RESEARCH ARTICLE Agricultural Science Digest, Volume 42 Issue 3: 260-265 (June 2022)

Physio-biochemical Parameters of Ajwain (*Trachyspermum ammi* L.) Induced by Gamma Rays and EMS

Saima Malik, Roshan Jahan, Shazia Bi Ansari, Ruhul Amin, Samiullah Khan

10.18805/ag.D-5442

ABSTRACT

Background: Induced mutagenesis has proved as a crucial implement to create genetic variability for various essential traits. *Trachyspermum ammi* is one of the most important medicinal seed spices and its oil exhibits various pharmacological properties. Ajwain has been overlooked owing its narrow genetic base and little study has been performed to create genetic variations.

Methods: Dry and healthy seeds of ajwain were treated with the different concentration of gamma rays (25, 50, 75 and 100Gy), EMS (0.1%, 0.2%, 0.3% and 0.4%EMS) and different doses of combination treatments (25+0.1%, 50+0.2%, 75+0.3% and 100Gy+0.4%EMS). Among both the mutagens, gamma rays were found to be more effectual mutagens as compared to EMS.

Result: The aim of the present study was to find out the mutagenic consequences of gamma rays, EMS and combination treatments on growth and physio-biochemical parameters of ajwain. Studies show that higher doses of both the mutagens caused significantly negative effect on the growth parameters whereas lower doses have positively influenced the parameters. Result shows lower doses of mutagens (25, 50Gy, 0.1%, 0.2%EMS and 25+0.1%, 50Gy+0.2%EMS) proved to be more effective as it caused less biological damage and therefore would be suitable for inducing the desirable mutations and improving the agronomic traits in ajwain.

Key words: Ajwain, EMS, Gamma rays, Induced mutagenesis, Physio-biochemical parameters.

INTRODUCTION

Since time immemorial, the drugs made from the plant kingdom have been used to alleviate or treat human diseases. The indigenous system of medicine is gradually gaining popularity day by day due to lesser or no noxious side effects of herbal drugs. In the world, almost two-thirds of the plant species have medicinal value and their different parts like stem, leaves, seeds, bark and fruits are used for the treatment of various diseases (Amin *et al*. 2019). According to the World Health Organization (WHO) majority of the human being, for primary health care chiefly depends on herbal drugs as well as traditional medicine. Approximately, more than hundred plants-based drugs have been introduced in markets and provide a valuable contribution to current therapeutics (Zehra *et al*. 2019). India is the land of spices with spice, herbs contributing a significant portion of the country's economy and because of the valuable pharmaceutical properties of its oil, they have a vital role in the humankind's life (Malik *et al*. 2019).

One of the most prominent Indian spice is *Trachyspermum ammi* L, commonly known as ajwain which belongs the family Apiaceae. It is an annual cross-pollinated, diploid crop (2n=2x=18) (Joy *et al*. 2001). The plant has profuse branches, erect stem and 2-3 pinnately divided tender leaves (She *et al*., 2005) and can widely grow in arid and semi-arid regions (Joshi, 2002). Ajwain is famous throughout the world for its medicinal and commercial value and in India; it is used as traditional ayurvedic medicine. Thymol (35-60%) is a chief constituent of essential oil of ajwain which exhibiting various pharmacological activities (Kumar and Dwivedi, 2015). Pertaining to the presence of thymol and non-thymol content in ajwain oil, a large amount of work has been done on antimicrobial activity, antiseptic, antitussive and expectorant properties in ajwain (Chahal *et al*. 2017).

In recent years, induced mutation is an immensely potent tool for crop improvement and day by day, it is gaining popularity to overcome reduction in yield of the crops caused by the global climatic changes such as drought, storms, floods, salinity and rise in sea level which are highly problematic and thus use of mutagens (physical and chemical) are increasingly being used to prevail over these problems (Takeda and Matsuoka, 2008). In the development of new varieties showing enhanced specific traits without changes in the original genetic makeup of the cultivar, induced mutagenesis has played a significant role (Khursheed *et al*. 2018). In very short period of time, mutagenesis is most beneficial method to enhance the polygenic variability of crop plants (Jahan *et al*. 2021). The developed mutant varieties have been cultivated by the

Department of Botany, Aligarh Muslim University, Aligarh-202 002, Uttar Pradesh, India.

