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Research Article

COMBINED EFFECT OF GA_3 AND GAMMA RADIATION ON SEED GERMINATION OF *Rheum emodi* Wall

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Abstract: Present study was aimed to determine the combined effect of gamma radiation and GA_3 on seed germination of *Rheum emodi* Wall. The seeds were pre-soaked in five different conc. of GA_3 for 48 h before irradiation with five different dosages (20.0 -100.0 Gy) of gamma radiation. Seeds were subsequently placed in Petri dishes with Whatman filter paper and moistened with dist. Water. Seeds treated with GA_3 (400.0 ppm) without irradiation showed maximum seed germination percentage (96.66%) and seed vigour index (3779.167). Germination index was also maximum (35.89) in seeds treated with GA_3 (500.0 ppm) without irradiation and minimum in the irradiated(100 Gy) seeds with GA_3(100 ppm). Gamma rays alone and in combination with GA_3 showed inhibitory effect on various parameters of seed germination even at the lowest dosage (20 Gy). Contrary to gamma radiation, GA_3 exhibited stimulatory effect on seed germination.

Keywords: *R. emodi, gamma radiation, doses, seed germination,* GA_3

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Introduction

Gamma rays are ionizing radiations which produce free radicals on reacting with atoms or molecules. These free radicals cause oxidative stress which leads to damage in the cell or sometimes modified the cells or components. So, depending upon the dosage of gamma radiations, damages and modifications of cells and components varies. Gamma radiations are known to cause various changes in plants at the morphological, physiological, anatomical and biochemical levels [1]. These radiations sometimes also play an important role in seed germination of various species, as it produces reactive oxygen species which play a central role with hormones especially abscisic acid (ABA) in releasing dormancy and completion of germination process. During imbibition in dormant seeds, high amount of ABA reduces the level of ROS by maintaining a high level of ROS scavenging enzymes. Therefore, accumulation of ROS block ABA signalling and stimulate GA signalling. Thus, gamma radiation plays a crucial role in breaking physiological dormancy and promoting seed germination [2]. Which seed germination is a crucial developmental phase of a plant's life cycle. Infact, determines the successful establishment of the plant under natural conditions [3]. Most of the endangered medicinal plants show poor seed germination due to the various limiting factors.

Rheum emodi Wall. belonging to family Polygonaceae is one of the important medicinal plants of the Himalayan region. The plant is used throughout the world in various ailments such as jaundice, headache, migraine, paralysis, sciatica, asthma, diarrhoea, cancer and liver disorders *etc.* [4]. *R. emodi* can be propagated through seeds as well as by vegetative means. However, the plant shows seed dormancy due to which its propagation through seeds is poor. The age factor of seeds also affects their germination. The older seeds show less germination percentage and are also more prone to infection. Even, one-year-old seeds show poor germination [5]. Therefore, the present study was aimed to investigate the effect of various germination inducing factors *viz*., doses of gamma radiation and GA_3 on seed germination, seed vigour index and germination index of *R. emodi*.

Materials and Methods

Plant material (seeds) of *R. emodi* was collected from Barjikang area of Pithoragarh district (30º18'03.9"N; 80º15'08.7"E and 4630m asl) of Uttarakhand and stored at 4-6 ºC for further use. Two parallel sets of seeds were presoaked (48 h) in five different conc. of GA_3 (0- control, 100 to 500 ppm). One set of seeds was then irradiated with different doses (20 - 100 Gy) of 60Co gamma rays (Source: Department of Radiations & Isotopic Tracers Laboratory, C.B.S.H, G.B. Pant University of Agriculture and Technology, Pantnagar) and the 2nd set was used without irradiation. Treated seeds were subsequently placed in Petri dishes (9.0 cm) on two layers of Whatmann (No. 1) filter paper moistened with 4ml dist. water and then incubated at 20ºC under 16:8 h alternate light: dark condition. The moisture content of the filter paper was maintained by adding dist. water as and when required. Germination was defined as radicle emergence (>2 mm) and observations were made on radicle emergence on daily basis. Subsequently, seed germination percentage, seed vigour index and germination index were calculated using following formulae.

