	5'-ACGACATGGCGACCCAC-3'
SCoT -10	5'-ACGACATGGCGACCGCG-3'
SCoT -11	5'-AAGCAATGGCTACCACCA-3'
SCoT -13	5'-ACGACATGGCGACCATCG-3'

Table 2: Interaction between effect gamma irradiation and 2,4-D 2 mg/L, Kin 0.5 mg/L on callus color, rigidity, fresh weight, callus dry weight and callus dry matter before subculture of *Atropa belladonna* L. after 45 days.

Treatments after 45 days.	Fresh weight (mg)	Dry weight (mg)	Dry matter content (%).	Color	Rigidity
Control	2.04	0.166	8.13	Light yellow	Friable
30 GY	2.46	0.193	7.84	Light yellow	Friable
60 GY	2.32	0.206	8.87	Light yellow	Friable
90 GY	3.77	0.196	5.19	Light yellow	Friable
120 GY	2.65	0.426	16.07	Light yellow	Friable
150 GY	2.72	0.136	5.00	Dark yellow	Friable
P ≤ 0.05	0.0011 **	0.000 ***			
LSD at 0.05	0.6190	0.0410			

Table 3: Effects of different γ-rays doses (0.0, 30, 60, 90, 120 and 150 Gy gamma rays) in calls *Atropa belladonna* on potassium (K^+), sodium (Na^+) calcium (Ca^{2+}), chloride (N^-) and magnesium (Mg^{2+}).

Elements Content%	γ-rays doses (Gy)	*Atropa belladonna* callus	Mean (b)
K^+	Control (0.0)	3.300±0.39	1.650
	30	3.897±0.41	1.948
	60	2.190 ±0.39	1.095
	90	2.350±0.05	1.175
	120	0.707 ±0.50	0.353
	150	1.790±0.69	0.895
	Mean(a)	2.372	
	L.S.D. (0.05)	a=n.s a=0.333 b= 0.576	a*b= 0.815
Na^+	Control (0.0)	3.717±0.35	1.858
	30	5.19 ± 0.44	2.598
	60	3.453±0.05	1.727
	90	3.193± 0.36	1.596
	120	0.080 ±0.51	0.040
	150	1.085± 0.26	0.543
	Mean(a)	2.787	
	L.S.D. (0.05)	a=n.s a=0.658 b= 1.139	a*b= 1.611
N^-	Control (0.0)	3.457±.032	1.728
	30	3.213±.054	1.607
	60	0.920±0.05	0.460
	90	0.640±.084	0.320
	120	1.010±0.05	0.505
	150	3.040±0.02	1.520
	Mean(a)	2.047	
	L.S.D. (0.05)	a=n.s a=0.205 b= 0.355	a*b= 0.501
Ca^{2+}	Control (0.0)	1.174±0.01	0.587
	30	0.646±0.05	0.323
	60	0.083±0.45	0.042
	90	0.063±0.07	0.032
	120	0.545±0.10	0.273
	150	0.819±0.21	0.409
	Mean(a)	0.555	
	L.S.D. (0.05)	a=n.s a=0.130 b= 0.225	a*b= 0.319
Mg^{2+}	Control (0.0)	0.685±0.11	0.342
	30	0.410±0.05	0.205
	60	0.456±0.07	0.228
	90	0.318±0.06	0.159
	120	0.325±0.07	0.162
	150	0.506±0.09	0.253
	Mean(a)	0.450	
	L.S.D. (0.05)	a=n.s a=0.067 b= 0.117	a*b= 0.165

LSD(a): *Atropa belladonna* callus, LSD(b): Gamma rays' doses (Gy), LSD (a*b): *Atropa belladonna* callus *Gamma rays' doses (Gy

Table 4: Summary of callus gamma dose 0.0, 30, 60, 90, 120 and 150 Gy *Atropa belladonna* protein banding pattern by SDS-PAGE analysis.