Corresponding Author: Saima Malik, Department of Botany, Aligarh Muslim University, Aligarh-202 002, Uttar Pradesh, India. Email: saimamalik.amu@gmail.com

How to cite this article: Malik, S., Jahan, R., Ansari, S.B., Amin, R. and Khan, S. (2022). Physio-biochemical Parameters of Ajwain (*Trachyspermum ammi* L.) Induced by Gamma Rays and EMS. Agricultural Science Digest. 42(3): 260-265. DOI: 10.18805/ag.D-5442.

Submitted: 07-07-2021 **Accepted:** 06-10-2021 **Online:** 26-11-2021

farmers in a large area, contributing to food security and overall boost in food production (Amin *et al*. 2019). In crop improvement, selection of suitable doses of mutagens is a significant challenge for inducing maximum variation in particular plant and variety. LD_{50} dose is an optimum dose for mutation induction, but the frequency of mutation arising in plants depends on the higher and lower doses of mutagen (Bashir *et al*. 2013). Amongst physical mutagen, gamma rays are the most accessible mutagen due to its high mutation frequency and good penetration property (Sarada *et al*. 2015), but in case of chemical mutagens, particularly EMS (Ethyl methane sulphonate) are widely used for the improvement of various traits of the crop (Verma *et al*. 2017). EMS is an alkylating, most potent, effective mutagen and generally causes high frequency of gene mutations and low frequency of chromosome aberrations in plants (Bashir *et al*. 2013). The main aim of the present study is to assess the mutagenic effect of gamma rays and EMS on the growth, physiological and biochemical parameters of ajwain.

MATERIALS AND METHODS

Germplasm and mutagen treatment

Seed samples of the *Trachyspermum ammi* var. AA1 was procured from the National Research Center of Seeds, Spices (NRCSS), Rajasthan, India. A set of 300 healthy and dried seeds of ajwain were used separately for each treatment along with control. For experimental treatment using both physical and chemical mutagen and in physical mutagen exposure was performed by irradiation with different doses *viz*., 25, 50, 75 and 100Gy of gamma rays from Gamma chamber-5000 with a radioisotope 60Co (cobalt-60) at Indian Agricultural Research Institute (IARI), Pusa Campus, New Delhi, India. For chemical treatments, seeds were pre-soaked for 6 hours and treated with different doses of ethyl methane sulphonate (EMS) *viz*, 0.1%, 0.2%, 0.3% and 0.4% EMS at pH 7 for 6 hours at room temperature 25±2°C. Untreated pre-soaked seeds were used as control.

Determination of seed germination, plant survival, pollen fertility and seedling height

Seed germination

Germination percentage of treated as well as untreated seeds was recorded on the basis of the total number of seeds sown in field.

$$\text{Germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{No. of seeds sown}} \times 100$$

Plant survival

In different treatments and control, plant survival was estimated at the time of the maturity and the survival was computed as percentage of the germinated seeds in the field.

$$\text{Percentage inhibition/ Percentage reduction} = \frac{\text{Control- treated}}{\text{Control}} \times 100$$

Pollen fertility

Pollen fertility was determined for each treatment and control at the time of flowering. Pollen grain were stained with 1% acetocarmine solution and pollen fertility was determined. Pollen grains which retain the stain and showing regular outline were considered as fertile, while unstained and sunken were sterile.

Seedling height

In laboratory conditions, seedling height (root+ shoot) were recorded on 30 randomly selected seedlings of each treatment and control on 14th day after sowing.

