Induction of *Alternaria* blight resistance using *in vivo* and *in vitro* mediated mutagenic techniques in Ethiopian mustard (*Brassica carinata* A. Braun)

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Abstract— *Rapeseed-mustard crops in general, show low average productivity due to the prevalence of various biotic and abiotic stresses. Among biotic stresses, Alternaria blight caused by Alternaria brassicae (Berk.) Sacc. is an important and a widespread destructive disease. In the present study, an attempt was made to isolate Alternaria blight resistant mutants in the susceptible variety 'Jayanti' of Ethiopian mustard (Brassica carinata A. Braun) using in vivo and in vitro mutagenic techniques. Dry and uniform coloured seeds were mutagenised by gamma rays (50, 60, 70, 80, 90, 100 and 110kR) in ^{60}Co gamma cell and Ethyl Methyl Sulphonate (EMS, 0.3%, 0.4% and 0.5%). About 46 mutants in M_2 generation were isolated which showed segregation for Alternaria brassicae resistance. Ten mutants showed very less sporulation intensity along with less halo and concentric ring diameter. Screening of different mutagenised population under Alternaria-sick plots resulted in the isolation of two mutants viz., $P_{(4)2}$ in 80kR and P_{13} in 100kR doses exhibiting moderate resistance/tolerance (PDI <25.0 %, scale 2) compared to the parental check 'Jayanti'. The resistance of theses mutants was further confirmed by in vitro studies using cultural filtrate and detached leaf techniques. The Alternaria blight tolerant mutants also exhibited dwarfness and earliness in comparison to parental variety while the yield potential of both tolerant mutants remained at par with the checks used in the study. Thus, the induced mutagenesis through irradiation and EMS treatments could be effectively used for the induction of Alternaria blight tolerance and the isolated mutants can be the potential genetic stocks for breeding Ethiopian mustard lines with tolerance to Alternaria blight coupled with earliness and dwarfness.*

Keywords— Culture filtrate, irradiation, EMS, Alternaria brassicae (Berk.) Sacc., mutation, resistance, Brassica carinata A. Braun.

I. INTRODUCTION

Rapeseed-mustard crops are the important oilseed crops grown on North western Himalayan region of India comprising the states of Himachal Pradesh, Jammu and Kashmir, and Uttarakhand. In Himachal Pradesh, the crops account for about 57% of total oilseeds area and contribute nearly 60% to total oilseeds production. In general, all *brassica* crops are attacked by a variety of pathogens culminating in huge losses in seed yield. *Alternaria* blight caused by *Alternaria brassicae* (Berk.) Sacc. is an important and a widespread destructive disease of rapeseed-mustard which causes considerable reduction in quantity and quality of harvested *brassica* products (Kumar *et al*., 2014). Depending upon its severity in India, the yield losses to the extent of 70 % have been reported (Kolte, 1985; Chahal, 1986; Gupta *et al*., 2003). The present grown commercial varieties of rapeseed-mustard are susceptible to *Alternaria* blight and no resistant source has been identified so far amongst a wide array of rapeseed-mustard germplasm. Consequently, development of resistant cultivars appears to be the most efficient, sustainable and eco-friendly approach for the management of this disease. There are a number of reports on the existence of variability in this pathogen on the basis of morphology, sporulation, growth and cultural characteristics and reaction on a set of host differentials.

Ethiopian mustard (*Brassica carinata* A. Braun) (BBCC, 2n=4x=34) is a natural allopolyploid between *B. nigra* L. (BB, 2n=2x=16) and *B. oleracea* L. (CC, 2n=2x=18). The variety 'Jayanti' is high yielding and very well suited to dry land farming but, is susceptible to *Alternaria* blight caused by *Alternaria brassicae.* Induced mutagenesis is one of the important tools to create genetic variability not available in the gene pool or to correct specific deficiency of an otherwise outstanding genotype (Bhat *et al*., 2001). Induced mutations have been extensively used for genetic enhancement of the oilseed crops and for improvement of some economic and quality traits in short duration of time (Manjaya and Nandanwar, 2007; Singh and Verma, 2015). *In vitro* mutagenesis mediated selection technique involves the use of pathogen toxins to select resistant

