Range Mgmt. & Agroforestry 36 (2) : 141-145, 2015
ISSN 0971-2070

Effect of pre-sowing irradiation of *Bromus inermis* (L.) seeds on germination, growth and some biochemical parameters

Ghasem Ali Dianati Tilaki*[1], Arezu Alizadeh[1], Behnam Naserian Khiabani[2]
[1]Rangeland Management Department, Tarbiat Modares University, Noor City, Mazandaran province, P.O.Box 46414-356, Iran
[2] Nuclear Agriculture Research School, Karaj City, Alborz province, P.O. Box 31465-1948, Iran
*Corresponding author e-mail: dianatig@modares.ac.ir
Received: 26th February, 2014 Accepted: 3rd November, 2015

Abstract

Present study was conducted to evaluate the effect of pre sowing exposure of gamma rays on seeds of *Bromus inermis* with doses of 15, 20, 30, 50, 100 and 150 gray, on germination, morphological traits and biochemical parameters. Irradiated seeds were sown in a completely randomized design in laboratory and greenhouse conditions. The seed germination, emergence parameters, growth, yield, chlorophyll, proline and total soluble protein content of leaves were measured. The results showed that the 15 gray doses caused the most positive effects on emergence parameters, growth, yield and chlorophyll content. Also gamma ray exposure on seeds of *B. inermis* caused significant decrease (P<0.01) in proline concentration. There was no significant differences between control and 15 gray treatment in their proline content but as gamma ray intensity increased proline concentration showed decrease and total soluble protein increased (P<0.01). As overall result, 15 gray can be advised as optimum doses for irradiation treatment in *B. inermis*.

Key words: Chlorophyll, Gamma ray, Growth, Proline, Protein, Seed germination.

Abbreviations: Chl a: Chlorophyll a; **Chl b:** Chlorophyll b; **Chl a+b:** Chlorophyll a+b; **DW:** Dry weight; **EC:** Emergence capacity; **ER:** Emergence rate; **EV:** Emergence value; **FW:** Fresh weight; **GC:** Germination capacity; **GV:** Germination value; **MDV:** Mean day of germination; **PV:** Peak value; **RWC:** Relative water content; **S:** Germination rate; **TW:** Turgid weight

Introduction

Forage and turf grasses like Brome grass have a considerable role in sustainable agriculture, meat and dairy production, preventing soil loss and restoration of degraded lands (Majidi and Mirlohi, 2010). Seedling establishment as a critical stage in plant's life depends on successful germination so the techniques that trigger germination increases seedling establishment and growth (Liu *et al.,* 2008). Initial growth materials are stored in the seed, so activation of those materials in the cotyledons is adequate for growth. Low doses of Gamma-radiation increase the enzymatic activation and awakening of the young embryo, which leads to stimulation of germination and vegetative growth (Moussa, 2011). It was generally agreed that low doses of gamma rays have simulative effects on cell division that causes increase in growth and development of plants by inducing cytological, biochemical and physiological changes in cells and tissues (Thapa, 2004). However, high doses of gamma radiation produce deleterious effects, such as inhibition of growth and genetic damage (Amjad and Akbar Anjum, 2007; Micco *et al*., 2011). Seed treatment with low doses of gamma rays resulted in a significant increase of germination traits, plant vigor and yield attributes of wheat (Zaka *et al*., 2004; Melki and Marouani, 2010; Singh and Datta, 2010). Also elongation of the root system by irradiating seeds with low doses of gamma ray have been reported in chickpea and wheat plants (Melki and Sallami, 2008; Melki and Dahmani, 2009). It was reported that, biochemical processes in plants are significantly affected by gamma-irradiation (Amjad and Akbar Anjum, 2007). *B. inermis* is a cool season grass species that produce valuable forage and grazing material in pasture and rangeland of Iran, but it is not resistant to high drought stress (Karimi, 2006). Present work is aimed at probing into the potential of low doses of gamma rays as pre-sowing treatment on dry seeds in modulating growth and development of *B. inermis*.

Materials and Methods

Dry seeds of *B. inermis* with moisture content of 9.5 per cent and viability of 82 per cent were divided into seven groups (4 g each). The first group was kept as control while the rest were exposed to 15, 20, 30, 50,100 and

150 gray gamma irradiation doses with dose rate of 0.18 Gys^{-1} using a source of cobalt-60 at agricultural, medical and industrial research school, Nuclear Science and Technology Research Institute, Iran. To find out the effect of gamma ray on germination, 50 seeds of each treatment were taken in a sterile Petri plates using top of paper method (ISTA, 1985) and each experiment was replicated three times. Petri plates were placed in germinator at 25°C temperature with relative humidity of 80 per cent. Seeds were considered as germinated when radical protrusion was 1-2 mm. Germination of seeds was calculated on 12th day. Germination capacity (GC), germination rate(S) and germination value (GV) was evaluated using following equations:

GC (%) = (n/N) * 100

Where, 'n' is number of germinated seeds, and 'N' is total number of sown seeds.

$$S\ (\text{seeds day}^{-1}) = (N_1 \times 1) + (N_2 - N_1) \times 1/2 + (N_3 - N_2) \times 1/3 + \ldots + (N_n - N_{n-1}) \times 1/n$$

Where, N_1, N_2, N_3… N_n are number of germinated seeds observed after 1, 2, 3….n days, (Chiapuso *et al.*, 1997).

GV = MDG × PV

Where, 'MDG' is mean day of germination and is calculated by, GC/total days of germination and 'PV' is peak value, which equals to maximum of means of germination in each days of experiment (Czabator, 1962).

To study morphological and biochemical parameters, 50 irradiated seeds along with control, in three replications were germinated in plastic pots of 22 cm height and 19 cm diameter. Each pot was filled with 3 kg of *B. inermis* natural habitat's soil from Kiasar in Sari province of Iran. The soil texture was clay loam, with 7.43 pH, 161.83 μscm^{-1} EC, 0.92% organic matter, 0.064% total nitrogen and 27.3 ppm available phosphorus. Pots were kept in greenhouse with temperature of 28±4 °C in day and 22±4 °C in night, with natural light and all pots were irrigated every 2nd day with equal amount of water. All the emerged seedlings were counted and germination per cent, germination rate and germination value were estimated from equations mentioned above. After two months the plants height, root and shoot biomass were measured by removing the plants. Green leaves were kept in liquid nitrogen and stored at -80°C for biochemical analysis. Chlorophyll-a, chlorophyll-b, and total chlorophyll quantities were calculated according to Arnon (1949). Total soluble protein contents were measured using Bradford's method (Bradford, 1976). Free proline was determined according to the method described by Bates *et al.* (1973). Relative water content (RWC) was measured using the following equation:

RWC (%) = 100× (FW-DW) / (TW- DW)

Fresh weight (FW) was taken after immediately cutting the leaves and, Turgid weight (TW) was obtained after 6h soaking in distilled water at room temperature, under low light conditions. The dry weight (DW) was obtained after drying the leaf samples at 60°C for 48 h in oven, (Schonfeld *et al.*, 1988). The results obtained were submitted to analysis of variance through the F test and Duncan test was performed to compare the means with the use of the SPSS 17 software.

Results and Discussion

***Seed Germination*:** The results of laboratory experiments showed that exposure to gamma rays caused significant difference in seed germination rate. However, no significant differences were noticed between 15, 20 and 30 gray treatment in case of germination rate. The highest germination speed and germination value were observed in 20 Gy treatments (Table 1). There was no significant difference in germination and emergence capacity of irradiated seeds, but radiation doses of 15, 20 and 30 Gy increased germination rate and germination value (a compound parameter of germination speed and germination capacity) at both laboratory and green house conditions, and higher doses decreased these germination traits. These increases in lower doses might be due to their stimulating effects on activating RNA synthesis or protein synthesis or it could be due to the elimination of germinating bacterial populations (Abou El-Yazied, 2011). Higher exposures of gamma rays may cause injury in seeds and usually shows inhibitory effects on seeds (Majeed and Muhammad, 2010). These results are in line with Toker *et al.* (2005) and Kim *et al.* (2004).

Seedlings emergence and growth: In glass house condition, the evaluation of the parameters showed that the highest value for evaluated parameters of seed germination like seedling emergence capacity (GC %), seedling emergence rate (S) and seedling emergence value (GV) relates to 15 Gy treatments. The plant height, shoot biomass and root biomass were considered as morphological parameters and the highest value for shoot biomass and root biomass belong to 15 Gy treatment and for plant height, 15 Gy and 20 Gy treatments showed the highest values (Table 2).

Table 1. Effect of various doses of Gamma-irradiation on seed germination

Gamma irradiation doses (Gy)	Parameters		
	GC (%)	**S (seed day^{-1})**	**GV**
0	81.3±5.2^{a}	7.1±1.0^{b}	38.40±1.7^{d}
15	89.6±6.2^{a}	8.4±0.3^{ab}	45.74±0.7^{bc}
20	90.6±1.3^{a}	9.8±0.1^{a}	56.56±2.2^{a}
30	90.6±4.0^{a}	8.7±0.6^{ab}	48.41±2.3^{b}
50	84.0±5.0^{a}	7.9±0.4^{b}	41.66±0.8^{cd}
100	84.6±3.7^{a}	8.0±0.3^{b}	42.30±1.3^{cd}
150	74.6±4.3^{a}	6.9±0.3^{b}	33.34±0.7^{e}
Mean	85.095	8.159	44.013
LSD	12.04	1.64	5.15
CV	0.094	0.149	0.169
SD	8.011	1.223	7.438

Means followed by the same letter in each column are not significantly different at 5% level

Table 2. Effect of various doses of Gamma-irradiation seedlings emergence and plant growth

Gamma irradiation doses (Gy)	Parameters					
	GC (%)	**S (seed day^{-1})**	**GV**	**Height (cm)**	**Shoot biomass (g)**	**Root biomass (g)**
0	81.6±1.6^{a}	6.63±0.1^{cd}	25.12±2.2^{c}	13.95±1.6^{b}	4.02±0.2^{c}	0.71±0.06^{dc}
15	88.6±2.9^{a}	8.87±0.5^{a}	46.0±1.7^{a}	19.76±2.2^{a}	7.5±0.5^{a}	0.81±0.07^{abc}
20	82.0±1.1^{a}	8.62±0.3^{ab}	39.9±1.7^{ab}	20.75±1.4^{a}	6.47±0.2^{b}	0.75±0.05^{bcd}
30	88.0±4.1^{a}	7.73±0.3^{abc}	46.2±3.7^{a}	16.69±1.9^{b}	5.57±0.2^{b}	0.86±0.02^{ab}
50	90.6±4.3^{a}	7.06±0.7^{dc}	37.0±2.7^{b}	15.47±0.7^{b}	4.13±0.3^{c}	0.92±0.05^{a}
100	89.6±0.8^{a}	7.45±0.1^{bcd}	36.7±2.2^{b}	16.95±1.3^{b}	4.46±0.1^{c}	0.66±0.03^{d}
150	82.0±5.2^{a}	6.29±0.2^{d}	24.8±3.2^{c}	14.96±1.0^{b}	3.97±0.1^{c}	0.71±0.02^{dc}
Mean	86.095	7.523	36.586	16.935	5.165	0.779
LSD	10.111	1.2750	9.007	2.732	0.9704	0.1356
CV	0.071	0.146	0.250	0.159	0.270	0.137
SD	6.155	1.101	9.176	2.706	1.398	0.107

Means followed by the same letter in each column are not significantly different at 5% level

Table 3. Effect of gamma irradiation on biochemical parameters

Gamma irradiation doses (Gy)	Parameters					
	Chl a	**Chl b**	**Chl a+b**	**Proline**	**Soluble protein**	**RWC (%)**
0	23.83±0.38^{a}	8.63±0.12^{a}	32.8±0.25^{a}	1.03±0.1^{a}	0.15±0.006^{d}	80.2±1.07^{a}
15	23.53±2.25^{ab}	9.36±1.00^{a}	33.23±3.32^{a}	0.99±0.027^{a}	0.18±0.011^{cd}	89.4±1.71^{a}
20	24.96±0.36^{a}	9.53±2.00^{a}	34.86±2.13^{a}	0.18±0.01^{b}	0.21±0.008^{bc}	85.9±1.24^{a}
30	24.26±0.7^{a}	8.7±0.2^{a}	33.36±0.95^{a}	0.17±0.01^{b}	0.21±0.006^{bc}	83.4±1.30^{a}
50	19.5±1.1^{bc}	7.06±0.24^{a}	26.83±1.31^{a}	0.13±0.01^{b}	0.23±0.008^{b}	85.2±2.94^{a}
100	16.33±1.73^{c}	9.00±3.33^{a}	25.53±4.74^{a}	0.02±0.00^{b}	0.22±0.007^{b}	82.7±3.47^{a}
150	16.96±1.64^{c}	8.33±3.32^{a}	25.53±3.65^{a}	0.018±0.00^{b}	0.28±0.02^{a}	79.3±3.16^{a}
Mean	21.342	8.661	30.309	0.366	0.215	83.759
LSD	4.105	5.990	8.443	0.341	0.00	7.0793
CV	0.186	0.342	0.185	1.241	0.197	0.056
SD	3.986	2.965	5.626	0.455	0.042	4.714

Means followed by the same letter in each column are not significantly different at 5% level

In morphological parameters of yield and height 15 and 20 gray showed the most positive effects. Low doses of ionizing radiation can stimulate growth by affecting the network of hormonal signals in plant cells that causes increase in cell division rate or by increasing the anti-oxidative capacity of the cells that leads to increasing plants tolerance to daily stress factors such as fluctuations of light intensity and temperature in the growth condition (Kim *et al.*, 2004; Jan *et al.*, 2010; Moussa, 2011). These beneficial effects resulted in higher germination; higher growth and yield in treated plants of *Capparis spinosa* L. (Al-Safadi and Elias, 2011), *Eruca vesicaria* sub sp. sativa (Moussa, 2006), Safflower (Srivastava and Kumar, 2011) and *Hibiscus sabdariffa* L. (El-Sherif *et al.*, 2011) are reported.

***Biochemical analysis*:** Biochemical parameters evaluation showed that the gamma irradiation with doses of 100 and 150 gray decreased chlorophyll 'a' significantly ($P<0.05$) but had no effect on chlorophyll 'b' and total content of chlorophyll (a+b). Proline content of leaves decreased significantly ($P<0.01$) in treatments of 20, 30, 50, 100 and 150 Gy. Increase in gamma ray intensity caused significant and direct increase in total soluble protein concentration in leaves and the highest amount observed in 150 gray treatments, but there was no significant difference in relative water content of leaves (Table 3).

About chlorophyll 'a', chlorophyll 'b' and chlorophyll 'a+b' content, doses lower than 50 gray had no significant effect on these photosynthetic pigments contents, but significant ($p<0.05$) decrease in chlorophyll 'a' content was observed in treatments of 50, 100 and 150 gray, and in total chlorophyll content there was no difference between the gamma ray doses but these pigment contents was numerically lower in 50, 100 and 150 gray of gamma irradiated seeds. The results obtained about photosynthetic pigments are in contrast with results of increasing of chlorophyll content in okra (Hegazi and Hamideldin, 2010). However, similar results were recorded in irradiated seeds of red pepper (Kim *et al.*, 2004) and safflower (Srivastava and Kumar, 2011). Irradiation promotes the accumulation of osmo-protectants such as protein and proline (Pick Kiong Ling *et al.*, 2008).The decreases in proline content of leaves in *B. inermis* is in contrast with results of Moussa (2011) reported in soybean. Increase in gamma ray doses caused a significant ($P<0.01$) direct increase in total protein content of leaves. As the results show the dose of 15 gray had no significant difference with controls in proline and protein content but at higher doses the proline content decreased and total soluble protein content increased.

Conclusion

Overall the 15 Gy treatments can be advised as optimum dose for *B. inermis* to reach higher germination and higher values of seedling emergence and height. The 15 Gy treatment resulted in better physiological traits like chlorophyll, proline and protein content which can help the plants to be more tolerant to fluctuations in the growth conditions resulting in more successful establishment of plants and more forage yield of *B. inermis*.

References

Abou El-Yazied, A. 2011. Growth, biochemical constituents and yield of snap bean as influenced by low gamma irradiation doses under different sowing dates. *Australian Journal of Basic and Applied Sciences* 5: 518-530.

Al-Safadi, B. and R. Elias. 2011. Improvement of caper (*Capparis spinosa* L.) propagation using in vitroculture and gamma irradiation. *Scientia Horticulturae* 127: 290–297.

Amjad, M. and M. Akbar Anjum. 2007**.** Effect of post-irradiation ageing on onion seeds. *Acta Physiologiae Plantarum* 29:36-69.

Arnon, D. L. 1949. A copper enzyme is isolated chloroplast polyphenol oxidase in *Beta vulgaries. Plant Physiology* 24: 1-15.

Bates, L. S., R. P. Waldren and I. D. Teare. 1973. Rapid determination of free proline for water stress studies. *Plant and Soil* 39: 205–208.

Bradford, M. M. 1976. A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein dye binding. *Annual Journal of Biochemistry* 72: 248–254.

Chiapusio, G., A. M. Sanchez, M. J. Reigosa, L. Gonzalez and F. Pellissier. 1997**.** Do germination indices adequately reflect allelochemical effects on germination process. *Journal of Chemical Ecology* 23: 2445-2453.

Czabator, F.G. 1962**.** Germination value: An index combing speed and completeness of pine seed germination. *Forest Science* 8: 386-396.

El-Sherif, F., S. Khattab, E. Ghoname, N. Salem and K. Radwan. 2011. Effect of gamma irradiation on enhancement of some economic traits and molecular changes in *Hibiscus Sabdariffa* L. *Life Science Journal* 8: 220-229.

Hegazi, A. Z. and N. Hamideldin. 2010. The effect of gamma irradiation on enhancement of growth and seed yield of okra [*Abelmoschus esculentus* (L.) Monech] and associated molecular changes. *Journal of Horticulture and Forestry* 2:38-51.

ISTA. 1985. Handbook on Seedling Evaluation. International Seed Testing Association (ISTA).

Jan, S., T. Parween, T. O. Siddiqi and M. Zafar. 2010**.** Gamma radiation effects on growth and yield attributes of *Psoralea corylifolia* L. with reference to enhanced production of psoralen. *Plant Growth Regulation* 64: 163-171.

Karimi, H. 2006.*Rangeland Management*. Tehran, Tehran University Press.

Kim, J. H., M. H. Baek, B. Y. Chun, S.G. Wi and J. S. Kim. 2004. Alterations in the photosynthetic pigments and antioxidant machineries of Red Pepper (*Capsicum annuum* L.) seedlings from gamma-irradiated seeds. *Journal of Plant Biology* 47: 314-321.

Liu, G.X., P. S. Mao, S. Q. Huang, Y.C. Sun and J. G. Han. 2008. Effects of soil disturbance, seed rate, nitrogen fertilizer and subsequent cutting treatment on establishment of *Bromus inermis* seedlings on degraded steppe grassland in China. *Grass and Forage Science* 63:331-338.

Majeed, A. and Z. Muhammad. 2010. Gamma irradiation effects on some growth parameters of *Lepidium sativum* L. *World Journal of Fungal and Plant Biology* 1: 8-11.

Majidi, M.M. and A.Mirlohi. 2010. Genetic similarities among Iranian populations of Festuca, Lolium, Bromus and Agropyron using amplified fragments length polymorphism (AFLP) markers. *Iranian Journal of Biotechnology* 8:16-23.

Melki, M. and D. Sallami. 2008. Studies the effects of low dose gamma rays on the behavior of chickpea under various conditions. *Pakistan Journal of Biological Sciences* 11: 2326-2330.

Melki, M. and A.Marouani. 2010. Effect of gamma rays irradiation on seeds germination and growth of hard wheat. *Environmental Chemistry Letters* 8: 307–310.

Melki, M. and Th. Dahmani. 2009. Gamma irradiation effects on durum wheat (*Triticum durum* Desf.) under various conditions. *Pakistan Journal of Biological Sciences* 12:1531–1534.

Micco, V., C. Arena, D. Pignalosa and M. Durante. 2011. Effects of sparsely and densely ionizing radiation on plants. *Radiation and Environmental Biophysics* 50: 1–19.

Moussa, H. R. 2006**.** Role of gamma irradiation in regulation of NO_3 level in rocket Plants (*Eruca versicaria subsp. Sativa*). *Russian Journal of Plant Physiology* 53: 193-197.

Moussa, H.R. 2011**.** Low dose of gamma irradiation enhanced drought tolerance in soybean. *Bulgarian Journal of Agricultural Science* 17: 63-72.

Pick Kiong Ling, A., J. Yi Chia, S. Hussein and A. R. Harun. 2008. Physiological responses of *Citrus sinensis* to gamma irradiation. *World Applied Sciences Journal* 5: 12-19.

Schonfeld, M.A., R. C. Johnson, B. F. Carver and D.W. Mornhinweg. 1988. Water relations in winter wheat as drought resistance indicator. *Crop Science* 28: 526–531.

Singh, B. and P. S. Datta. 2010. Gamma irradiation to improve plant vigour, grain development, and yield attributes of wheats. *Radiation Physics and Chemistry* 79: 139–143.

Srivastava, P. and G. Kumar. 2011. Gamma rays induced alterations in some morphological and biochemical indices of Safflower (*Carthamus tinctorius* L.). *Journal of Plant Science Researches* 27: 113-116.

Thapa, C. B. 2004. Effect of acute exposure of gamma rays on seed germination and seedling growth of *Pinus kesiya* Gord and *P. wallichiana*. *Our Nature* 2:13-17.

Toker, C., B. Uzen, H. Canci and F. O. Ceylan. 2005. Effects of gamma irradiation on shoot length of Cicer seeds. *Radiation Physics and Chemistry* 73: 365-367.

Zaka, R., C. Chenel and M. T. Misset. 2004. Effect of low doses of short term gamma irradiation on growth and development through two generation of *Pisum sativum*. *Science of the Total Environment* 320: 121-129.