MW	Cont.	30 Gy	60 Gy	90 Gy	120 Gy	150 Gy	Polymorphism
154.238	+	-	+	+	+	+	Polymorphic
151.384	-	-	-	-	+	-	Unique
126.060	-	-	-	-	+	+	Polymorphic
113.540	+	-	-	-	-	-	Unique
110.197	-	-	+	+	+	+	Polymorphic
108.157	-	+	-	-	-	-	Unique
97.415	-	-	-	-	+	+	Polymorphic
88.068	+	-	-	-	-	-	Unique
83.269	-	-	-	-	+	+	Polymorphic
82.341	-	-	+	-	-	-	Unique
76.128	-	-	-	+	-	-	Unique
60.841	-	-	-	+	+	+	Polymorphic
57.097	+	-	-	-	-	-	Unique
54.390	-	-	-	+	-	-	Unique
53.985	-	+	-	-	-	-	Unique
53.384	-	-	+	-	-	-	Unique
48.624	-	+	-	-	-	-	Unique
47.016	-	-	-	-	+	+	Polymorphic
42.031	-	+	-	-	-	-	Unique
40.793	-	-	-	+	+	+	Polymorphic
40.039	+	-	-	-	-	-	Unique
30.029	-	-	-	-	+	+	Polymorphic
28.182	+	-	-	-	-	-	Unique
24.727	-	-	-	+	-	-	Unique
23.643	-	+	-	-	-	-	Unique
21.295	-	-	-	-	+	+	Polymorphic
12.717	-	-	-	-	+	+	Polymorphic
9.154	-	-	-	-	-	+	Unique
Total	6	5	4	7	12	12	46

Table 5: Similarity Matrix according to SDS-PAGE protein control and gamma radiation callus *Atropa belladonna.*

	Control	30 Gy	60 Gy	90 Gy	120 Gy	150 Gy
Control	0،000	3،317	2،828	3،317	4،000	4،000
30 Gy	3،317	0،000	3،000	3،464	4،123	4،123
60 Gy	2،828	3،000	0،000	2،646	3،464	3،464
90 Gy	3،317	3،464	2،646	0،000	3،317	3،317
120 Gy	4،000	4،123	3،464	3،317	0،000	1،414
150 Gy	4،000	4،123	3،464	3،317	1،414	0،000

Table 6: List of primers used for SCoT analysis with their sequences, GC content and (TB) total number of bands, (MB) monomorphic band, (PB) polymorphic band, (UPB) unique positive band, (UNB) unique negative band and (BS) band of Size generated in PCR reaction with DNA from control and gamma radiation callus *Atropa belladonna*.

P N	P S	GC	TB	MB	PB	P%	UPM	UNM	B S
SCoT-01	5'-CAACAATGGCTACCACCA-3'	52.9	11	9	2	18	700bp	940bp	940 -240
SCoT-02	5'-CAACAATGGCTACCACCC-3'	52.9	6	6	0	00	-	-	530 -260
SCoT-06	5'-CACCATGGCTACCACCA-3'	58.8	11	3	8	73	760bp-390bp	400bp	1100 - 280
SCoT-08	5'-ACGACATGGCGACCCAC-3'	64.7	8	5	3	38	650bp	-	1100 - 260
SCoT-10	5'-ACGACATGGCGACCGCG-3'	70.6	14	4	10	71	260bp	-	630 -180
SCoT-11	5'-AAGCAATGGCTACCACCA-3'	70.6	6	6	0	00	-	-	550 -260
SCoT-13	5'-ACGACATGGCGACCATCG-3'	61.1	10	2	8	80	1000bp 788bp 610bp	-	1350 - 320
Total			66	35	31		8	2	940-320

Table 7: Similarity Matrix according to SCoT markers in control and gamma radiation callus *Atropa belladonna*.

	Control	30 Gy	60 Gy	90 Gy	120 Gy	150 Gy
Control	100					
30 Gy	90	100				
60 Gy	86	85	100			
90 Gy	85	84	93	100		
120 Gy	80	81	82	82	100	
150 Gy	80	81	80	84	83	100

