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Screening of chilli genotypes against *Colletotrichum truncatum* causing anthracnose in chilli

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Abstract
Anthracnose disease of chilli (*Capsicum annuum* L.) caused by *Colletotrichum* spp. has been a serious constraint to chilli production worldwide. Two species *Colletotrichum truncatum* and *Colletotrichum gloeosporioides* are known to cause anthracnose of chilli in India of which the former is the most predominant one. In the present study, 22 chilli genotypes were screened for anthracnose resistance under both *in vitro* and field conditions against the pathogen. The study indicated that disease development started at 3 days after inoculation (DAI) on 10th DAI the observations were taken for calculating percent disease index. Anthracnose symptoms were visible in both matured green and red ripened fruits and the disease resistance was assessed by using 0-5 disease index scale. All the genotypes reacted differently and significantly to the pathogen. Screening results revealed that none of the genotypes were completely resistant to anthracnose. Hence, the new resistant lines need to be developed by breeding programmes for anthracnose resistance in chilli.

Keywords: Anthracnose, chilli, *colletotrichum truncatum, colletotrichum gloeosporioides,* resistance, genotype

Introduction
Chilli (*Capsicum annuum* L.) is an important vegetable cum spice crop which belongs to *Solanaceae* family (2n = 24). It is extensively cultivated in India under rain fed as well as irrigated conditions covering an area of of 7.32 lakh hectares with an annual production of 19.88 lakh tonnes (www.dasd.gov.in, 2020-21).

India is the largest producer and exporter of chilli in the world. In India, Andhra Pradesh is the largest producer of the chilli with an annual production of 7.97 lakh tonnes from 1.17 lakh hectares with a productivity of 4489 kg/ha (www.Indiastat.com). The other important chilli growing states are Telangana, Karnataka, Maharashtra, Orissa, Rajasthan, Tamil Nadu and Madhya Pradesh.

Anthracnose caused by *Colletotrichum* spp. is one of the major constraint for chilli production in the tropics and subtropics worldwide. The genus *Colletotrichum* also causes anthracnose in a wide range of hosts including fruits, vegetables, legumes, cereals, grasses and ornamental plants (Sharma *et al.*, 2005) [9]. Chilli anthracnose is mainly a problem on mature fruits causing severe losses due to both pre and post-harvest fruit decay (Hadden and Black, 1989) [1]. It can cause yield losses up to 10-60%. Chilli anthracnose usually develops under high humid conditions when rain occurs after the fruits have started to ripen with reported losses of 84% approximately (Thind and Jhooty, 1985) [11]. Typical anthracnose symptoms on chilli fruit include sunken necrotic tissues, with concentric rings of acervuli.

Since the screening of chilli genotypes under lab condition which helps in identification of resistance sources in genotypes in a short period of a time and it can be selected and used in breeding programmes to develop resistant varieties is the most efficient, non-hazardous, environmentally safe and economical way to manage plant diseases. The present investigation was aimed to identify the resistant genotypes against *Colletotrichum truncatum*, under *in vitro* and field condition by artificial inoculation. Where the 22 germplasm were screened against *Colletotrichum truncatum*, in search of resistant genotypes.

Materials and Methods
Experiments were conducted during the crop season 2021-22, field and laboratory experiments were carried out in Department of Plant Pathology, Dr YSRHU, Horticultural Research Station, Lam, Guntur district, Andhra Pradesh.

Isolation of the fungus

The diseased chilli fruit samples were collected from the field and the diseased parts cut into small bits with the help of sterilized blade. Then surface sterilized with 1% sodium hypochlorite solution under aseptic conditions in laminar air flow chamber and washed thoroughly for 3 times with sterilized water to remove traces of chemical. Excess moisture was removed by placing these in the fold of sterilized blotting paper. The pieces then transferred into Petri plates with the help of sterilized needles. The petri plates were previously sterilized and poured with PDA media and antibiotics like Streptomycin used to prevent bacterial contamination. The Petri plates will be kept at 25±2 °C for 7-10 days in an incubator. Single spore isolation technique was followed for getting pure culture.

Pathogenicity test

Red ripe chilli fruits were collected from field and sterilized using 1% sodium hypochlorite solution followed by drying using sterilized filter paper. After drying mild pin prick was made on the fruit with the sterilized needle and then conidia suspension 2µl of concentration 1×10^6 per ml was placed over the wound. Spore concentration was adjusted by haemocytometer. The inoculated fruits were observed for disease development and pathogen was re isolated and observed under microscope to prove Koch postulates. The morphological and molecular characters were studied, the pathogenicity was proved and the identification was confirmed before its use for experimental purpose.

***In vitro* screening**

A total of 22 genotypes along with a susceptible check CA 960 were obtained from chilli breeder, HRS Lam and screened against virulent isolates of *Colletotrichum truncatum*. By detached fruit method under laboratory conditions. Both red and green fruits were collected to study the disease reaction. A purified and pathogenic isolate of *Colletotrichum truncatum*. Was used for screening based on its pathogenicity. For screening the fruits, pin prick method of artificial inoculation is used.

