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Research Note

Induced mutagenesis on growth, yield and yield attributing traits in buckwheat (*Fagopyrum tartaricum*)

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Abstract
The present study was conducted to assess the impact of induced mutagenesis on the growth, yield, and seedling traits of buckwheat (*Fagopyrum tartaricum*) using the genotype IC59921, at SHUATS, Prayagraj. The research utilized a Randomized Block Design (RBD) for field trials and a Completely Randomized Design (CRD) for laboratory experiments, incorporating eleven treatments, including a control (T0), ethyl methanesulfonate (EMS) treatments (T1-T4) at concentrations of 0.3% and 0.5% for 3 and 6 hours, as well as gamma irradiation (T5-T10) with doses ranging from 5 to 30 kR. The results showed that EMS treatment at 0.5% for 6 hours significantly enhanced seedling growth and key yield parameters. Additionally, lower doses of gamma radiation positively impacted these traits, while higher doses resulted in reduced germination and overall growth. The study underscores the potential of mutagenesis to improve the growth and productivity of buckwheat, offering a valuable approach for its genetic enhancement in crop improvement programs.

Keywords: Buckwheat, Mutagenesis, EMS, Gamma irradiation.

Buckwheat (*Fagopyrum tartaricum*) is a versatile pseudocereal originating from the Himalayan region, known for its adaptability to diverse environmental conditions. It is a rich source of high-quality protein, essential amino acids, and bioactive compounds like rutin, which provide significant health benefits such as antioxidant, anti-inflammatory, and anti-cancer properties (Zhang *et al.,* 2020). Despite its nutritional value, buckwheat cultivation remains limited, primarily due to its relatively low yield potential and genetic constraints, making it crucial to explore strategies for enhancing its productivity.

Buckwheat exhibits a strong heteromorphic self-incompatibility system, which prevents self-pollination and maintains genetic diversity through obligate outcrossing. This is governed by the S-locus supergene complex, which controls both floral morphology and compatibility, posing significant challenges in stabilizing desirable traits across generations (Yasui *et al.,* 2016). The genetic pool of buckwheat is narrow, limiting breeding options. The crop's relatively short domestication history and limited use in large-scale breeding programs have contributed to its reduced variability, impeding efforts to select for traits like higher yield, disease resistance, or abiotic stress tolerance (Zhou *et al.,* 2019). Due to its outcrossing nature and the presence of heteromorphic SI, buckwheat presents difficulty in fixing complex quantitative traits such as yield, plant height, and bioactive compound content. Progeny from selected individuals often do not maintain the desired trait expression, slowing the breeding progress (Gupta & Gupta, 2020). *In vitro* regeneration in buckwheat is often hindered by high levels of phenolic compounds and genotype-specific responses, which impede callus formation and plantlet regeneration. These limitations restrict the use of genetic engineering and advanced tissue culture methods for crop improvement (Biesaga-Kościelniak & Filek, 2019). Despite recent progress in genomic resources, such as draft genome sequences and genetic linkage maps, buckwheat still lacks the robust infrastructure that exists for major crops. This hinders the integration of modern molecular tools into conventional breeding (Michiyama *et.al.,* 2021).

Induced mutagenesis is a promising approach for generating genetic variability and improving crop traits, especially in underutilized species like buckwheat. Chemical mutagenesis with Ethyl Methane Sulfonate (EMS) and physical mutagenesis using gamma radiation have been widely applied to enhance various agronomic traits. EMS induces point mutations that can improve seed germination, plant height, and yield-related traits (Ahmad *et al.,* 2022), while gamma radiation induces chromosomal alterations, which can result in the development of more resilient and higher-yielding varieties (Rao *et al.,* 2021).

Both EMS and gamma radiation have shown significant potential in improving crop performance, though their effects are dose-dependent. Lower doses of EMS and gamma radiation tend to enhance desirable traits, whereas excessive doses can result in negative effects such as reduced germination and growth (Ahmed *et al.,* 2023). These mutagenic methods offer an efficient way to overcome genetic bottlenecks and enhance the agronomic traits of buckwheat, thus addressing its yield constraints.

