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DEVELOPMENT AND CHARACTERIZATION OF GAMMA IRRADIATED MUTANTS OF *Trichoderma* SPECIES, ISOLATED FROM APPLE RHIZOSPHERE OF NORTH-WESTERN HIMALAYAN REGION OF INDIA

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ABSTRACT

Six 10-day-old cultures of *Trichoderma harzianum, T. viride* and *T. asperellum* were irradiated with cobalt-60 γ-radiation @ 0.25 Gy min^{-1} (0-500 Gy) at BARC-Zakura, Srinagar, J&K, and a total of 60 mutants obtained. The mutants were assessed for stability up to seven generations based on morphocultural characteristics. Almost all the characters in mutants and wild types varied significantly. The mutants were divided into five groups based on their colony texture (cottony, fluffy, velvety, woolly and granular), two groups based on margins (regular or irregular), four groups based on colony colour (dark green mycelia with a white centre, light green mycelia with a white centre, green with a dull white centre, and blackish green with a white centre) and four groups based on colony shape. Spore characters also categorized them into four groups. Colony diameter ranged from 42.00 to 89.55 mm with maximum in mutant F9 (89.55 mm), followed by F10 (85.22 mm), A9 (83.00 mm) and A7 (81.00 mm) with a growth rate of 3.315, 2.747, 2.819 and 2.90 mm h^{-1}, respectively, after 2 days incubation at 28±1°C. Mycelial dry weight ranged from 47.41 to 348.18 mg with maximum in F10, followed by F9 (284.64 mg) and A7 (263.43 mg). Spore germination ranged from 0.0 to 7.69% with maximum in F9, followed by D2 (7.23%). Maximum CFU (21.5) was observed on PDA in mutant F9, followed D0 (20.5) and D4 after 24 h incubation at 28±1°C. The maximum length: breadth ratio of spores (1.7) was observed in F9, followed by A8 (1.62) and A6 and A9 (1.61). Variation in phialide shape was also noticed in these mutants as compared to their wild ones. The mutants F9, A7 and A9 belonging to *T. asperellum* were more efficient and stable than other mutants and wild types.

Keywords: Biological control, mutagenesis, *Trichoderma* species, morpho-cultural characterization

INTRODUCTION

Plant diseases caused by seed and soil-borne fungal pathogens adversely impact the crop productivity. Although chemical strategies are widely used to combat plant diseases, their future use is constrained

by the emergence of pesticide-resistant strains, the deregistration of certain pesticides, and growing public concerns over the potential adverse impacts of agrochemicals on both human health and environment (Heimpel and Mills, 2017). A range of chemicals is hoarded in agricultural soil due to their continuous use in intensive farming (Tudi *et al.*, 2021; Chauhan *et al.*, 2023a,). Carbendazim, which has a half-life of 3-12 months and does not percolate into the soil due to its strong adsorption to soil like other pesticides (Chauhan *et al.*, 2023b).The continuous and rigorous planting of crops in the same farming fields can lead to ecosystems that are susceptible to disease outbreaks. Up to 50% of plant output might be lost as a result of the disease's rapid spread (Rai *et al.*, 2024).

Biological control, which simply refers to a strategy for plant protection that employs biological control agents (BCAs) to manage the pest populations, including diseases. One of the most commonly used BCAs is *Trichoderma* whose different species are known for their effective biocontrol properties and positive plant interactions (Nawrocka and Małolepsza, 2013). *Trichoderma* genus is widely distributed and found in almost all soil types and acts as a mycoparasite, saprotroph, and plant symbiont. Some species are effectively used for the management of various plant diseases and to produce industrial enzymes (Benítez *et al*., 2004). *Trichoderma* has emerged as one of the prominent biocontrol agents due to its ability to suppress phytopathogenic fungi through both direct and indirect mechanisms (Sumaira Hamid *et al.*, 2024). The commercial success of *Trichoderma*-based BCAs may be because of all the factors responsible for its ability to perceive and suppress or kill other fungi, resistance to exogenous toxins, and priming plant immunity against plant pathogens (Mukherjee *et al.*, 2013). Nonetheless, despite a comprehensive study on *Trichoderma*-based biocontrol agents and their vast and exceptionally promising applications, the mechanism of biocontrol is not fully understood. Inconsistent efficiency in the field confines the broader use of BCAs and has kept research gaps to understand the underlying mechanisms fully. Concurrently, several strategies can enhance the BCA's antagonistic capabilities. One among such strategies is mutagenesis, which diversifies the genetic makeup of targeted organism. Depending on the mutagenesis method, the induced changes can either be targeted or random. Through effective screening and analysis of mutants obtained, the traits associated with biocontrol can be improved significantly (Alfiky, 2019). Gamma radiation is one of the mutagenic tools that is widely used in fungi, including *Trichoderma* species, for enhancing their beneficial traits, inducing mutations by altering DNA structures, which leads to genetic variability that can be used for enhancing biocontrol abilities, enzymatic activity, and stress tolerance (Mohamadi *et al.*, 2014). Gamma rays are electromagnetic waves with high energy that cause point mutations, rearrangements, or deletions in fungal DNA, which leads to increased genetic diversity (Manici *et al.*, 2018). The efficiency of mutation relies on the radiation dose; high doses can lead to growth defects or lethal mutations, while low doses can improve biocontrol traits (Sharma, 2020). Mutations can increase the efficiency of fungal secondary metabolite biosynthesis, enzyme production, and stress resistance (Baharvand *et al.*, 2014). This technique has been efficiently used to improve the fungal adaptability, allowing improved antagonistic potential and better rhizospheric competition against plant pathogens (Dutta *et al.*, 2023).