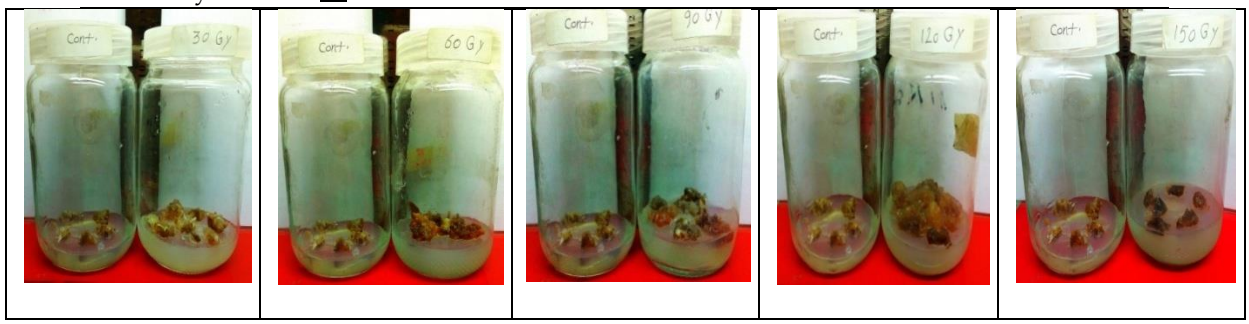

Figure 1: Callus formation from leaf explants on MS medium add up to with 2 mg/L (2,4-D) in combination with 0.5 mg/L (Kin) after 45 days under effect gamma irradiation.

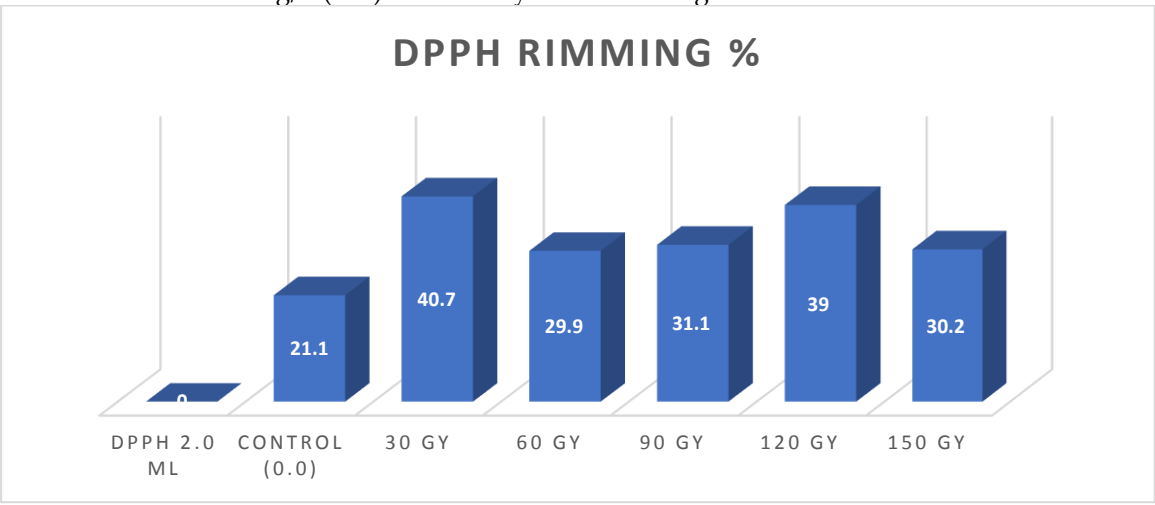

Figure 2: DPPH rimming % of *Atropa belladonna* callus and gamma dose 0.0, 30, 60, 90, 120 and 150 Gy.

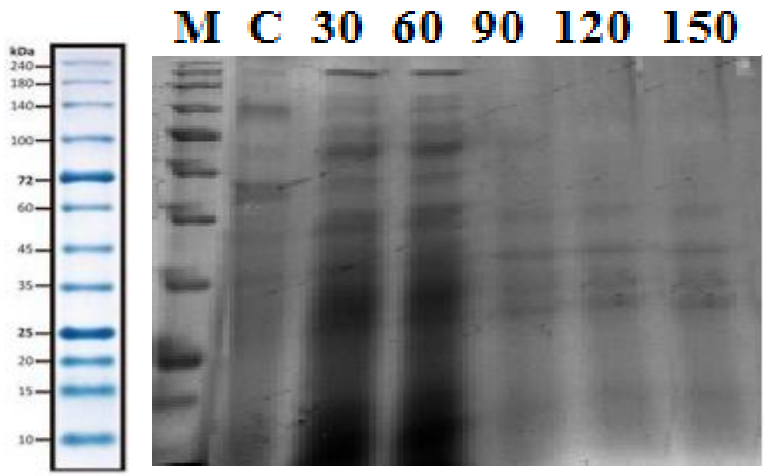

Figure 3: SDS-PAGE of *Atropa belladonna* protein extracts irradiated calluses in varying gamma ray concentrations. Lane (M) = Marker. Lanes: 1=Control, 2= 30 Gy, 3= 60 Gy, 4=90 Gy, 5= 120 Gy, 6= 150 Gy.