The scope of this research lies in evaluating the effects of EMS and gamma radiation on the growth, yield, and yield-related attributes of the buckwheat genotype IC599211. While gamma irradiation doses in this study were set between 5 and 30 kR. This range was selected based on previous research that demonstrated effective mutation induction and trait variability in pseudocereals and minor crops within this exposure window (Wani, 2009). These doses are known to generate desirable mutations while minimizing lethal effects, making them suitable for studying growth and yield-related traits in buckwheat. This study aims to identify optimal mutagenic treatments to improve the productivity of buckwheat, providing valuable insights into its genetic improvement.

The experiment was conducted during the Rabi season of 2023 at the Field Experimentation Centre and Notified Seed Testing Laboratory, Department of Genetics and Plant Breeding, Sam Higginbottom University of Agriculture, Technology, and Sciences, Prayagraj, Uttar Pradesh, to evaluate the effects of induced mutagenesis using EMS (Ethyl Methanesulphonate) and gamma rays on seedling growth, plant development, and yield parameters in buckwheat (*Fagopyrum tartaricum*) genotype IC599211. A factorial experiment was employed, utilizing a Completely Randomized Design (CRD) for laboratory trials with four replications and a Randomized Block Design (RBD) for field trials with three replications. The study involved eleven treatments: T0 (control, untreated seeds), T1 (EMS 0.3% for 3 hours), T2 (EMS 0.3% for 6 hours), T3 (EMS 0.5% for 3 hours), T4 (EMS 0.5% for 6 hours), T5 (gamma irradiation 5 kR), T6 (gamma irradiation 10 kR), T7 (gamma irradiation 15 kR), T8 (gamma irradiation 20 kR), T9 (gamma irradiation 25 kR), and T10 (gamma irradiation 30 kR). The experiment was conducted using a completely randomized design (CRD) for laboratory trials and a randomized block design (RBD) for field evaluations. CRD was selected for the laboratory phase due to its simplicity and suitability for homogeneous environments where experimental units are considered uniform—ideal for controlled germination and early growth assessments. For field trials, RBD was employed to account for environmental heterogeneity across the field site, as it effectively controls for variability due to soil conditions, light exposure, and other external factors, thereby increasing the precision of treatment comparisons. A factorial design was incorporated to evaluate the interactive effects of mutagen type (EMS and gamma irradiation) and dose levels. This approach not only allows for assessing the individual effects of each factor but also reveals any synergistic or antagonistic interactions between mutagens and their respective doses, offering a more comprehensive understanding of their influence on buckwheat growth and yield traits.

EMS solutions were prepared by dissolving the specified quantities of Ethyl Methanesulphonate (EMS) in distilled water to achieve concentrations of 0.3% and 0.5%. The solutions were thoroughly mixed to ensure homogeneity before being applied to the seeds for exposure durations of 3 and 6 hours, respectively. Gamma irradiation treatments were administered at incremental doses of 5, 10, 15, 20, 25, and 30 kR. These treatments were carried out at the National Botanical Research Institute (CSIR-NBRI), Lucknow, using the GIC-1200 model with a Cobalt-60 radioactive source. Both EMS and gamma irradiation treatments were conducted under controlled laboratory conditions to maintain consistency and precision in the mutagenesis process.