Physio-biochemical parameters

Total chlorophyll and carotenoid content

By the method of MacKinney (1941), chlorophyll and carotenoid content of the leaves were estimated. For chlorophyll and carotenoid extraction, 1 g fresh leaf tissue of each treatment along with control were grounded separately by using a mortar and pestle containing 80% acetone and centrifuged the mixture at 1500 rpm for 5 minutes. The supernatant was collected in a volumetric flask making the volume to 100 ml. The absorbance of the solution (chlorophyll and carotenoid extraction) was recorded at 663 nm and 645 nm, 510 nm and 480 nm respectively. The total chlorophyll and carotenoid content was calculated by using the following formula:

Total chlorophyll (mg g^{-1} leaf fresh mass) =

$$\{20.2\,(OD_{645}) + 8.02\,(OD_{663})\} \times \frac{v}{1000 \times w}$$

Carotenoid (mg g^{-1} leaf fresh mass) =

$$\frac{7.6\,(OD480) - 1.49\,(OD510)}{d \times 1000 \times w} \times V$$

Carbonic anhydrase activity (CAA)

In fresh leaf carbonic anhydrase activity was measured using Dewivedi and Randhawa (1974) method. Briefly, 200 mg of the fresh leaf piece were weighed and incubated in petriplates containing 10 ml of 0.2 M cysteine hydrochloride solution for 20 minutes at 4°C. To each petriplates 4 ml of 0.2 M sodium bicarbonate solution and 0.2 ml of 0.002% bromothymol blue were added and the reaction mixture was titrated against 0.05 N HCl using methyl red as an indicator. CAA activity was then estimated.

RESULTS AND DISCUSSION

Effect of mutagens on seed germination and plant survival

Data pertaining to seed germination and survival of ajwain under different doses of gamma rays and EMS in comparison to control is shown in Fig 1. Results shows, consistent decrease in seed germination and survival at higher doses of both mutagens, indicated higher doses had adverse effect

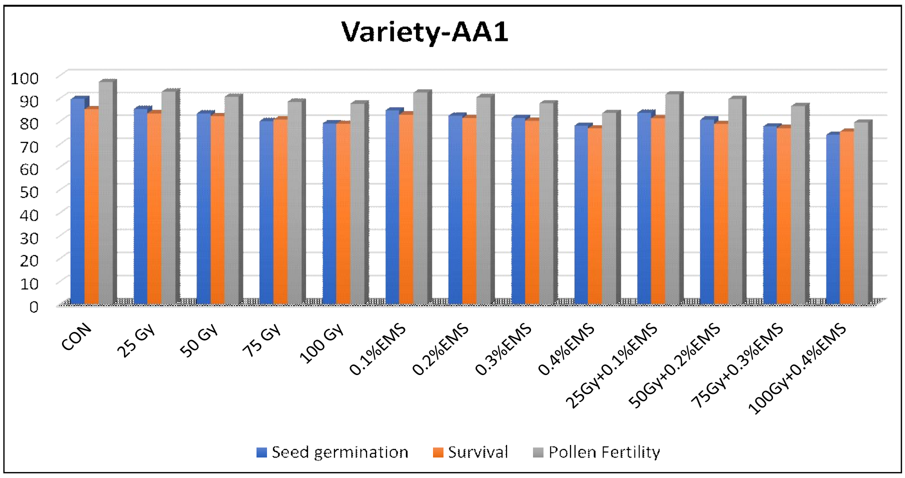

Fig 1: Effect of different doses of physical (gamma rays), chemical (EMS) and combination treatment (gamma rays + EMS) on seed germination, plant survival and pollen fertility of *Trachyspermum ammi* (ajwain) in M_1 generation.

as compared to control. Maximum germination and survival percentage were recorded as 85.33% (25Gy) and 83.42% (25Gy) while lowest as 74.00% (100Gy+0.4 EMS) and 75.43% (100Gy+0.4% EMS) respectively. Among different mutagenic treatments, maximum reduction in seed germination and survival percentage was observed at the higher dose of EMS as compared to gamma rays. Reduction in seed germination and survival was observed to be dose dependent in various umbelliferous crops like fennel (Verma *et al*. 2017) and coriander (Sarada *et al*., 2015; Salve and More, 2014; Singh *et al*. 1992). Similar effects lead to reduction in seed germination and survival percentage with increasing doses of mutagens were also found in fenugreek (Bashir *et al*. 2013 and Basu *et al*. 2008). Sikdar *et al*. (2013) and Bhosale and Mose (2013) reported similar results in tomato and in *Withannia somnifera* respectively. According to Chowdhury and Tah (2011) by using mutagens reduction in seed germination occur due to the alteration in enzyme activity and damage of cell constituent at molecular level. Several researchers also suggested that lower concentrations of mutagens give desirable mutation due to less biological damages. Lal *et al*. 2009, reported that seed germination, survival and seedling growth are some commonly use parameters to know the sensitivity of mutagens in plants.