Seed Germination %= (Number of germinated seeds)/(Total number of seeds) ×100 [6]

Germination index= n/d [7]

Where the n= number of seedlings emerging on the day 'd', d = days after planting

Seed vigour index= Germination % × Seedling length (cm) [8]

Statistical Analysis

All experiments were performed in replicates. The mean and ANOVA were calculated by using SPSS version 16 with two-way analysis followed by Duncan's multiple range test (DMRT) and significance was determined at $p<0.05$. Data represented in the graphs is as mean ± standard error (SE).

Results and Discussion

Effect of different doses of gamma radiations along with the different concentrations of GA_3 on seed percent germination, germination index and seed vigour index are presented in [Fig-1-3].

It is observed that the percent germination was maximum in the seeds treated with 400.0 ppm GA_3 without irradiation (96.66%) and minimum in the seeds treated with 100.0 ppm GA_3 along with 100.0 Gy gamma radiations (41.67%). At GA_3 (300 ppm), germination (%) at 80 Gy was similar to that of control. At high concentration of GA_3 (500 ppm), gamma radiation ranging from 20-80 Gy showed stimulatory effects on germination (%). Higher doses of gamma rays showed a negative effect on seed germination (%) [Fig-1]. The inhibitory effect of gamma radiation on seed germination percentage was also reported in *Hordeum vulgare* and different varieties of *Tagetes patula* at 0.1-0.4 kGy and 2.5-25KR respectively [9, 10]. However, Beyaz *et al*., (2016) also reported that low doses of gamma radiation showed stimulatory effect whereas high doses showed inhibitory effect on seed germination percentage [11]. The germination index was maximum in seeds treated with GA_3 (500.0 ppm) without irradiation (35.89) and minimum in seeds treated with 100ppm GA_3 at 100Gy gamma radiation (11.67). At GA_3 (100 ppm), germination vigour at 20Gy was stimulatory and rest all doses were inhibitory whereas at GA_3 (200 ppm) all doses were inhibitory. At GA_3 (300 ppm), 60 Gy showed a similar germination index as the control and rest were inhibitory. At GA_3 (400 and 500 ppm) all gamma doses were inhibitory. Thus, it is observed that the germination index increases with increasing concentration of GA_3 whereas decreases with the increasing doses of gamma radiation [Fig-2]. Marcu *et al* (2013), also reported similar results in *Zea mays*, where germination index decreased with increasing doses of gamma rays at 0.1-1.0 kGy [12]. Contrary to this, Aynehband and Afsharinafar (2012) reported the stimulatory effect of gamma radiations (0-250 Gy) on the germination index of Amaranth seeds [13].

Seeds treated with 400 ppm GA_3 without irradiation showed maximum seed vigour index (3779.167) while seeds treated with high doses of gamma radiation (100 Gy) along with the high concentration of GA_3 (500 ppm) showed minimum (437.77) seed vigour index. Seed vigour index increased with increasing conc. of GA_3 without irradiation. At all levels of GA_3, increasing doses of gamma radiation proved inhibitory for seed vigour index in a dose-dependent manner [Fig-3]. Thus, seed vigour index showed a similar pattern as shown by germination index *i.e.* seed vigour index increased with the increasing concentration of GA_3 but decreased with the increasing doses of the gamma radiation. Boranayaka *et al*., (2010), Anbarasan *et al*., (2013) and Ariraman *et al*., (2014) also reported the dose-dependent inhibitory effect of gamma radiation on seed vigour index in various plant species [14-16]. In the present study, gamma rays showed a negative effect on seed germination, germination index as well as on seed vigour index. However, high concentration of GA_3 (400-500 ppm) showed stimulatory effect on germination index and seed vigour index.