To evaluate the mutagenic effects on growth and development, a mutagenized population of buckwheat seeds was established. Laboratory analyses involved 100 seeds per replication, with four replications per treatment, resulting in 400 seeds per treatment and a total of 4,400 seeds across all treatments. Field experiments utilized to evaluate the mutagenic effects on growth and development, a mutagenized population of buckwheat seeds was developed. In the laboratory, 100 seeds per replication were used with four replications per treatment, amounting to 400 seeds per treatment and a total of 4,400 seeds across all treatments. For the field experiments, 27 seeds were sown per replication with three replications per treatment, resulting in 81 seeds per treatment and 891 seeds in total. The selection of 27 seeds per replication was based on the standard planting geometry for a 1 m × 1 m plot, with a spacing of 30 cm between rows and 10 cm between plants. This spacing allowed for proper growth, aeration, and management. Furthermore, only 27 healthy and viable seedlings were suitable for transplanting in each replication after initial screening, which further justified this number. This approach ensured uniformity in plant density and facilitated a consistent, manageable comparison of mutagenic effects under both controlled and field conditions, providing robust insights into the induced variability and potential agronomic improvements in buckwheat.This methodology enabled a comprehensive assessment of mutagenic effects under both controlled and natural conditions, offering valuable insights into the potential benefits and variations induced by these mutagens in buckwheat.

Field data were collected from five randomly selected plants per replication for each treatment, focusing on key parameters such as field emergence rate, days to 50% flowering, plant height at 20 and 40 days after sowing (DAS), number of branches at 20 and 40 DAS, days to maturity, number of spikes per plant, number of seeds per spike, seed yield per plant (g), seed yield per plot (kg), seed yield per hectare (q), biological yield (kg), harvest index, and test weight. Post-harvest, laboratory experiments were conducted to evaluate the optimal mutagenic dose for parameters including germination percentage, germination energy, shoot length (cm), root length (cm), seedling length (cm), seedling fresh weight (g), seedling dry weight (g), seed vigor index I, and seed vigor index II. Statistical analysis of the field data was carried out following the methodology outlined by Panse and Sukhatme (1985), ensuring a thorough and systematic evaluation of the results. This comprehensive approach provided a robust framework for assessing the impact of induced mutagenesis on the growth, yield, and yield-attributing traits of buckwheat.

This study presents a comprehensive analysis of the effects of EMS and gamma irradiation on the growth, yield, and seedling traits of buckwheat (*Fagopyrum tartaricum*) genotype IC599211. The experimental data revealed significant impacts of these treatments on multiple agronomic parameters **(Tables 1 and 2)**. Seeds treated with 0.5% EMS, soaked for six hours, exhibited the highest significant improvements across all evaluated traits, while seeds exposed to a higher dose of gamma irradiation (30 kR) showed the lowest performance. These findings emphasize the potential of EMS-induced mutagenesis to enhance buckwheat cultivation, with implications for improving seedling vigor, growth rates, and yield characteristics (Panse and Sukhatme, 1985).

The effect of induced mutagenesis on growth and yield characteristics of buckwheat genotypes is presented in **Table 1**. The highest rate of field emergence (861) was recorded in T4 (0.5% EMS for 6 hours), followed by T6 (10 kR of gamma irradiation, 832). The lowest field emergence was observed in T10 (30 kR of gamma irradiation, 587). Similar results were observed by Jain *et al.* (2015), who found that EMS treatment significantly improved field emergence in mung bean, with the highest concentration (0.5%) yielding the best results. Conversely, Banu *et al.* (2018) reported that high doses of gamma irradiation (e.g., 30 kR) reduced field emergence, likely due to damage to seed structures and DNA, a phenomenon consistent with the findings in this study.

As shown in **Table 1**, the shortest time to flowering (32 days) occurred in T4, followed by T6 (36.33 days), while the longest time to flowering (59 days) was recorded in T10. Similar results were found by Kurokawa *et al.* (2018) in rice, where EMS and gamma irradiation treatments affected flowering time. In this study, EMS treatments also led to significant plant growth improvements. T4 exhibited the highest plant height at both 20 DAS (29.93 cm) and 40 DAS (61.77 cm), while T10 had the shortest plant heights. EMS-induced mutations, caused by the alkylation of guanine bases during DNA replication, can alter genes regulating growth, which supports the observed increase in plant height (Zhang *et al.,* 2024).