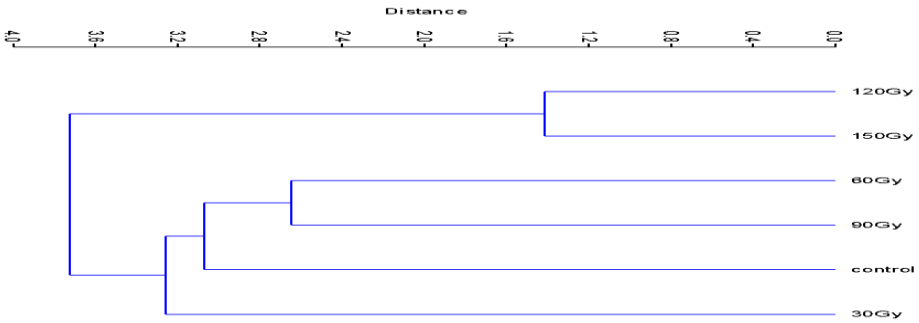

Figure 4: UPGMA based on Dice's resemblance, protein profiling estimates, shows a genetic link between control and various *Atropa belladonna* irradiated calluses.

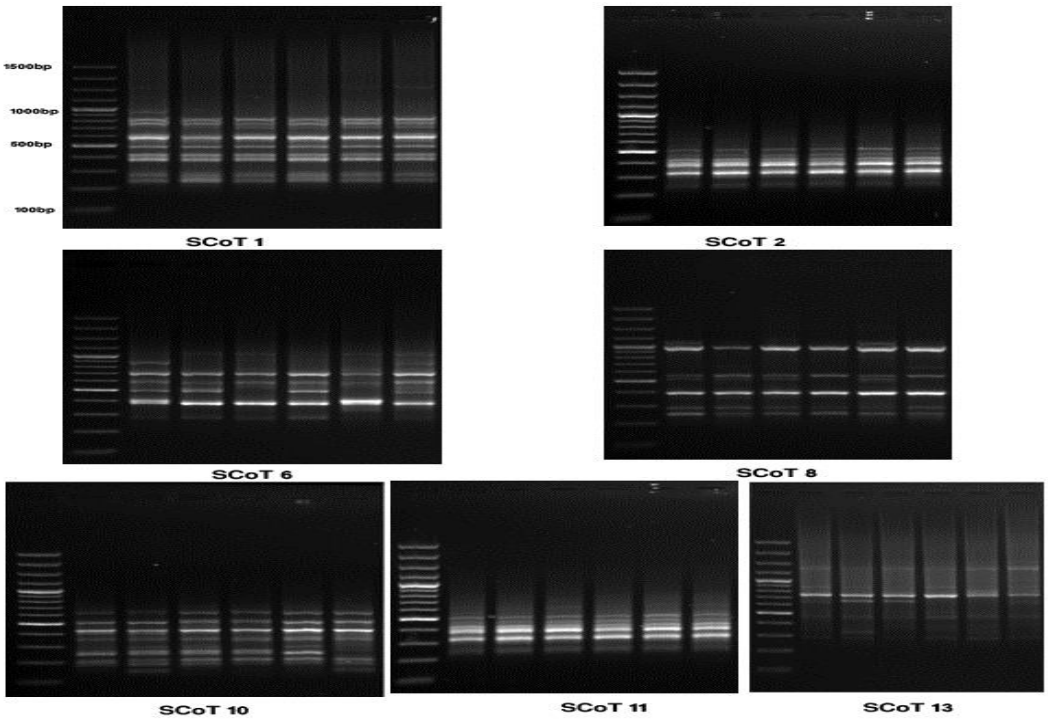

Figure 5: SCoT-based PCR fragments of seven primers in irradiated *Atropa belladonna*, callus M= DNA standard marker, 1= Control, 2= 30 Gy, 3=60 Gy, 4= 90 Gy, 5= 120Gy, 6= 150Gy.

