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INFLUENCE OF NACL STRESS AND GAMMA RADIATION ON IN VITRO SEEDS GERMINATION AND SOME PHYSIOLOGICAL PARAMETERS IN CALLUS PRODUCED FROM COWPEA (VIGNA UNGICULATA.L)

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ABSTRACT

Gamma radiation and *In Vitro* technique were applied in this study to evaluate some morphological and physiological parameters in callus of irradiated Cowpea (*Vigna ungiculata*. L) seedlings. Local variety seeds of Cowpea were exposed to (0, 25, 50 and 75) Gy of Gamma radiation (^{60}Co). Seeds were sterilized and germinated on Murashige and Skoog (MS) media. Results clarified that, in general, maximum seeds germination 93.0% at 25 Gy whereas maximum seedling height reached (4.53 and 3.40 cm) in addition to number of leaves (3.57,2.90) were observed at 25 and 50 Gy respectively. All mutagenic doses caused a significant reduction in photosynthetic chlorophyll pigment compared with control (non irradiated seeds). Callus was initiated from shoot tip explants and exposed to (NaCl) stress at the levels (6, 8 and 10 ds.l^{-1}). 4 weeks later, Proline, CHO and minerals were estimated. In general, increasing gamma radiation doses at 50 up to 75 Gy and NaCl at the level 10 ds. m^{-1} increased accumulation of Na^+ and Cl^- whereas highest accumulation of K^+ was found with 75 Gy when combined with 6 and 8 ds. m^{-1}. Moreover, highest content of CHO (8.75 mg. g^{-1}) was observed in 25Gy combined with the level 10 ds.m^{-1} and Proline (26.46 mg. g^{-1}) at 50 Gy combined with the level 10 ds.m^{-1}.

Keywords: Chlorophyll, Proline, CHO, ^{60}Co, Na^+, K^+

INTRODUCTION

Cowpea (*Vigna ungiculata*.L) is one of the most important human food sources due to its highly protein beside, all stages of plant growth are used in human food and animal feed as its provide a largest source of vegetable protein in human and livestock forages [Kang et al,,2020]. Moreover, Cowpea as a legume plant is able to form symbiotic association with N_2 fixing bacteria that form nodules (Rhizobium group) in the soil, which is supplies plants with N in a readily assimilable form and in sufficient quantity to make mineral-N fertilization unnecessary [Diop et al, 2015]. Legume family are also rich in phenolic compound which inhibit free radicals (ROS) which caused cardiovascular disease [Xiong et al. 2013]. In recent years, considerable progress have been made regarding the development and isolation of stress tolerant genotypes by Induced mutations through physical or chemical mutagenesis in combination with *in vitro* biotechnological techniques, which proved novel genetic variability and offers an efficient method for improving vegetative propagated plants [Nikam *et al*,2014] since these techniques allow induction of variation, selection and multiplication of the desired genotypes in a much shorter duration and smaller space compare with conventional methods [Afrasiab and Iqbal, 2012]. Also *In vitro* techniques allow to a large number of plants to grow in a small restricted area around the year without interfering other factors since there is a direct interaction of all cells with the stressor's agents in media components [Pérez-Clemente and Gómez-Cadenas.2012] so that many researchers had the opportunities to improve plants against biotic and abiotic stresses. Also, the researches in this field showed that plants regenerated from organ cultures, calli, and via somatic embryogenesis sometimes exhibit phenotypic and/or genotypic variations [Al Hattab *et al*,2017]. Available studies on using gamma radiation in combination with in vitro techniques to induced mutations have been used to improve major crops such as sugarcane, potato, wheat, rice, barley, cotton, peanuts, beans and creating crop varieties with desirable traits such as protein and starch quality, drought and salt tolerance or even diseases resistance have been reported [Yaycili, and Alikamanoglu. 2012]. However, despite the negative effect of stress on plant yield ,components, previous works on in vitro approaches for production of plants stress tolerance under various stresses have proved many advantages, for example a positive correlation were found in plant in related to antioxidant defense system enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) [Hossain *et al*,2007], Moreover, accumulation of secondary products with medicinal properties [El-Kaaby,2016].The aim of the present study is to determine the effect of gamma ray on some morphological and physiological parameters in stressed NaCl callus.