At 20 and 40 DAS, T4 also showed the highest number of branches (5.33 and 7.47, respectively), compared to T10, which had the fewest branches. EMS-induced point mutations, particularly G/C to A/T transitions, can influence branching patterns (Kim *et al*., 2020), which could explain the observed variability in branching. The maximum number of spikes per plant (6.47) was recorded in T4, followed closely by T6 (6.20), while T10 had the fewest spikes (2.07). Similar results were reported by Zhang *et al.* (2024), where EMS-induced mutations influenced phenotypic traits, including the number of spikes. **(Table 1)**

The days to maturity varied significantly, with T4 and T6 exhibiting the shortest maturity times (85 days), whereas T10 had the longest time to maturity (96 days). Similarly, T4 showed the highest number of seeds per spike (56.13), while T10 had the lowest (26.47), reinforcing the positive effects of EMS on flowering and seed production. **(Table 1)**

Regarding seed yield, the highest seed yield per plant (5.88 g) was observed in T4, followed by T6 (5.07 g), and the lowest yield was recorded in T10 (1.57 g). Singh *et al*. (2023) reported that moderate EMS concentrations enhance seed yield by improving yield-related traits, which is consistent with the results of this study. The highest seed yield per plot (0.16 kg) and seed yield per hectare (7.94 q) were also recorded in T4, with T6 following closely (0.14 kg and 6.84 q, respectively). In contrast, T10 showed the lowest values for these parameters (0.04 kg and 2.12 q). **(Table 1)**

As presented in **Table 1**, T4 exhibited the highest biological yield (16.10 g), followed by T6 (15.70 g), while T10 had the lowest (6.80 g). EMS-induced mutations significantly affect biological yield by altering both biomass and seed yield (Brown *et al.,* 2023). The harvest index ranged from 0.37 in T4 to 0.23 in T10, with the highest harvest

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Table 1.Mean performance of the effect of induced mutagenesis on growth and yield characteristics of buckwheat genotype.

Treatments	Rate of Field Emergence	Days to 50 % flowering	Plant height 20 DAS (cm)	Plant height 40 DAS (cm)	No. of branches (20 DAS)	No. of branches (40 DAS)	Days to Maturity	No. of spike per plant	No. of seeds per spike	Seed yield per plant (gm)	Seed yield per plot (kg)	Seed yield per hectare (q)	Biological yield per plot (kg)	Harvest index%	Test weight (gm)
T0	803	38.67	26.33	58.11	4.67	6.20	86	5.80	50.47	4.93	0.13	6.65	14.27	0.35	20.17
T1	697	46.00	21.77	46.03	3.13	5.53	91	4.27	42.07	3.96	0.11	5.35	13.53	0.29	15.77
T2	720	44.67	23.43	49.19	3.47	5.53	90	4.67	45.47	4.08	0.11	5.51	13.07	0.31	17.13
T3	773	42.67	25.23	55.32	4.00	5.93	87	5.47	48.80	4.63	0.13	6.26	14.40	0.32	19.57
T4	861	32.33	29.93	61.77	5.33	7.47	85	6.47	56.13	5.88	0.16	7.94	16.10	0.37	22.67
T5	745	44.33	24.19	51.79	3.87	5.80	88	5.20	48.20	4.34	0.12	5.86	13.60	0.32	18.77
T6	832	36.33	28.22	60.07	4.60	7.00	85	6.20	53.07	5.07	0.14	6.84	15.70	0.32	20.93
T7	659	49.00	19.10	43.95	2.67	4.40	93	3.47	39.80	3.97	0.11	5.36	12.73	0.31	14.10
T8	640	51.00	14.27	39.43	2.40	3.87	93	3.27	33.73	3.65	0.10	4.92	10.33	0.35	12.20
T9	620	58.00	12.09	35.47	1.73	3.13	94	2.67	29.73	2.56	0.06	3.21	8.80	0.29	11.43
T10	587	59.00	10.03	30.97	1.40	2.53	96	2.07	26.47	1.57	0.04	2.12	6.80	0.23	9.37
Grand Mean	**722**	**45.64**	**21.33**	**48.37**	**3.39**	**5.22**	**89.85**	**4.50**	**43.08**	**4.06**	**0.11**	**5.45**	**12.67**	**0.32**	**16.55**
Max.	**861.90**	**59.00**	**29.93**	**61.77**	**5.33**	**7.47**	**96**	**6.47**	**56.13**	**5.88**	**0.16**	**7.94**	**16.10**	**0.37**	**22.67**
Min.	**587.62**	**32.33**	**10.03**	**30.97**	**1.40**	**2.53**	**85**	**2.07**	**26.47**	**1.57**	**0.04**	**2.12**	**6.80**	**0.23**	**9.37**
S Em	**8.12**	**0.49**	**0.11**	**0.16**	**0.05**	**0.08**	**0.50**	**0.06**	**0.18**	**0.03**	**0.01**	**0.07**	**0.07**	**0.01**	**0.20**
S Ed	**11.48**	**0.70**	**0.15**	**0.23**	**0.07**	**0.11**	**0.71**	**0.08**	**0.26**	**0.05**	**0.01**	**0.10**	**0.10**	**0.01**	**0.29**
CD at 5%	**41.47**	**2.52**	**0.53**	**0.83**	**0.26**	**0.41**	**2.56**	**0.30**	**0.92**	**0.18**	**0.010**	**0.344**	**0.370**	**0.023**	**1.03**