variants in culture and has attracted considerable attention due to its simplicity and ease in exposing a large number of cells to a uniform dose of toxins (Chawla and Wenzel, 1987; Toyoda *et al*., 1988). Tissue and cell culture techniques have been utilized to induce variability in many crop plants including *brassica* crops (Kharb *et al*., 2002; Larkin and Scowcroft, 1981; Jain *et al*., 1990; Katiyar, 1997; Javier *et al*., 2011). Although, these approaches have resulted in the development of resistant plants in some crops but, there is no report on the induction of *Alternaria* blight resistance in Ethiopian mustard. Therefore, aim of the present study was to induce *Alternaria* blight resistance/tolerance in a susceptible variety 'Jayanti' through *in vivo* and *in vitro* mediated mutagenic techniques followed by *in vitro* screening of the induced mutants for disease resistance/tolerance.

II. MATERIALS AND METHODS

The present investigation was carried out at two distinct locations; Department of Crop Improvement, CSKHPKV, Palampur and Shivalik Agricultural Research and Extension Centre (SAREC), Kangra, HP. The generation advancement of putative mutants was carried out at High Land Agricultural Research and Extension Centre, Kukumseri, Lahaul & Spiti (off season nursery), Himachal Pradesh, India.

2.1 Experimental material

The experimental material comprised of one high yielding variety 'Jayanti' of Ethiopian mustard (*Brassica carinata* A. Braun) for the induction of *Alternaria* blight resistant mutants. The uniform colored (dull yellow), bold and dry seeds of variety 'Jayanti' were treated with gamma rays and Ethyl Methane Sulphonate (EMS) each below LD_{50} and above LD_{50} to raise M_1 generation. About 2500 dry seeds were got irradiated with gamma radiations of 90kR, 100kR and 110kR doses at Bhabha Atomic Research Centre (BARC), Trombay, Mumbai during 2008. Meanwhile, about 1000 dry seeds were again irradiated with gamma radiations of 50kR, 60kR, 70kR and 80kR doses at BARC, Trombay, Mumbai during 2009. For chemical mutagenesis, 1000 healthy, uniform and dry seeds were selected and divided into 2 equal parts. Half of seeds were soaked in distilled water for 12 hrs at room temperature prior to treatments. The presoaked (PS) as well as non-presoaked (WPS) seeds were treated with freshly prepared 0.3%, 0.4% and 0.5% ethyl methane sulphonate (EMS) for 6 hrs. The treated seeds were thoroughly washed in running water for 2 hrs to leach out excess EMS. Two control treatments were also maintained using non-irradiated seeds and seeds soaked in distilled water. The M_1 generation of 90kR, 100kR and 110kR doses was raised in the field at SAREC, Kangra during *rabi* 2008-09 and 50kR, 60kR, 70kR and 80kR doses at Kukumseri (off-season nursery) along with control 'Jayanti' during summer 2009. All the surviving plants were harvested individually to obtain M_2 generation. For the isolation of *Alternaria* blight resistant mutants under field conditions in M_2 generation, plant-to-row progenies of individual plants were raised at SAREC, Kangra (hot-spot for the disease) and Palampur during *rabi* 2009-10. Field plots of SAREC, Kangra were already sick plots but, in order to avoid any escape, plots were inoculated with *in vitro* multiplied inoculum of virulent isolates of *Alternaria brassicae* during both vegetative and reproductive stages. Severity of *Alternaria* blight was recorded on five randomly selected leaves of each plant using 0-6 scale. Disease severity on each plant was converted into Percent Disease Index (PDI) which facilitated the easy selection of resistant plants. The selected mutants were categorised as moderately resistant or tolerant mutants (PDI<25%, scale 2).

