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RESEARCH ARTICLE

Induced Physical mutagenesis on seed germination, lethal dosage and morphological mutants of Ashwagandha (*Withania somnifera* (L.) Dunal)

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Abstract

Induced on physical mutagenesis of gamma rays the seed germination, (LD_{50}) value and morphological character of Ashwagandha (*Withania somnifera* (L.) Dunal) were studied. The seeds were irradiated with different dose of gamma rays *viz.*, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50KR. The untreated seeds were taken as control. The physical mutagen gamma rays treatment in seed germination and seedling survival percentage were calculated. In morphological parameters was analyzed in above 120 days. The germination percentage was calculated on 15^{th}day and seedling survival proportion at 30^{th}day. The 50 per cent reduction of seed germination was observed as LD_{50} value (lethal dosage). The maximum 50 per cent reduction of seed germination in 25KR of gamma rays treatment. The seedling survival also increased with in decreased below 50 per cent observed in 25KR of gamma rays treatment. The Ashwagandha (Amukkra kizhangu) morphological parameters days to first flower, plant height (cm), number of branches per plant, number of leaves per plant, number of berries per plant, seed yield per plant (gm) and root yield per plant (fresh weight(gm) were analyzed in all the different dose of gamma ray treatments.

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Introduction

Withania somnifera (L.) Dunal, commonly known as Ashwagandha belonging to the family (Solanaceae) (English name: winter cherry), (Tamil name: Amukkra kizhangu) is a perennial herb (Ali *et al*., 1997) Plant species with immense therapeutic uses in traditional (Ayurvedha, Siddha and Unani) and also in modern system of medicine. It is also called as `Indian Ginseng' due to its properties similar to `panax ginseng' (Tripathi *et al*., 1996). The medicinal properties of Ashwagandha are due to its chemical constituents (alkolides and withanolides) primarily in root, stem and also in leaves. The root powder and paste of the species are important in treatment of rheumatic pain, arthiritis and cardiopulmonary disorder (Asthana and Raina, 1989). Constipations and loss of memory (Watt, 1972). Pulmonary tuberculosis (Maithani, 1973), abortion (Sahu, 1982). The formulations of root were also reported to be aphrodisiac, diuretic, restorative and rejuvenative in nature (Tripathi *et al*., 1996, Patra *et al*., 2004). Ulcers and tumors (Kapoor, 2001).

In ancient times *Withania somnifera* is being exploited in wild habitats, but in past few decades attention has been directed towards proper exploitation under sustainable cultivation. Cultivation of elite genotype of Ashwagandha is an appreciable proposition, but environmental factors play a significant role on synthesis and accumulation of phytomedical compound. Therefore, it would be a difficult task to gain control over secondary metabolite production in field grown plants. So one can think for an alternative strategy to obtain steady production of alkolides and withanolides throught the year, over fluctuation of environmental factors, in Ashwagandha plant. The Ashwagandha seed variety Jawahar-20 improves through induced on physical mutagen of gamma rays treatments.

Mutation is a sudden heritable change in an organism generally the structural change in genes. The term mutation was first introduced by Hugo De

Vries, 1901 in Oenothera lamarkiana. Mutation produced by change in base sequence of genes (as a result of base pair transition or transversion, deletion, duplication or inversion etc.,) are known as gene or point mutation. The mutation is produced by change in chromosome structure or even in chromosome number is known as chromosomal mutation. The induced mutation caused artificially by mutagenic factors. The agents that induce mutation are called as mutagens. Gamma ray is one of the physical mutagen used in the field of mutation breeding. Mutation can be induced spontaneously or artificially in seed propagated in medicinal plants.

Gamma rays are ionizing radiation having low wavelength with high penetrable power, interact with atoms or molecules to produce free radicals in the cells. The radicals can damage or modify important components of plant cells and have been reported to affect the seed germination, morphology, anatomy, physiology, biochemical and photochemical characters of plants differentially depending upon the level of irradiation. The effects include changes in cellular structure and metabolism of the plants. Irradiation it is on considered that among the physical mutagen, gamma rays stand first in its effectiveness in the induction of mutations.

Mutation breeding is one of the conventional breeding methods in plant breeding. It is relevant with various fields like morphology and morphological characters. (Gustafsson, 1947) advocated that mutation approach was superior to other methods of crop improvement. (Brock, 1977) induced mutation causes manipulation of genetic variations. Induced mutagenesis exploring the already existing varieties followed by controlled autogamy would therefore be a potent and economic tool for development of elite plant types of commercial significance. (Swaminathan, 1972) suggested that induced mutation provides ample opportunity for reconstruction of plant ideotypes. However, the drastic changes of the phenotype brought about by mutational event among the genes, (Scossiroli, 1965).