Conclusion

Based on the collective data of this study, it can be concluded that all the dosage of gamma ray showed inhibitory effect on different parameters of seed germination. On the contrary, increasing dosage of GA_3 showed stimulatory effect for all the seed germination parameters. The combined effect of gamma-ray and GA_3 also showed inhibitory effect on seed germination.

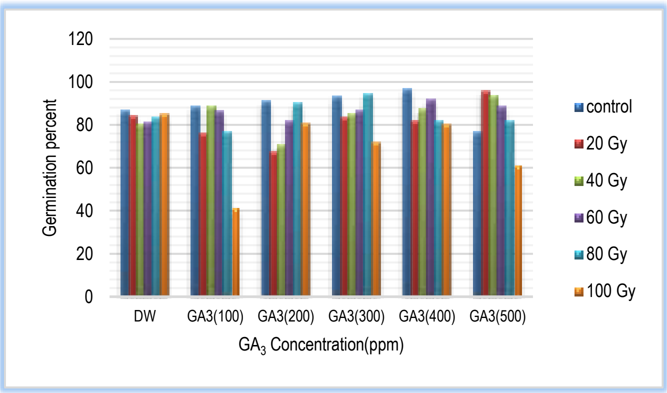

Fig-1 Effect of different doses of gamma-ray and GA_3 on seed germination percentage of *R. emodi* under *in vitro* conditions.

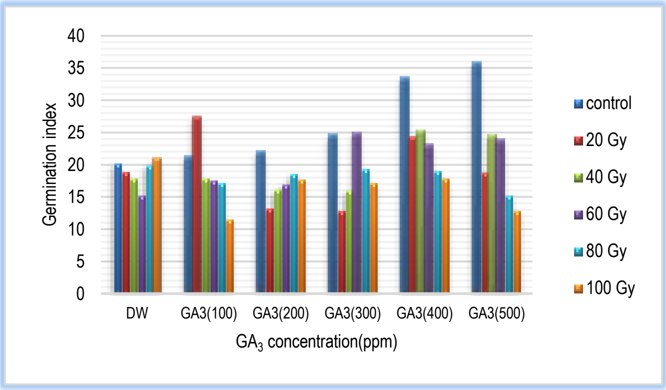

Fig-2 Effect of different doses of gamma-ray and GA_3 on germination index of *R. emodi* under *in vitro* conditions.

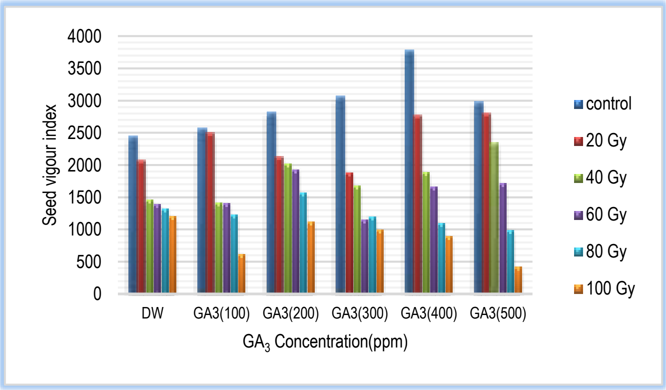

Fig-3 Effect of different doses of gamma-ray and GA_3 on seed vigour index of *R. emodi* under *in vitro* conditions.

Application of research: Study, gamma radiation was found to be inhibitory for seed germination. Based on the results, Gibberellic acid (400ppm) alone without irradiation could be recommended to overcome seed dormancy in *R. emodi*.

Research Category: Medicinal plants

Abbreviations: GA3: Gibberellic acid (GA), Gy: Gray, ABA: Abscisic acid, ROS: Reactive Oxygen Species, KR: Kilo Roentgen, dist.: distilled water, conc.: concentration

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