In order to study the variability in *Alternaria brassicae* isolates, *Alternaria* blight infected leaves showing characteristic symptoms of the disease were collected from four different *Brassica* species such as *Brassica juncea*, *Brassica campestris*, *Brassica napus* and *Brassica carinata* during *rabi* 2010-11. The pathogen was isolated and purified as per the method of Toussoun and Nelson (1976) and maintained on PDA/V_8 slants. The isolates collected from different species were designated on the basis of host species of collection *viz.*, *Brassica juncea* ($A.B_j$), *Brassica campestris* ($A.B_c$), *Brassica napus* ($A.B_n$) and *Brassica carinata* ($A.B_{car}$). To determine the pathogenic variability, a laboratory method; detached leaf technique (Bansal *et al*., 1990) was followed. About 25µl spore suspension (2000-2500 spores/ml) of each isolate was injected on the punctured side of the leaves and each leaf petiole was swabbed with wet cotton. The leaves were placed inside the wet chambers and incubated at 25±1°C for 48-72 hrs. The observations on incubation period, halo formation, concentric ring size and sporulation intensity were recorded periodically to categorize the virulence of four isolates. The most virulent isolate from *B. napus* ($A.B_n$) was used further to screen the putative mutants of *Brassica carinata* for their reaction against *Alternaria brassicae.*

The resistant/tolerant mutants were further screened using *in vitro* cultural filtrate method to confirm their reaction against *Alternaria brassicae*. Such an evaluation of disease resistance is dependent upon the positive correlation between *in vitro* resistance to cultural filtrate and whole plant disease resistance (Willmot *et al.*, 1989). M_3 generation plants were again raised at Kukumseri (off-season nursery) during summer 2010 to advance generation. The individual plant progenies were

harvested to raise M_4 generation. About 45 putative mutants in M_4 generation were raised at SAREC, Kangra for screening against *Alternaria* blight resistance under both natural and artificial epiphytotic conditions under field as well as laboratory during *rabi* 2010-11.

2.2 Evaluation for yield and related agronomical traits under non sick and sick plots

Only two mutants were identified to be tolerant against *Alternaria* blight during 2010-11 both under natural field and artificial laboratory conditions. These mutants were raised along with twenty six other promising mutants and two checks viz., RCC-4 (*Brassica juncea* variety for earliness and dwarfness) and Jayanti (*Brassica carinata*) to confirm their breeding behaviour at Palampur during *rabi* 2011-12. The experiment was laid out in randomized complete block design with three replications. The mutants along with check varieties were raised in 4 rows with row length of 4.0m each having inter-and intra-row spacings of 30 and 10cm, respectively. Standard cultural practices were followed to raise the crop. The observations on characters such as plant height (cm), number of primary branches per plant, number of secondary branches per plant, pods per plant, seeds per pod, days to first flower, days to 75% maturity, 1000-seed weight (g), seed yield per plant (g), biological yield per plant (g) and harvest index (%) were recorded at appropriate stages of crop growth. The mean data were subjected to analysis of variance as per standard procedure (Gomez and Gomez, 1983).

2.3 *In vitro* selection for *Alternaria* blight resistant mutants

2.3.1 Plant material and culture conditions

Cotyledons and hypocotyls segments excised from *in vitro* grown 8-10 days old seedlings of mutants obtained from all doses in M_0 and M_4 generation along with parent (control), were used to initiate calli. MS basal medium containing 2% sucrose and 0.8% agar along with 0.2mg/l BAP and 0.2mg/l NAA hormonal combination were used for callus induction (Murashige and Skoog, 1962). All cultures were exposed to constant light (1500 lux) at 25±1°C temperature for 16 hrs light and 8 hrs dark cycles. White and fragile, proliferating callus appeared within a week.

2.3.2 Isolation and purification of cultural filtrate of *Alternaria brassicae*

The pure culture of highly virulent *Alternaria brassicae* isolate was sub cultured on potato dextrose agar. Small bits (5mm diameter) of *Alternaria brassicae* culture were transferred to the conical flasks (250 ml) containing 30 ml of sterilized potato dextrose broth and incubated in a rotary shaker (150 rpm) at 22±1°C for 15 days. Fungal mycelium was separated by passing fungal culture through sterilized whatmann filter paper (No.1). The cultural filtrate was further sterilized by passing through millipore filter of 0.22 µm size. The pH of the cultural filtrate was adjusted to 5.8 before filter sterilization.