Materials and methods

Ashwagandha variety of Jawahar-20 seeds was collected from TNAU (Tamil Nadu Agricultural University) Coimbatore. 5gm of well matured seeds were taken from zip cover for irradiation. The mutagen of gamma irradiation was given at Sugarcane Breeding Institute Coimbatore. Ten sets of 5gm seed were taken as irradiated with different dosage of gamma rays (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50KR) were given inside the gamma chamber in source of Cobalt 60. The gamma ray irradiated seeds were sown in the pot culture in the botanical garden, Department of Botany, Faculty of Science, Annamalai University, Annamalainagar. The untreated seeds were taken as control.

The assessment of seed germination percentage and seedling survival percentage of treated and control seed of *Withania somnifera* were recorded in laboratory at room temperature (24 ±2). Growth of the radical following bursting of the seed coat was taken as an index for germination in petri plates. The extent of injury was assessed by determining the relative reduction in growth in the treated samples under controlled condition as compared to control. A gradual reduction of seed germination and seedling survival in Ashwagandha seeds under similar condition in moist filter paper kept on petri plates, because the gamma irradiation affects the seed germination and seedling survival as the consequence of mutagenic effect on growth phenomenon. Lethality was determined from reduction in germination with respect to control. Seed germination was recorded at 15^{th}day after sowing. Seedling survival percentage was determined from 30^{th}day after sowing.

Results and Discussion

Seed germination and Seedling survival

Generally, gradual reduction in germination percentage observed from lower to higher dosage of gamma ray treatments. 5KR treated seeds showed the highest germination percentage was observed in lower dosage and lowest germination was observed in higher doses. The dosage of gamma rays increased and it was also reported in Ashwagandha by (Mohsina *et al*., 2007b). 25KR of gamma rays treated seeds showed on 50 per cent of germination was observed in (51.32) per cent and seedling survival (50.12) per cent of Ashwagandha as given in (Table-1). Hence, it was considered as LD_{50} value for gamma rays irradiated seeds of Ashwagandha. Same results have been reported in Ashwagandha by (Mohsina *et al*., 2006). Therefore, indicated that mostly the dose of treatments, sub lethal dose 20 and 30KR of gamma rays.

Ashwagandha seed reduction in seed germination percentage and gradually increase in the production of active radicals responsible for seedling survival (lethality) and increasing dosage of gamma rays was immediately damage the physiological activities of seeds. Hence, all the morphological parameters were decreased in higher dose of gamma rays irradiated plants. A maximum reduction of germination per cent (08.45) observed in 50KR of gamma rays (Table -1). A maximum seed germination reduction in high dosage has also been reported in Ashwagandha by (Mohsina Iqbal and Datta, 2005). The decrease in germination percentage

and survival, when the concentration of EMS and time duration of treatment increased, has also been reported by (Ananya Das and Animesh Datta, 2010).

The extent of chromosome aberration occurring in the cells (Datta and Biswas, 1986) and (Datta *et al*., 1982) and also the structural changes (Gray and Read, 1950), (Evanas, 1965) blockage of cells into mitosis is the most important cellular event after mutagenic treatments, which results in cessation of growth. Retardation of growth due to mutagen is been primarily due to destruction of auxin of meristamatic cells and physiological and biochemical disturbances (Gunkel and Sparrow, 1954), (Raj *et al*., 1972, and Singh, 1974).

The seed germination, seedling survival, days to first flower, plant height, number of branches per plant, number of leaves per plant, number of berries per plant, seed yield per plant(gm) and root yield per plant (fresh weight (gm)) were gradually decreased with increase in various dosage of gamma rays. Similar results in morphological parameters were analyzed in Cowpea (Gnanamuthy *et al*., 2013) and also in Sesame (Anbarasan *et al*., 2013). It was noted by (Hameed *et al*., 2008) that final germination percent was decreased significantly after higher irradiation dose ranging from 350-500Gy. A maximum decreased in seed germination percentage was observed after 500Gy doses. Gamma radiation had inhibitory effect on physiological and quantitative traits (Khan and Goyal, 2009) and (Iqbal, 2007b).

Days to first flower

The minimum days to first flower was observed in lower dose of gamma rays. While the maximum days flowering stage observed in higher dosage of gamma rays. A minimum day was observed in (55.20) and maximum days was observed in (74.50) Physical mutagen gamma ray treatments (Table-2).

Plant height (cm)

In general, all the mutagenic treatments caused a reduction in the plant height compared with control. The effect of gamma radiation, there was an increase in the plant height at lower doses of gamma rays. A maximum plant height was observed in 5KR of gamma rays (69.12cm). The minimum plant height was observed in (36.73cm) at 50KR of gamma rays. The plant height increased in lower dose of gamma ray treatments, while plant height decreased with higher dose of gamma rays treatment.

Number of branches per Plant

A maximum number of branches were observed at 5KR of gamma rays. The minimum branches were observed in 50KR of gamma rays. The number of branches decreases with increase in doses of gamma radiation. The maximum number of branches was observed in 5 KR of gamma rays treatment (5.48), while the minimum branches was observed in (2.53) in 50KR of gamma rays treatment.