Trichoderma mutants irradiated with γ rays have shown increased production of protease, cellulase, and chitinase, which helps in pathogenic cell wall degradation (Rostami *et al.*, 2024). Various mutants show enhanced rhizospheric competence by exhibiting higher colonizing ability in the rhizosphere, outcompeting soil-borne pathogens (Singh *et al.*, 2013). Certain *Trichoderma* mutants exhibit induced systemic resistance (ISR) in plants by triggering plant defense mechanisms to reduce pathogenic susceptibility (Dutta *et al.*, 2023). *Trichoderma* mutants have shown higher resistance to extreme temperatures, drought, and salinity, which makes them more efficient in agricultural practices (Fazeli-Nasab *et al.*, 2022). Gamma radiation is being used in breeding superior biocontrol strains for commercial biopesticides. Improved *Trichoderma* mutants have been combined with bioformulations to combat fungal pathogens in crops like rice, wheat, and other field and fruit crops. This technique promotes soil health improvement and sustainable disease management by offering an eco-friendly alternative to chemical fungicides (Manzar *et al.*, 2022). The development of γ-irradiated mutants in *Trichoderma* species is lacking in isolates from the apple rhizosphere;

therefore, the present study was aimed to develop and characterize γ-irradiated mutations in *Trichoderma* species isolated from the apple rhizosphere of Jammu and Kashmir, India.

MATERIALS AND METHODS

Collection of Trichoderma species

Six strains of *Trichoderma* belonging to different species *viz., Trichoderma harzianum, T. viride* and *T. asperellum* isolated from apple rhizosphere were obtained from the Plant Virology and Molecular Pathology Laboratory, Division of Plant Pathology, SKUAST-Kashmir. The biocontrol cultures were purified by single-spore technique on *Trichoderma* specific medium (TSM) [Tuite, 1969]), and the purified colonies were maintained on potato dextrose agar slants (HiMedia, Mumbai) till further use.

Induction of mutation for mutant development

The mutation was induced by γ-radiation at the Bhabha Atomic Research Center, Zukura, Srinagar, Kashmir, following a standardized procedure (Gadgil *et al.*, 1995a). The seven-day-old cultures of *Trichoderma* species were irradiated with cobalt-60 γ-irradiation @ 0.25 Gy sec^{-1}. The applied dose rates were 0, 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 Gy. The obtained mutants (60) and 6 wild types were transferred to PDA slants and incubated at 25±1°C. These cultures were maintained in a refrigerator (4°C) till use.

Morphological characterization

The *Trichoderma* mutants, along with their wild types, were grown on PDA medium. A 5-6 mm mycelial disc from a 7-day-old culture of each mutant and wild type was aseptically obtained with the help of a sterilized cork borer from an actively growing culture and transferred to the centre of 90 mm Petri-plates containing PDA medium, and incubated at 28±1°C.

Colony characteristics, such as texture, margins, colour, and shape, were recorded during a 5-day incubation period. Each wild and mutant strain's conidial morphology, including septation, shape, colour, and size (width and length), was examined under a microscope model CX31 (Olympus, Tokyo, Japan) using spore suspension from a 7-day-old culture. After two days incubation, the phialides formation was observed, and after ten days of incubation at 28±1°C, the presence or absence of chlamydospores (Fig. 4) was recorded. For colony-forming units (CFU), the spore suspension with a concentration of 10^{-7} spores mL^{-1} was aseptically spread on the Petri-plates containing PDA and TSM in a laminar airflow cabinet and incubated at 28±1°C for 48 h, and then colonies present were counted. The per cent spore germination of *Trichoderma* mutants and wild types was observed in spore suspension at 10^{-6} mL^{-1} and incubated at 28±1°C. The observations on spore germination were recorded for each mutant and wild type after every three hours under a microscope with, 25 to 30 microscopic fields to for minimizing the error.