2.3.3 Culturing of callus on MS medium + cultural filtrate

The crude concentrated fungal liquid cultural filtrate was mixed to the autoclaved MS medium amended with BAP-2.0mg/l + NAA-0.1mg/l during cooling under aseptic conditions at 20, 60 and 100% concentration of fungal filtrate and dispensed in sterilized petriplates. The medium was stored at 25±1°C and used for inoculation. The hypocotyl segments of 5-6 mm length were excised from 8-10 days old seedlings of different doses viz., M_0 (50 kR, 60 kR, 70 kR, 80 kR, 90 kR, 100 kR and 110 kR) and $P(_4)_2$ in 80 kR and P_{13} in 100 kR (two M_4 generation mutants that showed moderate resistance in field screening) along with the control Jayanti. These hypocotyl segments were implanted on MS medium with or without cultural filtrate and the petriplates were sealed with parafilm and incubated at a temperature of 25±1°C for one month under 16 hours light (1500 lux) and 8 hours dark cycles. In each petriplate, 10 explants were cultured and whole experiment was replicated twice. After one month, the observations were recorded on percent callus induction, color of callus, fresh and dry weights of individual calli and shooting response (morphogenetic response) to screen them for their resistance.

III. RESULT AND DISCUSSION

3.1 *In vivo* mutagenesis for the selection of *Alternaria* blight resistance (sick plots)

Selections for *Alternaria* blight resistance were made in the plant-to row progenies of M_1 generations of different mutagenised populations of both physical and chemical mutagens in susceptible variety Jayanti at Kangra and Palampur during *rabi* 2009-10. A total of 46 mutants in M_2 generation were isolated in different mutagenised population which showed segregation for *Alternaria brassicae* resistance (Table 1). The highest number of progenies (11) with *Alternaria* blight tolerant plants was observed in 0.3% EMS (PS) followed by 50kR (10). The M_3 generation was raised at Kukumseri (off-season nursery) for generation advancement during summer 2010 and M_4 generation was again raised at Kangra (hot-spot for *Alternaria* blight) during *rabi* 2010-11.

TABLE 1
NUMBER OF *ALTERNARIA* BLIGHT RESISTANT/TOLERANT PROGENIES SELECTED IN DIFFERENT GENERATIONS OF THE MUTAGENISED POPULATION OF THE ETHIOPIAN MUSTARD VARIETY JAYANTI

Mutagen and dose	*In vivo* screening			*In vitro* screening
	M_1 plants harvested at Kangra (2008-09)	Tolerant progenies in M_2 at Kangra (2009-10)	Tolerant mutants in M_4 at Kangra (2010-11)	Mutants showing tolerance under laboratory conditions
50kR	1921	10	4	0
60kR	2127	6	0	0
70kR	1611	6	3	0
80kR	1190	1	1	1
90kR	1129	4	1	0
100kR	867	5	1	1
110kR	287	1	0	0
Subtotal	9132	33	10	2
0.3 % EMS (PS)	2472	11	0	0
0.4 % EMS (PS)	1947	1	0	0
0.5 % EMS (PS)	1831	1	0	0
Subtotal	6250	13	0	0
Grand Total	15, 382	46	10	2

3.2 *In vitro* selection for *Alternaria* blight resistance through detached leaf technique

In the juvenile stage of M_4 generation, putative mutants were screened against *Alternaria* blight resistance through detached leaf technique. The isolate *A.*B_n recorded less incubation period, larger diameter of concentric ring and halo and higher sporulation intensity exhibiting higher virulence towards the host *Brassica carinata*. This most virulent isolate of *Alternaria brassicae* isolated from *Brassica napus* (*A.*B_n) was used further to screen putative mutants of *Brassica carinata.* The maximum incubation period (4.0-4.5 days) was recorded by 17 mutants but, 10 mutants showed very less sporulation intensity along with less halo and concentric ring diameter (Table 2). The mutants such as P_7, $(P_{10})_2$, P_{11} and P_{74} in 50kR, $(P_4)_2$, $(P_5)_2$ and P_2 in 70kR, $(P_4)_2$ in 80kR, P_{22} in 90kR and P_{13} in 100kR were found to exhibit moderate resistance (Kumari *et al.*, 2013). These mutants were further evaluated under natural field conditions at SAREC, Kangra to confirm their resistance against *Alternaria brassicae* during *rabi* 2010-11. Out of 45 mutant progenies obtained in M_2 generation, only 2 mutants such as $(P_4)_2$ in 80kR and P_{13} in 100kR showed moderately resistance/tolerance to *Alternaria* blight (Figure 1) in M_4 generation (Kumari *et al*., 2013).