MATERIALS AND METHODS

The study was carried out at Genetic Engineering Department, tissue culture Unit and Soil and Water Resources Center during the years 2018-2020.

Plant material

Seeds irradiation

Mature seeds of Cowpea (*Vigna ungiculata*.L) were obtained from local market and then irradiated at 0, 25, 50 and 75 Gy respectively. Irradiation was done at IAEA laboratories (Seibersdorf), using a cobalt - 60 source ((Dose rate 2 Gy/Sec.).

In Vitro Seeds surface sterilization and establishment of culture

Irradiated and non-irradiated seeds of Cowpea were surface sterilized under aseptic conditions in a laminar air flow cabinet with 70% ethanol for 1 min following with 2% commercial bleach sodium hypochlorite (NaOCl) for 10 min (with continuous shaking) and rinsed 3 times with distilled water 5 min for each [Khare *et al*, 2016]. Afterwards, seeds were germinated on full strength of basal media Murashige and Skoog (MS) [1962]. pH was adjusted to 5.70 and media Solidified with 7 $g.l^{-1}$ agar before autoclaved. The cultures were stored in controlling growth room at 25±2°C and light photoperiod 18 /6 darkness for 2 weeks. Seeds germination%, seedling height, Number of leaves was recorded as well as Chlorophyll in leaves of plantlets contains were determined according to [Abdelgawad *et al*, 2019].

Callus induction media

After 2 weeks of seed germination, shoot tips excised and transferred to MS media containing 5 $mg.l^{-1}$ 5.0 kinetin concentration [Dadmal, and Navhale, 2011] for callus induction media. The cultures were incubated at the same conditions previously described in seed germination stage.

Callus stressed media

For investigation of salinity stress in cowpea, 100 mg of callus fresh weight of each radiation dose was cultured in vitro on media supplemented with Three levels of NaCl (6, 8 and 10 $ds.l^{-1}$). 4 weeks later, proline was determined by [Bates *et al*1973], Minerals such Na^+ according to [Page *et al* 1982], Cl^- estimated according to [Richared *et al*,1954], K^+ estimated based on [Chapman and Pratt. 1961] and CHO according to [Dubois *et al*, 1956] as total carbohydrates was expressed as mg glucose/g dry weight.

Statistical Analysis

All experiments were design in completely randomized with three replicates for each. Data were statistically analyzed using GenStat software and means were compared at 5% of probability

RESULTS

Effect of Gamma radiation on germination%, shoot length and number of leaves and chlorophyll in leaves contain

Based on the data displayed in the table (1) some effects were observed on the germination rate and phenotypic characteristics of seedling resulted from cowpea seeds subjected to mutagenesis by gamma radiation. A gradual decline was observed in seeds germination % and the lowest% was achieved at the dose 75 Gy reached (13.33%) compare to higher germination (93.0%) at 25 Gy. For seedling height and number of leaves**,** 25 and 50 Gy affected significantly on both parameters with average of (4.53 and 3.40 cm), (3.57 and 2.90) respectively. For chlorophyll, data at same table revealed decrease at all doses as compare with 31.83 mg. g^{-1} at control treatment (non irradiated seeds).

Table (1) Effect of Gamma radiation on in vitro seeds germination%, shoot

height and number of leaves and chlorophyll contain

Radiation doses Gy	Seeds germination%	seedling height (cm)	Number of leaves	Chlorophyll mg. g^{-1}
0	40.0 b	3.07 b	2.47 b	31.83 a
25	93.0 a	4.53 a	3.57 a	20.32 b
50	46.67 b	3.40 ab	2.90 a	15.6 c
75	13.33 c	1.27 c	1.10 c	11.85 c

Means followed by different letter in the same column significantly differ according to Duncan's Multiple Range (DMRT) test at 5% significance differences

Effect of gamma radiation and salt stress on total CHO and Proline in callus contents derivative from shoot tips explants

Figure (1) shows the total carbohydrate content in irradiated stressed callus of cowpea plant was significantly increased (6.154 mg. g^{-1}) at low dose of gamma radiation (25Gy). On the other hand, high level of NaCl (10 $ds.m^{-1}$) caused highly significant increase (4.828 mg. g^{-1}) for CHO in callus content. While highest accumulation of CHO was recorded (8.75 mg. g^{-1}) when interactions of (25 Gy at 10 $ds.m^{-1}$)