Pollen fertility

In control plants, the pollen fertility was recorded as 97.12% whereas, in treated plants, the fertility ranged between 92.86% (25Gy) to 79.46% (100Gy+0.4%EMS). Maximum reduction in pollen fertility was observed in combination treatment (100Gy+ 0.4%EMS) whereas, minimum reduction in pollen fertility was observed in 25Gy (Fig 1). According to Sikder *et al*. (2013) in tomato plant, reduction in pollen fertility was found to be highest in EMS treatment followed by gamma irradiation. Muthusamy and Jayabalan (2002), also suggested that reduction in pollen fertility with increase in the doses of mutagenic treatments may be attributed to increase in chromosomal aberration as well as physiological damage.

Shoot and root length

In general, reduction in root and shoot length depends on the concentrations of the mutagens means as the concentration of mutagens increases the length of root and shoot was decreased. Among the treatments, 25Gy recorded the maximum shoot (6.18 cm) and root length (4.76 cm) respectively as compared to the other concentrations, whereas maximum reduction in shoot (3.46 cm) and root length (3.08 cm) was observed in combination treatment (100Gy+0.4%EMS). In control, the shoot and root length were recorded as 7.08 cm, 5.16 cm respectively (Fig 2). Sarada *et al*. (2015) reported that reduction in shoot and root length in coriander depends on the concentrations of the mutagens so that, the mutagens concentration increases reduction in the shoot and root length. In fenugreek, Bashir *et al*. (2013) reported that reduction in shoot and root length was more in EMS treatments followed by gamma irradiation. Evan and Sparrow (1961), reported that the chromosomal damage and inhibition of cell division are the major causes of reduction in seedling growth (root and shoot length).

Physio-biochemical parameters

Study of the physio-biochemical parameters are considered as an important indicator for validating the genetic variation and selection of superior mutant genotype for breeding purpose.

Chlorophyll, carotenoids and carbonic anhydrase activity

Chlorophyll, carotenoid and carbonic anhydrase activity (CAA) in ajwain plant showed enhancement in lower concentration while in higher concentration it decreased. In control population, chlorophyll contents (1.52 mg-g^{-1} FW), carotenoid content (0.39 mg-g^{-1} FW) and carbonic anhydrase activity (195.07 µmol CO^2 kg^{-1} FWs^{-1}) were recorded. As compared to gamma rays and EMS combined treatment population shows a drastic reduction in chlorophyll, carotenoid and CAA activity. Higher chlorophyll and carotenoid activity were found at 25Gy (1.53 mg-g^{-1} FW), (0.40 mg-g^{-1} FW) respectively similarly, carbonic anhydrase activity (CAA) was higher in 50 Gy (194.26 μmol CO_2 kg^{-1} FW s^{-1}) (Table 1). According to Tomlekova *et al.* (2009), the enhancement in chlorophyll-a and β-carotenoid contents were mainly responsible for rise in total chlorophyll and carotenoid contents in the mutants.

Morphological abnormalities in cotyledonary leaves

A broad spectrum of cotyledonary leaves abnormalities was observed in mutagen-treated plants through the modification in number and morphology of cotyledons. Uni- seedling (one cotyledonary leaf), tri-seedling (one extra cotyledonary leaf) and a bifurcate cotyledon were recorded in both higher and

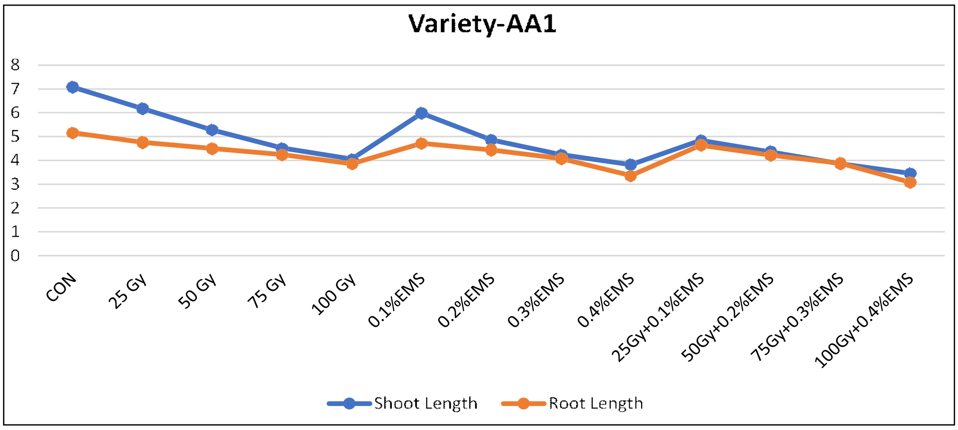

Fig 2: Effect of different doses physical (gamma rays), chemical (EMS) and combination treatment (gamma rays + EMS) on seedling height of *Trachyspermum ammi* (ajwain) in M_1 generation.

Fig 3: Cotyledonary leaves abnormalities in control and treated plant of *Trachyspermum ammii* L.
a- Normal Cotyledon, b- Tricotyledon c- Monocotyledon and d- Bifurcate.

Table 1: Effect of different doses of physical (gamma rays), chemical (EMS) and combination treatment (gamma rays + EMS on physio-biochemical parameter of *Trachyspermum ammi* L. (ajwain) in M_1 generation.

Treatment	Chlorophyll (mg-g^{-1} FW) Mean±S.E	Carotenoids (mg-g^{-1} FW) Mean±S.E	Carbonic anhydrase (CAA) (μmol CO_2 kg^{-1} FW s^{-1}) Mean±S.E
Con	1.52^{abc}±0.0093	0.39^{a}±0.0058	195.07^{a}±0.6927
25Gy	1.54^{a}±0.0051	0.40^{a}±0.0051	193.01^{b}±0.7585
50Gy	1.50^{bcde}±0.0102	0.36^{b}±0.0086	194.26^{ab}±0.6129
75Gy	1.48^{ef}±0.0051	0.34^{cd}±0.0051	193.68^{ab}±0.4554
100Gy	1.47^{f}±0.0051	0.31^{ef}±0.0058	190.14^{de}±0.5636
0.1%EMS	1.50^{cde}±0.0070	0.36^{b}±0.0121	192.52^{bc}±0.6522
0.2%EMS	1.51^{bcd}±0.0070	0.35^{bc}±0.0075	192.36^{bc}±0.6153
0.3%EMS	1.48^{def}±0.0086	0.33^{de}±0.0074	190.24^{de}±0.7366
0.4%EMS	1.45^{g}±0.0070	0.28^{gh}±0.0109	188.64^{ef}±0.5671
25Gy+0.1%EMS	1.52^{ab}±0.0067	0.34^{cd}±0.0092	190.78^{cd}±0.5276
50Gy+0.2%EMS	1.47^{fg}±0.0070	0.30^{f}±0.0068	189.88^{de}±0.4465
75Gy+0.3%EMS	1.47^{fg}±0.0051	0.29^{fg}±0.0071	187.10^{fg}±0.7720
100Gy+0.4%EMS	1.42^{h}±0.0092	0.26^{h}±0.0071	186.42^{g}±0.6666

lower doses of gamma rays and EMS. In control plants which shows normal dicotyledonary leaves (Fig 3). Similar results were also reported by some researchers in various crops like in black cumin (Tantray *et al*. 2017) and linseed (Jahan *et al*. 2020).

CONCLUSION

Ajwain is most eminent spice with innumerable medicinal properties. Amongst both the mutagens, gamma rays were found to be more effective as compared to the EMS. The present experiment showed the effect of mutagens (gamma rays and EMS) on the growth and Physio-biochemical parameters of ajwain in M_1 generation. Result showed lower and moderate doses of gamma rays and EMS were found to more effective and efficient to produce more positive deviation in growth and physio-biochemical parameters as compare to higher concentrations.

ACKNOWLEDGEMENT

Authors are thankful to the Chairman, Department of Botany, Aligarh Muslim University, Aligarh for providing research facilities and Indian Agricultural Research Institute (IARI), New Delhi. I also gratefully acknowledge to Dr. S.S. Meena, National Research Centre on Seed Spices (NRCSS), Rajasthan for providing seeds.

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