$P_{(4)2}$ (80kR)

FIGURE 1: ALTERNARIA BLIGHT TOLERANT MUTANT IN M_4 GENERATION AT KANGRA DURING 2010-11

TABLE 2
RESPONSE OF 45 PUTATIVE MUTANTS OF BRASSICA CARINATA TO THE MOST VIRULENT ISOLATE OF A. BRASSICAE ($A.B_N$) THROUGH DETACHED LEAF TECHNIQUE

Mutants	Dose	Mean incubation period (days)	Mean halo diameter (mm)	Mean concentric ring size (mm)	Sporulation intensity	Category
P_7	50 kR	4.5	40	38	very less	MR
$(P_{10})_2$	50 kR	4.0	37	37	very less	MR
P_{10}	50 kR	3.0	68	60	dark	HS
P_{46}	50 kR	3.9	64	56	dark	S
$(P_8)_2$	50 kR	4.2	42	41	medium	MS
P_{11}	50 kR	3.7	40	40	very less	MR
P_{74}	50 kR	4.0	45	42	very less	MR
$(P_{11})_2$	50 kR	4.4	48	40	medium	MS
P_{56}	50 kR	3.8	56	49	dark	S
$(P_3)_2$	50 kR	3.6	73	61	dark	S
P_{47}	50 kR	4.2	57	50	dark	S
P_{26}	60 kR	3.8	65	55	medium	MS
P_{39}	60 kR	3.5	56	51	medium	MS
P_{38}	60 kR	3.0	75	68	very dark	HS
$(P_1)_2$	60 kR	3.8	63	58	sparsely	MS
$(P_9)_2$	60 kR	3.9	65	55	dark	S
$(P_4)_2$	70 kR	4.4	45	36	very less	MR
$(P_5)_2$	70 kR	4.5	48	45	very less	MR
P_6	70 kR	3.7	78	70	dark	S
P_{22}	70 kR	4.0	58	50	medium	MS
$(P_2)_2$	70 kR	4.2	50	47	medium	MS
P_2	70 kR	4.0	47	43	very less	MR
$(P_4)_2$	80 kR	4.4	45	36	very less	MR
P_{15}	90 kR	3.0	72	70	dark	HS
P_2	90 kR	4.0	66	50	medium	MS
P_{22}	90 kR	4.5	35	35	very less	MR
$(P_2)_2$	90 kR	4.0	50	38	medium	MS
P_{22}	100 kR	3.9	58	50	medium	MS
P_4	100 kR	3.7	56	49	medium	MS
$(P_1)_2$	100 kR	3.8	60	55	medium	MS
P_{13}	100 kR	4.3	42	40	sparsely	MR
$(P_2)_2$	110 kR	3.5	68	54	medium	S
$(P_2)_2$	0.3% EMS-PS	3.0	75	70	very dark	HS
P_{34}	0.3% EMS-PS	3.0	72	61	very dark	HS
P_{31}	0.3% EMS-PS	3.0	74	60	medium	S
P_{13}	0.3% EMS-PS	3.7	60	52	medium	S
$(P_9)_2$	0.3% EMS-PS	3.1	68	59	dark	S
$(P_{17})_2$	0.3% EMS-PS	4.0	50	45	medium	MS
P_{27}	0.3% EMS-PS	3.0	56	50	medium	S
P_{14}	0.3% EMS-PS	3.5	54	50	very less	MS
P_{18}	0.3% EMS-PS	3.2	58	52	sparsely	MS
P_5	0.3% EMS-PS	3.0	69	65	very dark	S
$(P_{23})_2$	0.3% EMS-PS	3.0	74	70	very dark	HS
$(P_1)_2$	0.4% EMS-PS 0.5%	3.1	71	67	dark	HS
P_8	EMS- PS	3.5	62	55	medium	S

MR-Moderately Resistant; MS-Moderately Susceptible; S-Susceptible, HS-Highly susceptible; EMS-PS-Ethyl Methane Sulphonate with pre-soaking

3.3 Confirmation of *Alternaria* blight tolerance through tissue culture method

Two resistant mutants selected from field screening under sick plots showed resistance under laboratory conditions also. The resistance was further confirmed in M_4 generation using cultural filtrate method.