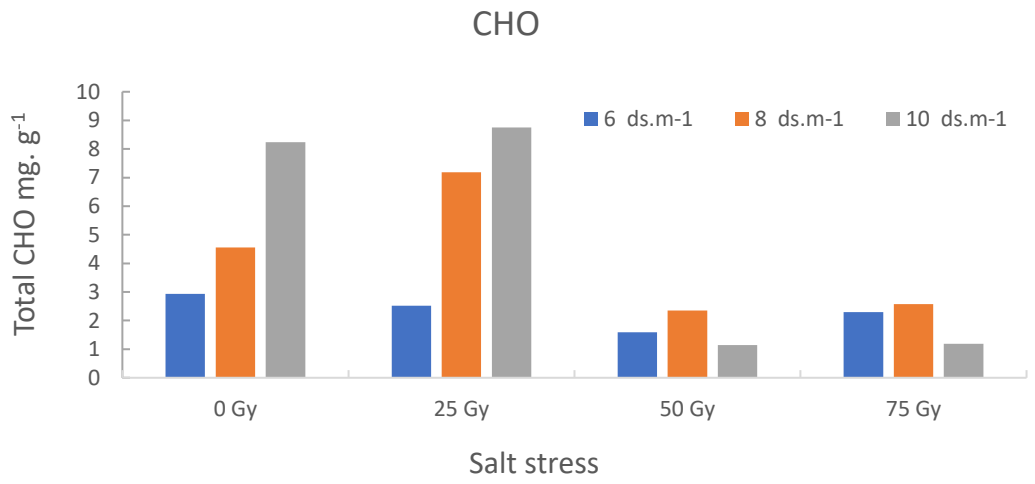


fig.1. Effect of gamma radiation and salt stress on total CHO mg. g^{-1} in callus contents derivative from shoot tips explants.

Where:

1. **Irradiation doses are 0, 25, 50 and 75 Gy.**

2. **Statistical Analysis**
   - **LSD for CHO of Irradiation: 0.5123**
   - **LSD for CHO of salt stress: 0.4437**
   - **LSD for CHO of Irradiation* salt stress: 0.8874**

Data in Fig. 2 revealed the positive role for high doses of gamma radiation to accumulate proline with average of (15.97 and 16.3 mg. g^{-1}) at 50 and 75Gy respectively. Also, the same results were found at high level of NaCl (10 $ds.m^{-1)}$ proline achieved high accumulation (21.35 mg. g^{-1}). In addition, the interaction between (50 Gy at 10 $ds.m^{-1}$) recorded highest accumulation reached (26.46 mg. g^{-1})

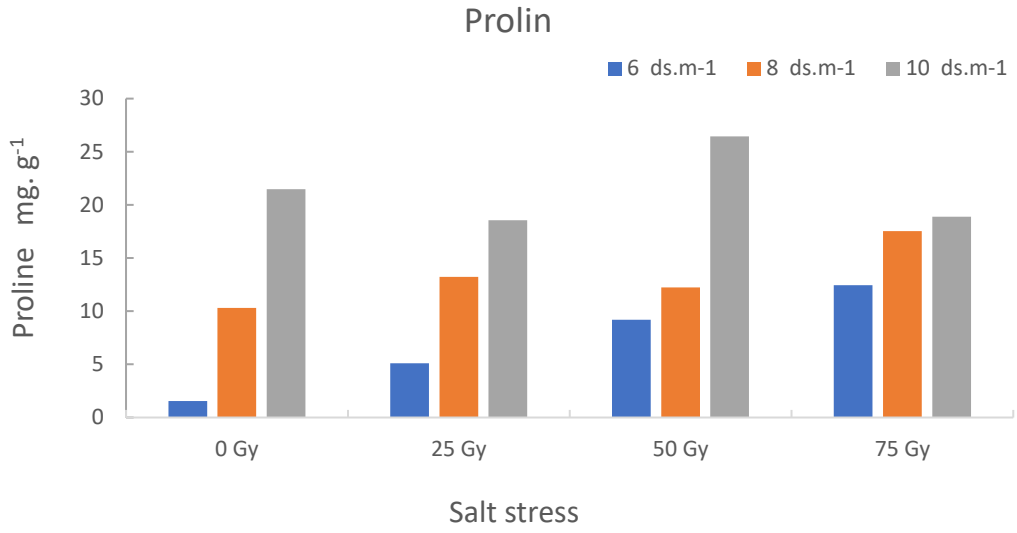


Fig 2. Effect of gamma radiation and salt stress on proline mg. g^{-1} in callus contents derivative from shoot tips explants