Whereas, T0- Control, T1- EMS (Ethyl Methanesulphonate) 0.3 % (3 hrs.), T2- EMS 0.3 % (6 hrs.), T3- EMS 0.5 % (3 hrs), T4- EMS 0.5 % (6 hrs), T5- GY 5 kR, T6- GY10 kR, T7- GY 15 kR, T8- GY 20 kR, T9-GY 25 kR, T10- GY 30 kR

Table 2.Mean performance of the effect of induced mutagenesis on Seedling characteristics of Buckwheat genotype.

Treatments	Germination %	Germination Energy	Shoot Length (cm)	Root Length (cm)	Seedling Length (cm)	Fresh weight (gm)	Dry weight (gm)	Seed Vigour Index-I	Seed Vigour Index-II
T0	79.25	43	8.68	11.91	20.58	1.35	0.22	1631	17.44
T1	74.75	37.25	7.46	10.47	17.93	1.30	0.17	1340	12.52
T2	75.75	39	7.86	10.90	18.76	1.33	0.19	1421	14.03
T3	78	42.5	8.36	11.51	19.87	1.38	0.21	1550	16.17
T4	90.5	49.25	9.20	12.37	21.57	1.64	0.28	1952	25.56
T5	76	40.5	8.14	11.17	19.30	1.32	0.19	1467	14.44
T6	86.75	44.5	9.06	12.10	21.16	1.58	0.26	1835	22.11
T7	71	31.75	7.09	10.14	17.23	1.26	0.16	1223	11.18
T8	68.25	30	6.67	9.74	16.41	1.15	0.15	1120	10.24
T9	66	29.25	6.35	9.35	15.70	1.12	0.15	1036	9.57
T10	65	27.25	6.09	8.83	14.92	1.08	0.13	970	8.62
Grand Mean	**74.09**	**35.65**	**6.80**	**6.76**	**13.56**	**1.32**	**0.15**	**1031.58**	**12.05**
S Em	**0.91**	**0.74**	**0.03**	**0.03**	**0.04**	**0.02**	**0.01**	**16.88**	**0.57**
S Ed	**1.29**	**1.05**	**0.04**	**0.05**	**0.05**	**0.03**	**0.01**	**23.88**	**0.81**
CD at 5%	**3.93**	**3.19**	**0.11**	**0.14**	**0.15**	**0.07**	**0.02**	**72.8**	**2.47**

Whereas, T0- Control, T1- EMS (Ethyl Methanesulphonate) 0.3 % (3 hrs.), T2- EMS 0.3 % (6 hrs.), T3- EMS 0.5 % (3 hrs), T4- EMS 0.5 % (6 hrs), T5- GY 5 kR, T6- GY10 kR, T7- GY 15 kR, T8- GY 20 kR, T9-GY 25 kR, T10- GY 30 kR

index observed in T4. These findings are in line with Brown *et al.* (2023), who reported that EMS treatments can significantly improve harvest index. Test weight was highest in T4 (22.67 g), followed by T6 (20.93 g), and lowest in T10 (9.37 g). EMS treatments have been shown to affect grain morphology and density, thus influencing test weight (Wang *et al.,* 2023).