3.3.1 Callus initiation

Callus initiation was observed within one week of cultured hypocotyl explants in almost all media (media without cultural filtrate, with 20% and 60% cultural filtrate), except in medium with 100% cultural filtrate. Results revealed that the early callus initiation and better callus growth which was indicated through percent callus induction, reduced drastically from lower to higher concentrations of cultural filtrate i.e. 20% to 100% as the higher concentration of cultural filtrate inhibited cell division and caused death to cultured cells. However, 80kR and 100kR dose-explants (both in M_0 and M_4 generations) showed greater extent of survival in all concentrations of fungal toxin viz, 20%, 60% and 100% cultural filtrate as compared to control Jayanti (parental explants). In addition to callus induction, color of callus also supported the above results. The mutant $P_{(4)2}$ (80kR) in M_4 generation also showed pale yellow color of callus in all three concentrations of fungal filtrate (Kumari *et al*., 2014). Earlier workers had demonstrated that the callus growth in mustard stimulated as the dose rate increased in cotyledon cultures obtained from seeds treated with higher doses and maximum growth was observed at 25kR dose (George and Rao, 1980). The highest inhibitory effect on shoot regeneration was also observed by Singh *et al*. (2007) in 5kR, 10kR, 20kR, 30kR, 40kR & 100kR doses except 54kR along with total suppression of morphogenetic response by 100kR doses.

3.3.2 Fresh and dry weight of callus

In general, both fresh and dry weights of calli were maximum in 80kR and 100kR dose-explants both in M_0 and M_4 generations as compared to 50kR, 60kR, 70kR, 90 kR and 110kR dose-explants in M_0 generation. This suggested that the 80kR and 100kR doses exhibited moderate resistance/tolerance to fungal toxin of *Alternaria* blight (Table 3).

TABLE 3
EFFECT OF CULTURAL FILTRATE ON DIFFERENT DOSE-EXPLANTS THROUGH TISSUE CULTURE METHOD IN ETHIOPIAN MUSTARD

Dose → / **Characters ↓**	**Control**	**50kR kR**	**60 kR**	**70 kR**	**80 kR**	**90 kR**	**100 kR**	**110 kR**	**80 kR $P_{(4)2}$**	**100 kR P_{13}**
Without CF										
Callus induction (%)	100	100	96	100	100	94	94	86	100	100
Color of callus	OW	PY	LB	PY	PY	PY	PY	LB	W	W
Fresh wt.(mg)	288	284	173	276	273	180	198	172	581	471
Dry wt.(mg)	68	82	30	33	46	38	63	36	76	80
Shoot response	S	S	S	S	S	NS	S	NS	S	S
20% CF										
Callus induction (%)	100	100	70	85	82	78	88	58	100	100
Color of callus	LB	LB	DB	B	LB	LB	LB	LB	PY	PY
Fresh wt.(mg)	258	257	76	194	248	230	170	93	329	230
Dry wt.(mg)	76	66	40	52	48	72	60	48	124	74
Shoot response	S	S	S	S	S	NS	S	NS	S	S
60% CF										
Callus induction (%)	65	50	50	40	67	43	71	40	75	76
Color of callus	B	B	B	B	B	B	B	B	PY	LB
Fresh wt.(mg)	156	120	38	68	232	160	110	74	234	206
Dry wt.(mg)	24	28	4	24	58	30	24	18	40	36
Shoot response	NS	NS	NS	NS	S	NS	NS	NS	S	S
100% CF										
Callus induction (%)	33	36	32	29	64	42	72	31	63	67
Color of callus	B	B	B	B	B	B	B	B	PY	LB
Fresh wt.(mg)	74	55	40	43	110	52	100	20	207	160
Dry wt.(mg)	14	12	9	14	26	18	14	19	22	28
Shoot response	NS	NS	NS	NS	S	NS	NS	NS	S	S