Where:

1. **Irradiation doses are 0, 25, 50 and 75 Gy .**
2. **Statistical Analysis**
   - **LSD for Proline of Irradiation: 1.878**
   - **LSD for Proline of salt stress: 0.627**
   - **LSD for Proline of Irradiation* salt stress: 3.253**

Effect of gamma radiation and salt stress on (Na^+, K^+) in callus contents derivative from shoot tips explants

From the results in Fig. 3. gamma radiation caused significant increased in Na^+ ion reached (14.66, 15.05 mg. g^{-1}) at high doses (50 and 75 Gy) respectively and no significant differences were found between both doses. Furthermore, 10 $ds.m^{-1}$ of NaCl was most effective in giving highest accumulation (21.8 mg. g^{-1}) for Na^+. For the interaction between Gamma doses and NaCl levels, 50 and 75 Gy at 10 $ds.m^{-1}$ were

superior in giving (23.79, 24.49 mg. g^{-1}) for Na^+ respectively.

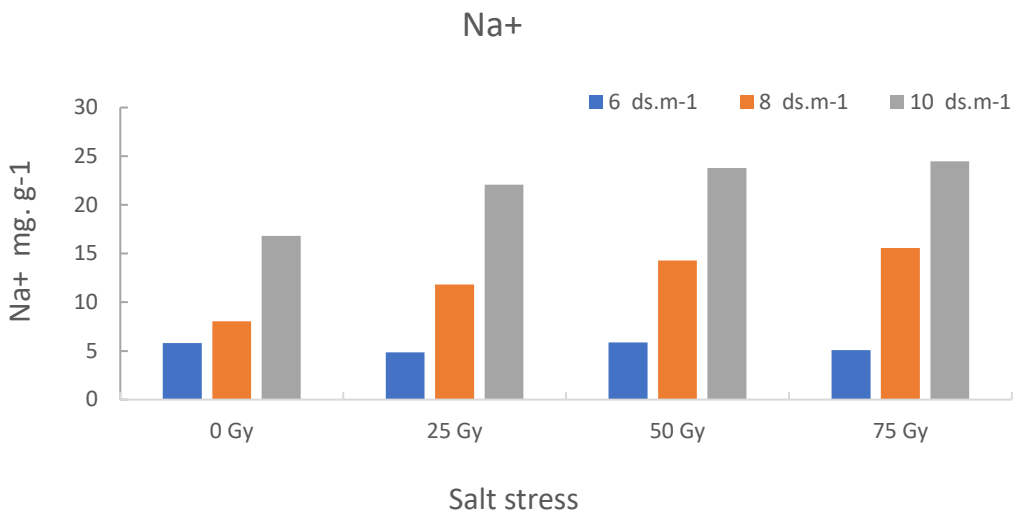


Fig 3. Effect of gamma radiation and salt stress on Na^+ mg. g^{-1} in callus contents derivative from shoot tips explants

Where:

1. **Irradiation doses are 0, 25, 50 and 75 Gy.**
2. **Statistical Analysis**

- **LSD for Na^+ of Irradiation: 2.106**
- **LSD for Na^+ of salt stress: 1.824**
- **LSD for Na^+ of Irradiation* salt stress: 3.647**

data in fig. 4 showed that K+ accumulation decreased (4.744 mg. g-1) with increasing salinity level to (10 ds.m^{-1}) compare to (6.673 mg. g^{-1}) at 6 ds.m^{-1} whereas highest accumulation (6.183, 5.933 mg. g^{-1}) was found at 0 and 75Gy Gamma radiation with no significant differences were found between both.

Furthermore, 0 and 75Gy Gamma radiation in combined with (6 and 8 ds.m^{-1}) affected significantly on increasing K^+ (fig. 4).