The effect of induced mutagenesis on seedling parameters of buckwheat genotypes is presented in **Table 2**. The highest germination percentage (90.50%) was recorded in T4 (0.5% EMS soaking for 6 hours), followed by T6 (10 kR Gamma irradiation) at 86.75%. In contrast, the lowest germination percentage was observed in the highest dose of gamma irradiation (T10, 30 kR) at 65%. Similar findings were reported by Mba *et al*. (2013) and Huszár *et al.* (2005), where moderate doses of EMS and gamma irradiation were found to enhance seed germination, while higher doses reduced seed viability. Correspondingly, the highest germination energy (49.25%) was also observed in T4, followed by T6 (44.5%). The lowest germination energy (27.25%) was found in T10, reinforcing the negative impact of higher irradiation doses on seed vigor. These findings align with the research by Minoia (2010), which emphasized that moderate stress treatments can enhance early seedling vigor.

In terms of seedling growth, the highest shoot length (9.20 cm) and root length (12.37 cm) were recorded in T4, followed by T6 with shoot and root lengths of 9.06 cm and 12.10 cm, respectively. On the other hand, the lowest values were observed in the highest dose of Gamma irradiation (T10), with shoot length at 6.09 cm and root length at 8.83 cm. These results are consistent with those of Kurokawa *et al.* (2018) and Kosambi *et al.* (2018), who noted that moderate doses of EMS and Gamma irradiation support seedling growth, whereas excessive irradiation negatively affects both shoot and root development. **(Table 2)**

As presented in **Table 2,** the highest seedling length (21.57 cm) was recorded in T4, followed by T6 at 20.58 cm. The lowest seedling length (14.92 cm) was noted in T10. Additionally, T4 also exhibited the highest fresh weight (1.64 gm) and dry weight (0.28 gm), with T6 following closely at 1.58 gm and 0.26 gm, respectively. The lowest values were observed in T10 (fresh weight: 1.08 gm, dry weight: 0.13 gm). These findings corroborate those of Li *et al.* (2018) and Chen *et al.* (2018), who highlighted the positive influence of moderate doses of EMS and Gamma irradiation on seedling biomass, while higher doses resulted in stunted growth.

Vigour index I and II are critical indicators of seedling performance. The highest Vigour Index I (1952) was recorded in T4, followed by T6 at 1835, while T10 showed the lowest Vigour Index I (970). Similarly, Vigour Index II was highest in T4 (25.56), followed by T6 (22.11), and lowest in T10 (8.62). These results are consistent with the findings of Abdel-Hady *et al.* (2016) and Bolat *et al.* (2018), who observed that treatments with moderate EMS concentrations and Gamma irradiation doses enhanced seedling vigor, while excessive doses reduced seedling performance. **(Table 2)**

In conclusion, this study highlights the significant potential of induced mutagenesis, particularly through EMS (Ethyl Methanesulphonate) treatment, in enhancing seedling growth and yield parameters in buckwheat. The results demonstrate that EMS treatment notably improved seedling vigor, growth rates, and yield-related traits compared to untreated controls, indicating the beneficial impact of EMS-induced mutations on buckwheat cultivation. Additionally, gamma irradiation at a dosage of 10 kR yielded positive alterations in seedling growth and yield parameters. However, higher doses of gamma irradiation were found to have detrimental effects, leading to a decline in plant characteristics such as seedling growth and yield. These findings underscore the effectiveness of moderate mutagenic treatments in improving buckwheat performance, while also emphasizing the need for careful optimization of irradiation doses.

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