Cultural characteristics

A 5-6 mm mycelial disc from *Trichoderma* wild and mutant cultures were obtained from 7 days old culture and inoculated on Petri-plates containing PDA medium at 28±1°C for 3 consecutive days. The cultural characteristics namely mycelial dry weight, growth rate and colony diameter were recorded after every 24 h and growth rate calculated in mutant and wild type cultures for comparison. The experiment was conducted under aseptic conditions in a completely randomized design with each treatment replicated five times. To determine the mycelial dry weight, 5 mm mycelial discs of each *Trichoderma* mutant and parent were inoculated into potato dextrose broth and incubated for 10 days at 28±1°C. The mycelium was harvested on pre-weighed and pre-sterilized Whatman No. 1 filter paper. The Whatman No. 1 filter paper containing mycelium was dried for 3 consecutive days at 60°C for 1 h in a Thermotech hot air oven (MAC-230, Punjab Biotechnology, Chandigarh, India) until constant weight was attained. A digital weighing balance was used to record the mycelial weight**.**

RESULTS AND DISCUSSION

Induction of mutation

The induction of mutation in *Trichoderma* isolates at ten different doses *viz.*, 0, 50, 100,150, 200, 250, 300, 350, 400, 450, and 500 Gy was achieved by irradiating 10-day-old sporulated cultures. The irradiation dose treatments resulted in significant changes in morphocultural characteristics of obtained mutants as compared to their wild-type cultures. These mutants were tested for stability up to seven generations by sub-culturing on PDA medium.

Morpho-cultural characterization

Trichoderma mutants belonging to different species *viz., T. harzianum*, *T. viride* and *T. asperellum* showed considerable differences in colony characters, such as colour, texture, shape, and margins, as compared to *Trichoderma* wild types (Table 1; Fig. 1). The mutants of *Trichoderma* were divided into

Table 1: Grouping of *Trichoderma* mutants and wild type based on morphological characteristics

Group	Colony characteristics[$]			
	Texture	Margins	Colour	Shape
I	Cottony (18.18%) A9, C1, C2, C3, C4, C5, C6, C7, C8, C9, & C10	Regular (87.88%) A0, A1, A3, A4, A6, A7, A9, A10, B0, B1, B2, B3, B5, B6, B7, B8, B9, B10, C0, C1, C2, C3, C4, C5, C6, C7, C8, C9 C10, D0, D1, D2, D3, D4, D5, D6, D7, D8, D9, D10. E0, E2, E3, E4, E5, E6, E7, E8, E9, E10, F1, F2, F3, F5, F6, F7, F8, & F9	Dull white centre with light green outer region (13.63%) A9, B1, B5, B10, D10, E4, E5, F5, & F6	White mycelia at centre with dense green conidial production dispersed near margins in 1-2 concentric rings along with dark green or amber pigmentation (48%) A1, A3, A6, B1, C0, C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, E0, E1, E2, E3, E4, E6, E7, E8, E9, E10, F1, F3, F5, F6, F7, F8, & F10
II	Fluffy (33.33%) B0, B1, B2, B3, B4, B5, B6, B7, B8, B9, B10, D0, D1, D2, D3, D4, D5, D6, D7, D8, D9, & D10	Irregular (12.12%) A2, A5, A8, B4, E1, F0, F4, & F10	White centre with dark green outer region (39.41%) A1, A3, A4, A6, A7, A10, C0, C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, E1, E6, E7, E9, E10, F1, F3, F7, & F10	Green conidial production dense at centre than towards margins in 1-2 concentric rings with yellow pigmentation (21%) A0, A2, A7, B0, B3, B4, B7, B8, B9, E8, F0, F2, F4, & F9
III	Velvety (35.84%) A0, A1, A2, A3, A5, A6, A7, A8, C0, E0, E6, E9, F0, F1, F2, F3, F4, F5, F6. F7, F8, F9, F10	-	Dark green centre with white outer region (15.15%) A0, A2, A5, A8, E0, E8, F0, F2, F4, & F9	White mycelia with less green conidial formation in concentric rings near centre or dispersed along margins (28%) A4, A5, A8, A10, B2, B6, B10, D0, D1, D2, D3, D4, D5, D6, D7, D8, D9, D10, & E5
IV	Wooly (6.06%) E2, E4, E7, & E10	-	Dull green centre with white outer region (31.81%) B0, B2, B3, B4, B6, B7, B8, B9, E2, E3, E6, D0, D1, D2, D3, D4, D5, D6, D7, D8, & D9	White mycelial production with limited or no conidial production throughout plate (3%) A9, & B5
V	Granular (9.09%) A4, A10 E1, E3, E5, & E8	-	-	-

four groups based on colony colour. Group I was dark green mycelium with a white centre, while group II had light green mycelium with white centre. The group III was green with a dull white centre and group IV was blackish green with white centre. These groups accommodated 45.83, 16.67, 20.83, and 16.67% mutants, respectively (Table 1). Based on texture, the mutants were grouped into five categories as group I (fluffy), group II (velvety), group III (cottony), group IV (wooly), and group V (granular) which accommodated 33.33 (22), 35.84 (23), 18.18 (11), 6.06 (4), and 9.09% (6) isolates, respectively (Table 1). The mutants were divided into four groups based on colony shape, comprising 48, 21, 28, and 3% mutants in groups I, II, III, and IV, respectively (Table 2). Based on mycelium texture, the mutants were divided into two groups with irregular margins (group I) and with margins (group II) (Table 1).