Number of leaves per Plant

In Ashwagandha leaves more level of chemical compound present. A gradual reduction of leaves per plant was observed in high to lower dose of gamma ray treatment, when compared with control. The maximum number of leaves was observed in 5KR of gamma rays treatment (91.39) at lower doses. The minimum number of leaves was observed in 50kr of gamma rays treatments (32.25) as in (Table-2).

Table-1: Effect of gamma rays on seed germination of *Withania somnifera* (L.) Dun

Treatment dose of gamma rays	Total number of seed sown	Seed germination (%)	Seedling survival (%)
Control	100	83.75	80.71
05KR	100	77.21	71.58
10KR	100	72.55	65.85
15KR	100	67.39	59.53
20KR	100	58.35	55.26
25KR	100	51.32	50.12
30KR	100	45.69	43.72
35KR	100	36.62	30.89
40KR	100	28.42	21.81
45KR	100	15.13	10.36
50KR	100	08.45	05.61

Table-2: Effect of gamma rays on morphological characters of *Withania somnifera* (L.) Dun in R_1 generation

Treatment dose of Gamma rays	Days to first flower (days)	Plant height (Cm)	Number of branches per plant	Number of leaves per plant	Number of berries per plant	Seed yield per plant (gm)	Root yield per plant Fresh weight (gm)
Control	55.08±1.65	71.55±2.14	5.94±0.18	95.49±2.86	53.84±1.61	6.86±0.20	15.87±0.47
5KR	55.20±1.65	69.12±2.07	5.48±0.16	91.39±2.74	52.33±1.57	5.79±0.17	14.36±0.43
10KR	58.99±1.77	68.28±2.05	4.89±0.14	89.55±2.69	51.65±1.55	5.59±0.16	13.29±0.39
15KR	60.27±1.80	66.13±1.98	4.56±0.13	87.30±2.61	50.88±1.53	4.36±0.14	11.85±0.35
20 KR	61.91±1.85	62.44±1.87	4.18±0.12	84.02±2.52	49.56±1.49	4.32±0.13	10.07±0.30
25 KR	63.15±1.89	60.40±1.81	3.70±0.11	80.19±2.40	43.30±1.29	3.96±0.12	09.43±0.28
30 KR	65.49±1.96	57.34±1.72	3.57±0.10	76.30±2.28	40.49±1.21	3.76±0.11	07.68±0.23
35KR	70.92±2.13	54.77±1.64	3.32±0.09	72.14±2.16	36.53±1.09	2.63±0.07	06.13±0.18
40KR	71.47±2.14	49.13±1.47	3.11±0.09	68.87±2.07	32.59±0.98	2.50±0.07	05.28±0.15
45KR	73.73±2.21	41.32±1.24	2.90±0.08	46.53±1.39	25.61±0.77	1.30±0.04	04.19±0.12
50KR	74.50±2.23	36.73±1.10	2.53±0.07	32.25±0.96	20.14±0.60	1.05±0.03	03.54±0.10

Number of berries per Plant

Ashwagandha a gradual reduction in number of berries per plant was observed in high to lower dose of gamma ray treatments compared with control. The maximum number of berries was observed in 5KR of gamma rays treatment (52.33). A minimum number of berries were observed in 50 KR of gamma ray treatment (20.14), (Table-2).

Seed yield per plant (gm)

In M_1 generation seed yield was gradually decreased with high to lower dose of gamma rays. The physical mutagen of gamma rays induced in seed treatments. The gamma radiation with low wavelength with high penetrable power affected the seed yield. The lower dose of gamma rays gradually increased in seed yield. While the high dose of gamma rays treatment was gradually decreased in seed yield parameters. 5KR of gamma rays treatment (3.79 gm) and 50KR of gamma rays treatment (2.25gm) were observed (Table-2).

Root yield per plant (gm) (Fresh weight)

The root yield per plant (fresh weight) was measured in grams. There was a gradual decrease in root yield when the dose of gamma rays increases. The maximum yield was observed in 5KR (14.36 gm). The minimum yield was observed in 50KR (03.54 gm) as given in (Table-2).

In conclusion, the effect of gamma irradiation the seed germination, seedling survival, days to first flower, plant height, number of branches per plant, number of leaves per plant, number of berries per plant, seed yield per plant (gm) and root yield per plant (fresh weight (gm)) were gradually decreased from lower to higher doses of gamma rays treatment. Then particular dose was gradually decreased especially 20, 25 and 30KR of gamma rays. The highest germination percentage and seedling survival were observed at lower dose of gamma rays when compared to other doses. The LD_{50} value for 50 per cent reduction of seed germination, (LD_{50} lethal dosage), seedling survival was observed in 25KR of gamma rays treatments.

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