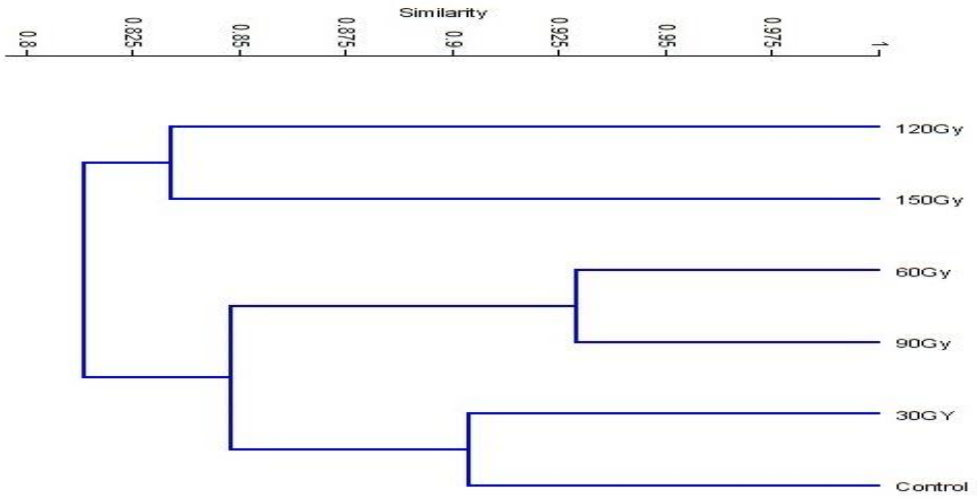

Figure 6: Dendrogram resulted from SCoT markers in control and irradiated *Atropa belladonna* callus.

تقييم الثبات الجيني باستخدام واسمات SCoT و SDS-PAGE بإشعاع جاما على الكالس (*Atropa belladonna* L) والنشاط المضاد للأكسدة.

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الملخص العربي

لتحسين الأصول الوراثية استخدمت الطفرات في إنتاج نباتات متحولة جديدة، كانت هذه الطفرات مفيدة في مواجهة تحديات حماية الغذاء والتغذية العالمية. ساهم التعرض للمطفرات في تربية النباتات ولمشاريع تطوير قلويدات الأدوية في مجموعة واسعة من التنوعات الجينية. المصدر التجاري الأساسي لقلويدات العقاقير مثل الأتروبين هو أتروبا بلادونا. تركز هذه الورقة على تأثير أشعة جاما على أتروبا بلادونا على المستوى الجزيئي والتغيرات الكيميائي الحيوي في الكالس. تم تعريض الكالس أتروبا بلادونا للإشعاع باستخدام 0.0 و 30 و 60 و 90 و 120 و 150 أشعة جاما بعد 6 أسابيع على الوسائط الحثية. تم تسجيل فروق ذات دلالة إحصائية بين تأثير أشعة جاما على الكالس في أتروبا بلادونا عند 2 mg/L 2،4 D و Kin 0.5 mg/L على التوالي على وزن الكالس الطازج ووزن الكالس الجاف. 120 جراي أعطت أعلي وزن من الكالس الطازج والوزن الجاف ومحتوى المادة الجافة (٪) بالمقارنة مع الغير معامل بالاشعاع. تم قياس الفرق في مستويات الأيونات في الكالس المعامل والغير معامل بالاشعاع. تنخفض النتائج بوضوح في مستويات الأيونات مع زيادة جرعة جاما. من ناحية أخرى ، تم قياس تأثير المواد الفعالة كمضادات للأكسدة من الكالس. أعلي فاعلية كمضادات للأكسدة كانت بجرعة 150 غراي والتي سجلت 30.20 مقارنة مع الكالس الغير معامل أعطي 20.10. تم استخدام البولي أكريلاميد الكهربائي للهلام البروتين في التفرقة. في النباتات المعرضة للإشعاع ، كان هناك العديد من نطاقات البروتين الجديدة التي يمكن استخدامها كواسمات لكل جرعة. باستخدام مقايسة تعدد الأشكال المستهدفة لكودون DNA-Start (SCoT) ، كان هناك نوعان من الواسمات الجزيئية السالبة التي وجدت فقط في الأصل بالمقارنة مع الكالس المشع بأحجام جزيئية 940 و 400 زوج قاعدي.

الكلمات الاسترشادية: