**Effect of Pre-sowing Irradiation of *Bromus inermis* (L.) Seeds on Germination, Growth and some Biochemical Parameters**

*Ghasem Ali Dianati Tilaki\*1,Arezu Alizadeh2, Behnam Naserian Khiabani3*

1. Associate Professor, Rangeland Management Department, Natural Resources Faculty, Tarbiat Modares University.

2. M.Sc. Student, Rangeland Management Department, Natural Resources Faculty, Tarbiat Modares University.

3. M.Sc. Plant breeding, Agricultural,Medical and Industrial Research School,Nuclear Science and Technology Research Institute, Atomic Energy Organization of Iran.

\*Corresponding author: Rangeland Management Department, Natural Resources Faculty, Tarbiat Modares University, Noor City, Mazandaran province, Iran , P.O.Box 46414-356

, *Tel: +98 -122-6253101; Fax: +98-122- 6253499 Email:dianatitilaki@yahoo.com*

**Abstract**

This study was conducted to evaluate the effect of pre sowing exposure of gamma ray on seeds of *Br.inermis* with doses of 15, 20, 30, 50, 100 and 150 gray, on germination, some morphological traits and biochemical parameters*.* for this regard an experiment of completely randomized design were done and irradiated seeds were sown in laboratory and greenhouse condition, then seeds germination and 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 exposuring on seeds of *Br.inermis* caused significant decrease (P<0.01) in proline concentration as there was no significant differences between control and 15 gray treat 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 *Br.inermis*.

**Key words**: Gamma ray, *Br.inermis*, Seed germination, growth, Chlorophyll, Proline Protein.

**Introduction**

Forage and turf grasses like Brome grasses have a considerable role in sustainable agriculture, meat and dairy production, preventing soil loss and restoration of degraded lands (Majidi and Milrohi, 2010). Seedling establishment is a critical stage in plants life that is result of successful emergence, germination triggering increases seedling establishment and plant 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 γ-radiation increase the enzymatic activation and awakening of the young embryo, and lead to stimulating germination and vegetative growth (Moussa, 2011). It was generally agreed that low doses of gamma rays have stimulative effects on cell division, that causes increase in growth and development of plants by inducing cytological, biochemical and physiological changes in cells and tissues (Charbaji and Nabulsi, 1999; Thapa, 2004; El-Bazza et al., 2001; El-Sherif et al., 2011), but high doses of gamma radiation produce deleterious effects, such as inhibition on growth and genetic damage (Amjad and 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 of 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 Anjum, 2007). Present work is aimed at probing into the potential of low doses of gamma rays pre-sowing treatment on dry seeds in modulating growth and development of one of the important forage grasses in Iran, *Br.inermis*.

**Materials and methods**

Dry seeds of *Br.inermis* with moisture content of 9.5 percent and viability of 82 percent were divided into eight groups of 4 gram samples, 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 0.18Gys-1 using a source of cobalt-60 at agricultural, medical and industrial research school, nuclear science and technology research institute, Atomic Energy Organization of Iran. To find out the effect of gamma ray on germination, 50 seeds of each treat were taken in a sterile petriplates using paper towel method (ISTA, 1985), and each experiment was replicated three times. Petri plates were placed in germinator at 25○C temperature with relative moisture of 80 percent, seeds were considered as germinated when radical protrusion was ≥ 1-2mm, germinated seeds was calculated for 12 day. Then germination capacities (GC), germination rate(S) and germination value (GV) were evaluated using the following equations:

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

n is number of germinated seeds, and N is total number of sowed seeds.

S (seeds day -1) = (N1\*1)+(N2 – N1)\*1/2 + (N3-N2)\*1/3+ …+ (Nn-Nn-1)\*1/n

N1, N2, N3,…, Nn are number of germinated seeds observed after 1, 2, 3,…, n days, (Chiapuso et al. 1997).

GV = MDG × PV

(Czabator, 1962), MDG is mean day of germination and is calculated by, GC/total day of experiment (12 day), and PV is peak value, which equals to maximum of means of germination in each day of experiment.

Also to study morphological and biochemical parameters, irradiated seeds along with controls, with three replication of 50 seeds were allowed to germinate in plastic pots 22cm height and 19cm diameter. Each pot was filled with three kg of *Br.inermis* natural habitat’s soil from Kiasar in Sari province of Iran. The soil texture was clay loam, with acidity of 7.43, electrical conductivity equal to 161.83 µscm-1, organic material of 0.92%, and total nitrogen of 0.064% and available phosphorus of 27.3 ppm. Pots were put in greenhouse with temperature of 28±4 in day and 22±4 in night, with natural light and all pots were irrigated every 2 day with equal amount of water. All the emerged seedlings were calculated and emerge percent, emerge speed and emerge value was evaluated from equations mentioned above for germination characteristics. after two month the plants height were measured, all the shoots in each pot were cut, then the soil was removed from roots with washing method, to measure shoots and roots biomass. Green leaves were putted in liquid nitrogen and saved in -80○C for biochemical analysis. Plant leaves were extracted in 80% ice-cold acetone, and the absorbance of the extracts was measured at 663 and 645 nm. Chlorophyll-a, chlorophyll-b, and total chlorophyll quantities were calculated according to Arnon's method (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), using the following equation:

RWC(%) = 100\*(FW-DW) /(TW- DW)

FW (fresh weight), (TW) was obtained after the leaves had been soaked in distilled water in test tubes for 6 h at room temperature, under low light conditions. (DW) was obtained after the leaf samples had been oven dried at 60○C for 48, (Schonfeld et al. 1988).

Statistical analysis

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**

The results of laboratory experiments showed that gamma ray caused significant difference in germination rate of seeds and the highest germination speed and germination value was observedin 20 Gy treat, and there was no significant difference between 15, 20 and 30 gray treats in these parameters (table 1).

Table 1 effect of various doses of ɣ-irradiation on germination capacity (GC), germination rate (GR) and germination value (GV) in Petri dishes

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| parameters | ɣ-irradiation doses (Gy) | | | | | | |
| 0 | 15 | 20 | 30 | 50 | 100 | 150 |
| GC (%) | 81.3±5.2a | 89.6±6.2a | 90.6±1.3a | 90.6±4.0a | 84.0±5.0a | 84.6±3.7a | 74.6±4.3a |
| GR  (seed\*day-1) | 7.1±1.0b | 8.4±0.3ab | 9.8±0.1a | 8.7±0.6ab | 7.9±0.4b | 8.0±0.3b | 6.9±0.3b |
| GV | 38.40±1.7d | 45.74±0.7bc | 56.56±2.2a | 48.41±2.3b | 41.66±0.8cd | 42.30±1.3cd | 33.34±0.7e |

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

In glass house condition, the evaluation of the parameters showed that the highest value of emergence and morphological parameters was observed in 15 Gy irradiation doses, but there was no significant difference in relative water content of leaves (table 2).

Table 2 The effect of various doses of ɣ-irradiation on emergence capacity (EC), emergence rate (ER) and emergence value (EV), mean height of plants in each pot (cm), shoot biomass (gram\*pot-1), root biomass (gram\*pot-1) and relative water content (RWC%)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| parameters | ɣ-irradiation doses (Gy) | | | | | | |
| 0 | 15 | 20 | 30 | 50 | 100 | 150 |
| EC (%) | 81.6±1.66a | 88.6±2.90a | 82.0±1.15a | 88.0±4.16a | 90.6±4.37a | 89.6±0.88a | 82.0±5.29a |
| ER  (seed\*day-1) | 6.63±0.13cd | 8.87±0.51a | 8.62±0.36ab | 7.73±0.32abc | 7.06±0.78dc | 7.45±0.18bcd | 6.29±0.25d |
| EV | 25.12±2.24c | 46.0±1.73a | 39.9±1.72ab | 46.2±3.73a | 37.0±2.73b | 36.7±2.20b | 24.8±3.22c |
| Height | 13.95±1.63b | 19.76±2.26a | 20.75±1.40a | 16.69±1.96b | 15.47±0.73b | 16.95±1.31b | 14.96±1.07b |
| Shoot biomass | 4.02±0.21c | 7.5±0.57a | 6.47±0.28b | 5.57±0.23b | 4.13±0.39c | 4.46±0.18c | 3.97±0.12c |
| Root biomass | 0.71±0.06dc | 0.81±0.07abc | 0.75±0.05bcd | 0.86±0.02ab | 0.92±0.05a | 0.66±0.03d | 0.71±0.02dc |
| RWC | 80.25±1.07a | 89.41±1.71a | 85.90±1.24a | 83.40±1.30a | 85.24±2.94a | 82.79±3.47a | 79.30±3.16a |

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

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), also proilne content of leaves decreased significantly (P<0.01) in treats 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 was observed in 150 gray treat (table 3).

Table 3 Effect of gamma irradiation on chlorophyll a , chlorophyll b and chlorophyll a+b content (µg g FW-1), proline (mg g FW-1), and total soluble protein content (mg ml-1)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| parameters | gamma irradiation doses (Gy) | | | | | | |
| 0 | 15 | 20 | 30 | 50 | 100 | 150 |
| Chl a | 23.83±0.38a | 23.53±2.25ab | 24.96±0.36a | 24.26±0.7a | 19.5±1.1bc | 16.33±1.73c | 16.96±1.64c |
| Chl b | 8.63±0.12a | 9.36±1.00a | 9.53±2.00a | 8.7±0.2a | 7.06±0.24a | 9.00±3.33a | 8.33±3.32a |
| Chl a+b | 32.8±0.25a | 33.23±3.32a | 34.86±2.13a | 33.36±0.95a | 26.83±1.31a | 25.53±4.74a | 25.53±3.65a |
| Proline | 1.03±0.1a | 0.99±0.027a | 0.18±0.01b | 0.17±0.01b | 0.13±0.01b | 0.02±0.00b | 0.018±0.00b |
| Soluble protein | 0.15±0.006d | 0.18±0.011cd | 0.21±0.008bc | 0.21±0.006bc | 0.23±0.008b | 0.22±0.007b | 0.28±0.02a |

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

**Discussion**

There was no significant difference in germination and emergence capacity of irradiated seeds, but radiation doses of 15, 20 and 30 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, 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); Kim et al., (2004); Wi et al., (2007), and Amjad and Anjum, (2007). 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 devision 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., 2011; Moussa, 2011). These beneficial effects resulted in higher germination; higher growth and yield in treated plants of *Capparis spinosa* L., Eruca vesicaria subsp. sativa, Safflower and *Hibiscus Sabdariffa* L. are reported by (Al-Safadi et al., 2000; Moussa, 2006;Srivastava and Kumar, 2011, El Sherif et al., 2011 ). 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 treats 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 (*Abelmoschus esculentus* (L.) Monech), (Hegazi and Hamideldin, 2010) and parallel with the results of irradiated seeds of Red Pepper (*Capsicum annuum* L.), (Kim et al., 2004) and irradiated seeds of safflower (Carthamus tinctorius L.,), (Srivastava and Kumar, 2011). Irradiation promotes the accumulation of osmoprotectants such as protein and proline, the decreases in proline content of leaves in *Br.inermis is*  in contrast with results of Moussa (2011) that was reported about soybean, and 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.

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