The mutants showed a significant variation in spore germination and colony-forming units (CFU) as compared to the wild types. The percent spore germination ranged from 0.0 to 7.69% with the highest in mutant F9 at 450 Gy, followed by D2 (7.23%) at 100 Gy and A7 (6.43%) at 350 Gy using spore suspension @ 10^{-6} spores mL^{-1}. The CFU ranged from 0.0 to 21.5 with the highest in mutant F9, followed by D0 (20.5 colonies) and D5 (20.0 colonies) using 10^{-7} spores L^{-1} spore suspension (Table 3; Fig. 2).

Fig. 1: Mycelial growth of different *Trichoderma* mutants (A-F [(*Trichoderma* species); 1-10 (Gamma radiation doses: 50-500 Gy)] and their wild types (A0-F0) on potato dextrose agar medium after 7 days of incubation at 28±1ºC

Table 2: Grouping of *Trichoderma* isolates based on spore colour, shape, ornamentation and the shape of phialides

Groups	Features	Isolates[$]	Percentage
I	Bluish green, smooth walled, sub-globose to obvoid conidia having flask phialide.	A0, A1, A2, A3, A4, A5, A6, A7, A8, A9, B5, B10, D10, & E5	21.21
II	Yellow green, rough walled, globose to sub-globose conidia with slender phialide	C0, C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, D0, D1, D2, D3, D4, D5, D6, D7, D8, & D9	31.81
III	Pale green, smooth walled, globose to sub-cylindrical conidia on elongated phialides	E0, E1, E2, E3, E4, E6, E7, E8, E9, E10, F0, F1, F2, F3, F4, F5, F6, F7, F8, & F10	30.30
IV	Dull green, smooth walled, globose conidia on swollen shaped phialide	A10, B0, B1, B2, B3, B4, B6, B7, B8, B9, & F9	16.68

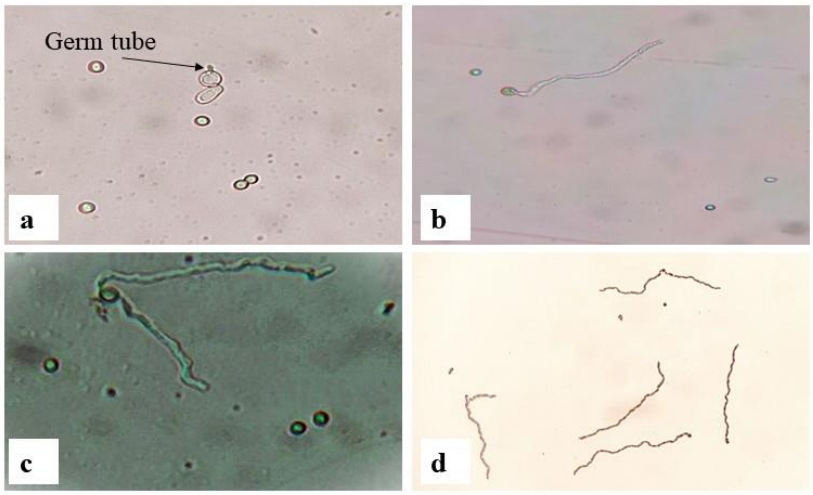

Fig. 2a: Spore germination of *Trichoderma* mutants at 10^{-6} mL^{-1} spores for 24 h at 28±1ºC; a) formation of germ tube in spores; b) germination of unipolar spore; c) germination of bipolar spore; d) germination of both unipolar and bipolar spores

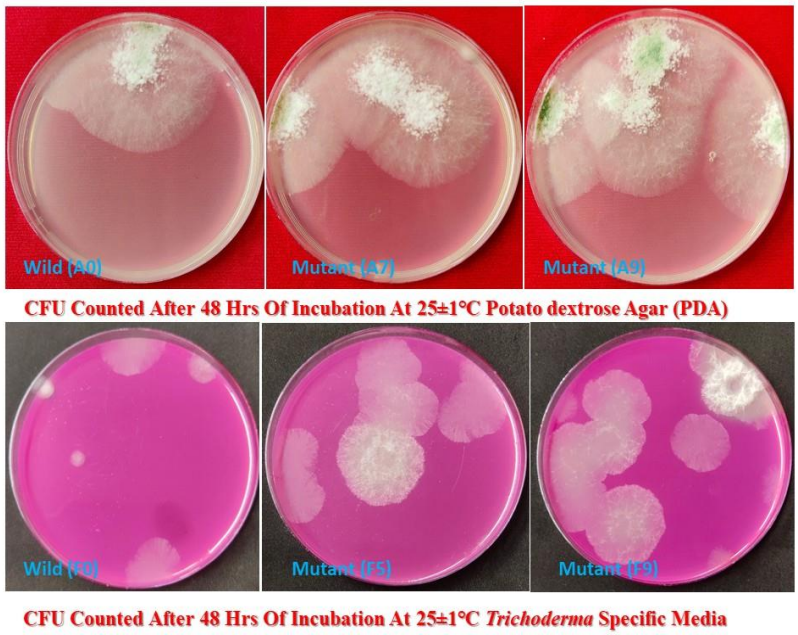

Fig. 2b: Colony-forming units of different *Trichoderma* mutants on PDA and TSM after 48 hrs of incubation at 25 ±1ºC.

Several mutants of *Trichoderma* species on PDA medium revealed considerable changes in spore characters such as colour, shape, ornamentation, and phialide formation. Based on conidial colour, four groups were formed: group I (bluish green spores), group II (pale green), group III (yellow green), and group IV (dull green), accommodating 14, 21, 20, and 11 mutants, respectively. Four groups were formed based on shape of spores: group I had sub-globose to obovoid conidia; group II with globose to sub-cylindrical; group III with globose to sub-globose; and group IV with slightly elongated to oval conidia, accommodating 21.21, 31.81, 30.30, and 16.68% of mutants, respectively. Based on the conidial orientation, the isolates were classified into two groups: group I comprised 69.70% isolates with smooth-walled conidia, and group II had 30.30% isolates with rough-walled conidia. Based on phialide shape, *Trichoderma* isolates were divided into four groups; group I had flask shaped phialides, group II with elongated ones, group III with cylindrical ones, and group IV with enlarged phialides, accommodating 14, 21, 20, and 11 isolates, respectively (Table 2; Fig. 3). However, Group II had the highest percentage of isolates (31.81%), followed by groups III (30.30%), and group I (21.21%), while group IV had the least number of isolates (16.66%).

The conidial length and width in *Trichoderma* mutants was in the range of 4.25-9.99 x 5.00-6.25 µm. The F9 mutant had largest average conidial size, measuring 9.95 µm in length, and 5.75 µm in

Table 3: Cultural characteristics of different mutants and wild types of *Trichoderma* species

Isolate/ mutant code	Colony diameter (mm)[$]	Growth rate (mm h^{-1})	Mycelial dry weight (mg)[&]	% Spore germination after 24 h	CFU 10^{-6} mL^{-1} after 48 h[#]	Conidial dimensions (µm)[*] after 7 days		
						Range (L x B)	Mean size (L x B)	L/B ratio[**]
A0	49.00	1.208	199.20	0.68	8.00	7.4-7.77 x 5.12-5.35	7.548 x 5.15	1.46
A1	47.13	1.283	150.53	2.07	8.00	7.4-7.77 x 5.25-5.55	7.66 x 5.15	1.48
A2	45.80	1.338	171.29	0.00	5.50	7.03-8.14 x 5.14-5.73	7.77 x 5.25	1.48
A3	52.60	1.317	184.29	0.44	3.50	7.4-7.77 x 5.34-5.55	7.54 x 5.4	1.39
A4	44.93	1.206	189.37	0.78	3.50	7.4-7.77 x 5.25-5.73	7.20 x 5.55	1.29
A5	43.50	1.104	183.51	1.38	3.00	7.5-8.14 x 5.55-5.75	7.63 x 5.55	1.37
A6	42.63	1.276	160.27	2.08	3.50	7.4-9.25 x 5.25-5.55	8.63 x 5.35	1.61
A7	81.00	2.903	263.43	6.43	16.50	7.77-9.25 x 5.25-5.75	8.43 x 5.55	1.51
A8	45.50	1.188	188.50	2.50	11.00	7.77-9.25 x 5.00-5.73	8.55 x 5.25	1.62
A9	83.00	2.819	260.40	1.25	17.00	7.4-9.99 x 5.00-5.75	8.88 x 5.50	1.61
A10	42.00	1.208	175.29	1.54	14.50	7.4-9.99 x 5.25-5.73	8.74 x 5.55	1.57
B0	73.80	2.158	163.38	1.90	10.50	7.4-7.77 x 5.25-5.55	7.47 x 5.45	1.37
B1	75.10	2.254	156.32	0.59	12.50	7.03-7.77 x 5.25-5.55	7.52 x 5.30	1.41
B2	72.63	2.179	165.30	0.80	8.00	7.4-7.77 x 5.14-5.65	7.54 x 5.55	1.35
B3	71.30	2.179	151.30	0.90	2.00	6.66-7.77 x 4.25-5.34	7.32 x 5.25	1.39
B4	77.13	2.256	155.50	0.64	4.50	7.03-8.14 x 5.55-5.65	7.47 x 5.45	1.37
B5	70.30	1.828	168.41	2.40	9.50	6.29-7.4 x 5.35-5.75	6.95 x 5.55	1.25
B6	75.80	2.182	125.39	1.36	13.00	7.03-7.77 x 5.25-5.55	7.40 x 5.45	1.35
B7	80.00	2.024	163.51	1.56	11.00	7.03-7.77 x 5.55-5.75	7.40 x 5.65	1.30
B8	72.30	2.258	130.38	0.94	0.00	6.66-7.4 x 5.35-5.65	7.03 x 5.55	1.26
B9	79.15	2.322	157.21	4.14	15.00	7.4-8.14 x 5.45-5.75	7.77 x 5.65	1.37
B10	78.15	2.223	171.43	1.79	14.50	5.365-8.14 x 5.55-5.75	6.73 x 5.65	1.19
C0	49.07	1.653	210.10	6.06	9.00	7.03-7.77 x 5.14-5.75	7.54 x 5.55	1.35
C1	46.67	1.571	181.33	0.66	6.50	7.03-7.77 x 5.25-5.55	7.40 x 5.45	1.35
C2	47.63	1.639	208.19	1.67	10.50	7.03-7.77 x 5.35-5.75	7.40 x 5.65	1.30
C3	62.63	2.186	181.18	0.93	12.50	7.40-8.14 x 5.14-5.55	7.77 x 5.55	1.40
C4	46.87	1.597	176.50	1.11	7.50	7.4-8.51 x 5.25-5.85	7.62 x 5.75	1.32
C5	48.77	1.557	200.40	1.88	9.00	6.66-7.4 x 5.14-5.75	7.10 x 5.65	1.25
C6	49.43	1.597	186.45	3.67	7.50	7.03-7.4 x 5.14-5.25	7.10 x 5.25	1.35
C7	51.20	1.658	179.28	0.91	7.00	7.40-7.77 x 5.25-5.75	7.47 x 5.65	1.32
C8	47.10	1.579	203.57	0.00	0.00	7.03-7.77 x 5.25-5.85	7.40 x 5.75	1.28
C9	54.10	1.714	195.45	3.29	9.00	7.40-7.77 x 5.25-5.75	7.47 x 5.75	1.29
C10	47.53	1.538	172.37	0.94	13.50	6.66-8.51 x 5.35-5.89	7.47 x 5.80	1.37
D0	73.20	2.018	155.63	3.66	20.50	7.40-8.14 x 5.25-5.55	7.84 x 5.45	1.43
D1	75.53	2.051	158.35	6.22	7.50	7.40-7.77 x 5.14-5.45	7.47 x 5.45	1.37
D2	64.53	1.913	159.11	7.23	19.50	7.40-8.14 x 5.26-5.55	7.84 x 5.45	1.43
D3	69.63	1.971	161.34	3.78	0.00	7.40-8.14 x 5.25-5.65	7.69 x 5.55	1.38
D4	74.53	2.004	142.67	3.20	20.00	7.40-8.14 x 5.25-5.75	7.84 x 5.65	1.38
D5	71.07	2.004	134.11	3.57	12.50	7.40-7.77 x 5.14-5.55	7.54 x 5.55	1.35
D6	74.40	2.051	180.23	3.19	3.00	7.40-8.14 x 5.25-5.65	7.54 x 5.65	1.34
D7	71.53	2.065	147.66	3.45	7.50	7.40-7.77 x 5.14-5.55	7.47 x 5.55	1.34
D8	71.53	1.949	157.46	5.19	12.50	7.40-8.14 x 5.45-5.75	7.77 x 5.65	1.37
D9	73.43	2.186	145.24	3.13	18.00	7.40-8.14 x 5.55-5.65	7.99 x 5.65	1.41
D10	69.40	1.981	180.20	3.17	10.00	7.40-8.51 x 5.55-5.77	7.69 x 5.65	1.36
E0	66.30	1.992	59.56	1.33	4.00	7.40-7.77 x 5.25-5.65	7.62 x 5.55	1.37
E1	60.13	1.876	90.17	0.50	8.50	7.03-7.77 x 5.55-5.75	7.32 x 5.55	1.31
E2	58.43	1.922	55.16	1.58	8.50	7.40-8.14 x 5.12-5.55	7.62 x 5.25	1.45
E3	60.93	1.917	75.41	1.03	12.00	7.40-7.77 x 5.25-5.75	7.47 x 5.65	1.32
E4	60.80	1.974	47.41	0.65	0.00	6.29-8.14 x 5.35-5.75	7.25 x 5.65	1.28
E5	49.73	1.635	92.56	3.03	2.00	7.40-8.51 x 5.25-5.75	7.77 x 5.75	1.35
E6	57.30	1.842	88.11	1.67	8.00	7.40-7.77 x 5.25-5.77	7.54 x 5.55	1.35
E7	50.43	1.513	101.56	2.22	9.00	7.40-8.14 x 5.35-5.95	7.77 x 5.85	1.32

E8	60.17	1.890	79.31	2.00	0.00	7.40-7.77 x 5.25-5.65	7.47 x 5.55	1.34
E9	49.93	1.756	197.42	1.67	5.50	7.03-8.14 x 5.35-5.98	7.54 x 5.85	1.28
E10	59.60	1.861	111.24	0.77	7.50	7.40-8.14 x 5.55-6.00	7.69 x 5.75	1.33
F0	47.07	1.281	143.16	2.40	4.00	7.40-7.77 x 5.14-5.25	7.52 x 5.22	1.44
F1	47.00	1.261	149.61	1.00	12.50	5.55-9.25 x 5.22-5.75	7.48 x 5.65	1.32
F2	58.07	1.621	148.44	2.52	16.00	7.40-8.14 x 5.34-5.77	7.62 x 5.75	1.32
F3	50.17	1.421	140.48	1.79	4.50	7.40-8.14 x 5.25-5.75	7.77 x 5.55	1.40
F4	62.53	1.779	139.16	0.87	12.00	7.03-8.14 x 5.30-5.77	7.54 x 5.65	1.33
F5	54.53	1.560	234.10	1.60	16.00	7.40-8.14 x 5.35-5.78	7.25 x 5.75	1.26
F6	48.67	1.301	158.17	2.21	14.00	5.55-8.14 x 5.25-5.65	7.14 x 5.55	1.28
F7	50.53	1.444	291.43	2.76	7.50	4.25-9.25 x 5.35-6.25	6.93 x 5.95	1.16
F8	56.77	1.565	149.35	5.60	9.00	6.66-7.77 x 5.25-5.77	7.08 x 5.55	1.27
F9	89.55	3.315	284.64	7.69	20.00	5.55-9.55 x 5.25-5.78	9.95 x 5.75	1.73
F10	85.22	2.747	348.18	3.45	10.50	6.29-7.77 x 5.25-5.77	7.32 x 5.75	1.27
$CD_{0.05}$	7.57	0.52	12.70	1.21	3.12	-	-	0.22

*Average of 25 microscopic observations (400X); **Length (L) and breadth (B) ratio; #Colony forming units; $observations recorded after 2 days of incubation; &observations recorded after 15 days of incubation

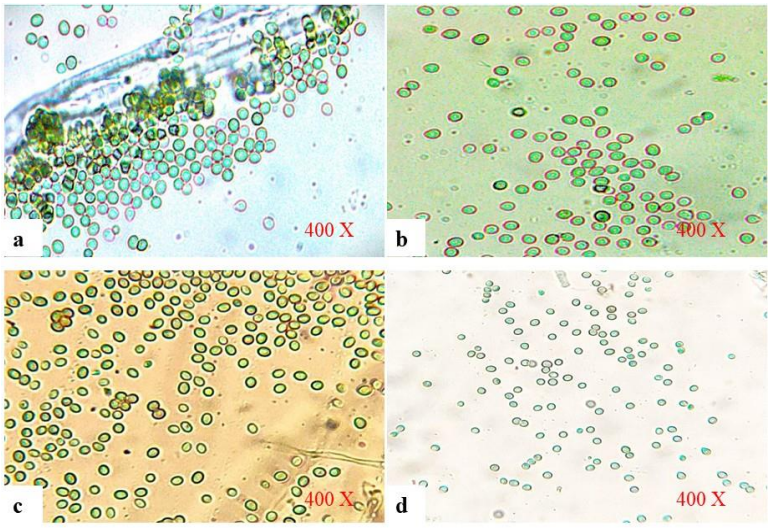

Fig. 3: Spore characteristics of *Trichoderma* mutants on PDA after seven days of incubation at 28 ±1°C; a) Bluish green, smooth walled, sub-globose to obvoid conidia having flask-shaped phialides; b) Yellow green, rough walled, globose to sub-globose conidia with slender-shaped phialides; c) Pale green, smooth walled, globose to sub-cylindrical conidia on elongated phialides; d) Dull green, smooth walled, globose conidia on swollen-shaped phialide

breadth, followed by A9 (8.88 x 5.50 µm) and A10 (8.74 x 5.55 µm) (Table 3). Mutant B10 had the lowest mean conidial dimensions, measuring 6.73 µm long and 5.65 µm wide (6.73 x 5.65 µm). Isolate F9 had the largest length-to-width ratio (1.73 µm), whereas isolate F7 had lowest ratio (1.16 µm). Conidial diameter also varied amongst *Trichoderma* mutants, ranging from 2-5 to 2-4 µm.

The growth parameters of various *Trichoderma* mutants varied significantly in terms of mycelial dry weight, colony diameter, and growth rate (Table 3). The colony diameter of mutants ranged from 42.00 mm to 89.55 mm, with mutant F9 having highest mean colony diameter (89.55 mm), followed by F10 (85.22 mm), A9 (83.00 mm) and A7 (81.00 mm). Conversely, mutant A10 had the lowest colony diameter, measuring 42.0 mm. Growth rates (mm h^{-1}) ranged between 1.104-3.315 and the mutants F9, A7 and A9, belong to *Trichoderma asperellum*, showed maximum growth rates of 3.315, 2.90 and 2.819 mm h^{-1} with better efficiency compared to other mutants and wild types. However, mutant A5 had lowest growth rate of 1.104 mm h^{-1}. It was observed that the dose rate of 450 followed by 350 Gy was found better for improved morph-cultural characteristics of *Trichoderma* species isolated from apple rhizosphere. Our findings are supported by Abbasi *et al.* (2016) who used gamma irradiations to modify *Trichoderma* spp., leading to the selection of 24 mutants. They reported variations in the morphological characteristics of *Trichoderma*, including colour, colony appearance,

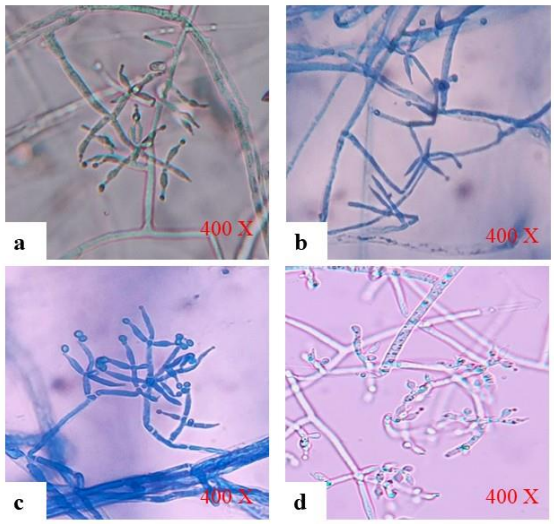

Fig. 3: Formation of phialides in different *Trichoderma* wild and mutants after 2 days incubation at 28±1ºC; a) Flask-shaped phialides; b) Elongated phialides; c) Slender-shaped Phialides; d) Swollen-shaped phialides

and sporulation, by the use of gamma radiation. Nine *Trichoderma viride* mutants were developed by chemical mutation employing hydroxylamine (HA) and ethylene methane sulfonate (EMS), followed by sub-culturing up to six generations to evaluate the durability of mutants and a significant morphological diversity in the mutants was observed as a result of mutagenesis (Vyawahare *et al.*, 2019). Mutagenesis in *T. koningii* and *T. harzianum* with 50 and 75 kilo-rad doses of gamma radiation resulted in four mutants that produced higher levels of chitinase enzyme and were stable and improved from their wild type in terms of sporulation, growth, and potential against *B. cinerea* (Haggag and Mohamed, 2002) . Gamma irradiated *T. viride* mutants were reported morphologically different from their mother culture in terms of colony colour, shape, growth rate, and sporulation (Baharvand *et al*., 2014). Morphological traits like mycelial growth rate, colony colour, shape, and sporulation were found to be altered using gamma

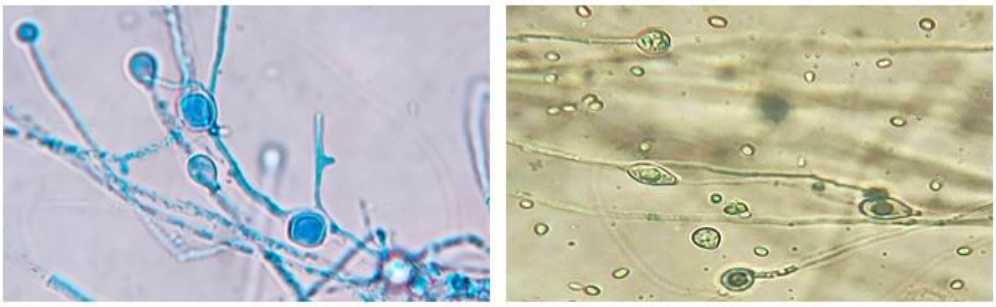

Fig. 4: Formation of chlamydospores of different shapes and sizes

irradiation (Moradi *et al.*, 2015). *T. harzianum* T334 strain-based 36 UV-induced mutants were obtained with different colony morphological traits (Szekeres *et al.*, 2007).

Conclusion: In present study 60 mutants were produced from six wild-type isolates of *Trichoderma* belonging to *T. harzianum, T. viride*, and *T. asperellum* by using γ-radiation at different doses ranging from 50-500 Gy. All the mutants showed significant variations in morpho-cultural characteristics and were grouped into different categories. Of the 10 tested dose rates, 450 Gy followed by 350 Gy proved best in improving the morpho-cultural characters. Mutants F9, A7 and A9 belonging to *T. asperellum* showed improvement in studied characteristics in comparison to other isolates, including wild types. Therefore, γ-irradiation at 450 and 350 Gy dose rates can be considered best dose for the production of mutants in *Trichoderma* species with improved biocontrol strategies.

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