OW-Off White, PY-Pale Yellow, W-White, LB-Light Brown, B-Brown and DB-Dark Brown
S-Shoot appears, NS- Shoot does not appear

3.3.3 Shoot regeneration

In general, the shoot regeneration potential decreased drastically as the concentration of cultural filtrate increased from 20% onwards. In 20% concentration of cultural filtrate, the shooting response was similar to medium without cultural filtrate. The shoot regeneration was observed in all concentrations of fungal toxin viz., 20%, 60% and 100% cultural filtrate in 80kR dose-explants while 100kR dose-explants showed regeneration in 20% cultural filtrate only. On the other hand, P_{13} in 100kR in M_4 generation recorded shoot regeneration in 20%, 60% and 100% cultural filtrate. Thus, the two mutants such as $P_{(4)2}$ in 80kR and P_{13} in 100kR in M_4 generation had moderate resistance/tolerance to *Alternaria* blight (Kumari *et al*., 2014) based on cultural filtrate method.

3.4 Performance of laboratory-confirmed mutants under non-sick field conditions

Two mutants confirmed as moderately resistant/tolerant against *Alternaria* blight in M_4 generation were raised in non-sick plots at Palampur and Akrot along with mutants to confirm their breeding behaviour in M_5 generation (Table 4). Based upon the data under natural field conditions, detached leaf technique and cultural filtrate method, two mutants such as $P(_4)_2$ in 80kR and P_{13} in 100kR doses were observed to be moderately resistant/tolerant (PDI <25.0 %, scale 2) against *Alternaria* blight. In addition, three mutants such as $P_{(3)2}$ (0.3% EMS PS), $P_{(4)2}$ (0.3% EMS PS) and $P_{(11)2}$ (0.3% EMS PS) showed earliness coupled with dwarfness (Figure 2). Thus, the tolerance to *Alternaria* blight was confirmed through both *in vivo* and *in vitro* screening methods. These *Alternaria* blight tolerant mutants also exhibited dwarfness and earliness in comparison to parental check Jayanti while the yield potential of both the tolerant mutants remained at par with the checks. Thus, the isolated mutants can be used as the potential genetic stocks for breeding Ethiopian mustard lines with tolerance to *Alternaria* blight coupled with earliness and dwarfness and inheritance of *Alternaria* blight resistance. Earlier studies on the genetics of *Alternaria* blight resistance indicated the presence of duplicate gene epistasis in Indian mustard (Chaurasia and Bhajan, 2015).

TABLE 4
PERFORMANCE OF *ALTERNARIA* BLIGHT TOLERANT MUTANTS ALONG WITH OTHER DESIRABLE MUTANTS UNDER NON-SICK PLOTS IN M5 GENERATION DURING *RABI* 2011-12

Mutants	Plant height (cm)		Days to maturity		Yield (g/plant)	
	Akrot	Palampur	Akrot	Palampur	Akrot	Palampur
Alternaria blight tolerant mutants						
$P(4)_2$ 80kR	186.1	164.3	165	189	7.3	18.4
P13 100kR	188.8	135.7	164	189	9.6	11.9
RCC-4 (check)	153.5	156	159	180	7.1	11.2
Jayanti (check)	189.4	181.7	166	194	9.5	11.9
Early and dwarf mutants						
$P(3)_2$ 0.3% EMS (PS)	141.3	117.8	160	181	10.1	13.2
$P(4)_2$ 0.3% EMS (PS)	143.0	144.3	160	181	9.1	9.3
$P(11)_2$ 0.3% EMS (PS)	140.4	118.7	159	182	8.0	12.6

$P(3)_2$ in 0.3 % EMS (PS)
FIGURE 2: EARLY AND DWARF MUTANT

IV. CONCLUSION

The induced mutagenesis through irradiation and EMS treatments could be effectively used for the induction of *Alternaria* blight tolerance and the isolated mutants can be the potential genetic stocks for breeding Ethiopian mustard lines with tolerance to *Alternaria* blight coupled with earliness and dwarfness.

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