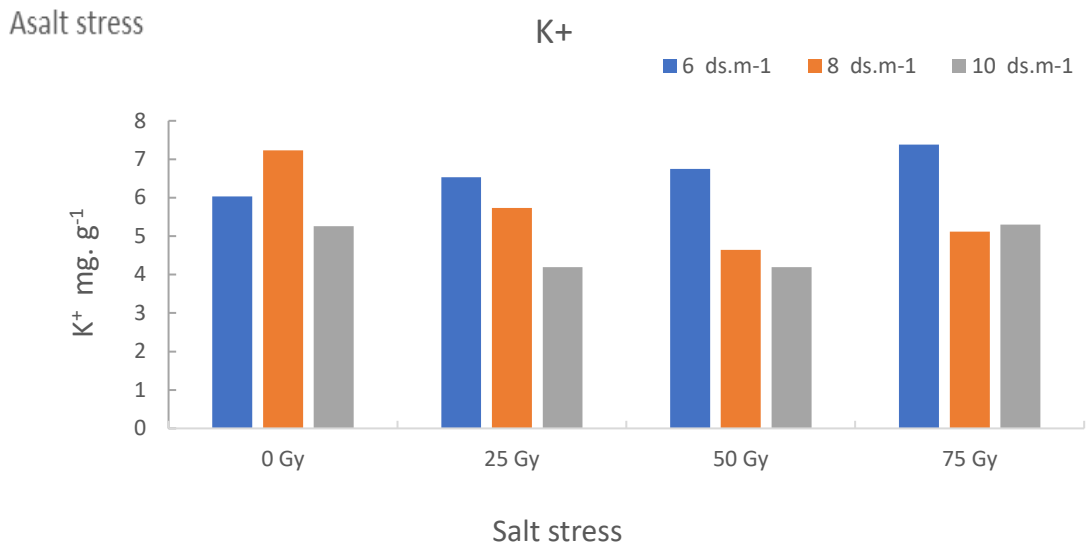


Fig. 4. Effect of gamma radiation and salt stress on K^+ mg. g^{-1} in callus contents derivative from shoot tips explants

Where:

1. **Irradiation doses are 0, 25, 50 and 75 Gy.**
2. **Statistical Analysis**

- **LSD for K^+ of Irradiation: 0.4054**
- **LSD for K^+ of salt stress: 0.3511**
- **LSD for K^+ of Irradiation* salt stress: 0.7021**

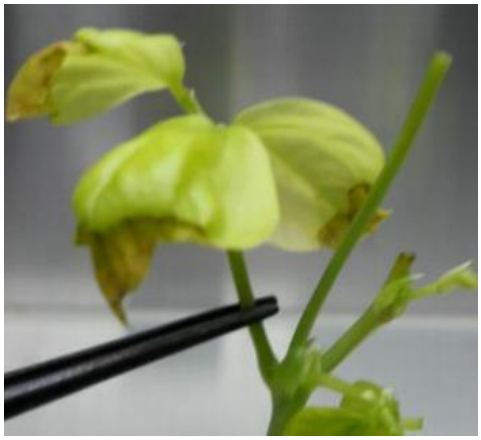
DISCUSSION

According to table (1) when seeds irradiated with (75Gy), high reduction of germination%, seedling height, chlorophyll content. Seeds exposed to gamma radiation modify plant metabolism which in turn made the plant faced severe effects led to reduction in photosynthetic pigments capacity (pict. 1) further the leaves are yellowing, wilting and falling (pict. 2) besides, in other seeds abnormalities growth in seedling was found (pict. 3) in this study. Our results are agreeing with previous studies by [El-Beltagie et al,2013]. Results of carbohydrate and proline presented in fig (1 and 2) revealed that increase in both parameters continents in stressed callus at the level 10 ds.m-1 in combined with (25 or 50 Gy). Our results are in accordance with (El-Beltagie et al,2013). they found interaction between gamma rays and salt in accumulation of proline and total carbohydrate. Also, our results are agreeing with radiation [Al-Rumaih and Al-Rumaih, 2008.; Aly et al,2018].

Form the results in fig (3 and 4) its clear salinity caused high significant increase in Na^+ in stressed callus on the other hand, the K^+ concentration was reduced in salt-treated calli. This contradiction in the increase for some ions and decrease for other ions were also observed by [Patade et al. [2008] in callus of sugarcane

and [Aly *et al*,2018] in wheat.

Pict. 1: produced from seeds irradiated with 75Gy gamma



pict 2: Pale leaves in plants produced from seeds irradiated with (75 Gy) with seeds irradiated with

pict 3: seedlings produced from seeds irradiated with (75 Gy)

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