Screening procedure

Conidia of the fungus were collected from actively growing ten days old culture with the help of scalpel. The spore mass was dissolved in distilled water and homogenised to get uniform concentration of spore suspension. The concentration of spore suspension was adjusted to 1 x 10^6 conidia per ml of water (Rajapakse, 1998) [8] with the help of haemocytometer. A set of three chilli fruits of both red and green fruits were taken in each line with two replications. The specific disease reaction for each genotype was assessed. Fruits were carefully detached from plants and washed with sterile distilled water (SDW) and then wiped with cotton wools soaked in ethanol to remove microbes on the surface. Fruits were slightly punched with small needle and were inoculated with 2 µl of conidial suspension. After inoculation, the chilli fruits were placed in a plastic tray lined with three layers of paper towel moistened with sterile distilled water to produce a humid environment and covered with another tray. Symptoms on the chilli fruit were examined for disease development and evaluated at 3, 7 and 10 days after inoculation by measuring the area of the lesion. The PDI (Per cent disease index) was calculated for infected fruits. The genotypes were rated as resistant and susceptible based on the range of the disease index. By using the 0-5 scale given by Jeyalakshmi and Seetharaman (1998) [4], grades were given based on the fruit area infected *i.e*., 0-no disease, 1-up to 5% infection, 2- >5-10% infection, 3- >10-25%, 4- >25-50%, 5- >50% the percent fruit area infected was calculated as per the following formula:

$$\text{Percent fruit area infected} = \frac{\text{Sum of numerical ratings}}{\text{Total no of fruits observed x maximum grade}} \times 100$$

The disease reaction of each genotype was categorized on the basis of following rating scale given by Singh *et al.* (1993) [10] presented in the Table 1.

Screening under field conditions

Seedlings of 22 genotypes along with a susceptible check CA 960 were raised in nursery for 40 days and transplanted in the main field at a spacing of 75 × 30 cm. Each genotype was planted in a plot of 2 x 1.5 m size in RBD replicated twice. Spore suspension having spore load of (1 x 10^6 conidia ml^{-1} water) sprayed on fruits. Standard agronomic practices were followed and the plant protection measures were taken up against sucking pests and pod borers. Observations were taken at the time of fruit ripening stage on five plants selected at random from each genotype. The per cent disease index was recorded by using the following 0-5 disease rating scale Jeyalakshmi and Seetharaman (1998) [4]. Per cent disease index was calculated by the following formula:

$$\text{Per cent disease index} = \frac{\text{Sum of numerical ratings}}{\text{Total no of fruits observed x maximum grade}} \times 100$$

Based on the PDI disease reaction of the genotypes was categorized on the basis of rating scale given in the Table 2.

Table 1: Scale used for calculating PDI

Grade	Fruit area covered by the disease
0	Healthy (no disease symptoms)
1	up to 5%
2	>5-10%
3	>10-25%
4	>25-50%
5	above 50

Table 2: Scale for categorizing disease reaction of genotypes.

Disease ratings	Reaction type
0%	Immune (I)
>0-5%	Resistant (R)
>5-25%	Moderately resistant (MR)
>25-50%	Susceptible (S)
>50%	Highly Susceptible (HS)

Results and Discussion

***In vitro* screening**

A total of 22 genotypes along with a susceptible check CA 960 were screened against virulent isolate CC-14 by pin prick method under *in vitro* conditions. Red and green chilli fruits were collected from all the genotypes and were inoculated with 2 µl of conidial suspension for each fruit with a spore load of 1 x 10^6 per ml. Data on the area of the lesion was recoded at 3,7 and 10 days after inoculation. Based on the standard scale, per cent fruit area infected was calculated and

the disease reaction of the genotypes were presented in the Table 3.

Field screening

For field screening, 22 genotypes along with a susceptible check CA 960 were planted in the field. Observations were taken at the time of fruit ripening stage on five plants selected at random from each genotype. The per cent disease index was calculated by using 0-5 disease rating scale and disease reaction of different genotypes presented in Table 6.

Table 3: Screening of chilli genotypes against *Colletotrichum truncatum* under *in vitro* conditions.

S. No	*In vitro* conditions							
	PDI (Red fruits)			Disease Reaction	PDI (Green fruits)			Disease Reaction
	R_1	R_2	Mean		R_1	R_2	Mean	
Gp-1	42.40 (40.61)	37.60 (37.81)	40.00 (39.22)	S	42.40 (38.14)	37.60 (35.56)	36 (36.86)	S
Gp-2	31.80 (34.31)	28.20 (32.06)	30.00 (33.20)	S	31.80 (31.65)	28.20 (29.62)	26 (30.65)	S
Gp-3	38.90 (38.57)	34.50 (35.96)	36.70 (37.27)	S	38.90 (35.61)	34.50 (33.25)	32 (34.44)	S
Gp-4	35.30(36.44)	31.30 (34.01)	33.30 (35.23)	S	35.30 (34.38)	31.30 (32.12)	30.1 (33.26)	S
Gp-5	14.10 (22.05)	12.50 (20.70)	13.30 (21.38)	MR	14.10 (20.89)	12.50 19.62)	12 (20.26)	MR
Gp-6	38.90 (38.57)	34.50 (35.96)	36.70 (37.27)	S	38.90 (35.61)	34.50 (33.25)	32 (34.44)	S
Gp-7	38.90 (38.57)	34.50 (35.96)	36.70 (37.27)	S	38.90 (34.96)	34.50 (32.66)	31 (33.82)	S
Gp-8	17.70 (24.87)	15.70 (23.33)	16.70 (24.11)	MR	17.70 (20.98)	15.70 (19.70)	12.1 (20.35)	MR
Gp-9	24.70 (29.79)	21.90 (27.89)	23.30 (28.85)	MR	24.70 (27.40)	21.90 (25.69)	20 (26.55)	MR
Gp-10	28.30 (32.13)	25.10 (30.05)	26.70 (31.10)	S	28.30 (28.86)	25.10 (27.04)	22 (27.96)	MR
Gp-11	24.70 (29.79)	21.90 (27.89)	23.30 (28.85)	MR	24.70 (25.89)	21.90 (24.28)	18 (25.09)	MR
Gp-12	31.80 (34.31)	28.20 (32.06)	30.00 (33.20)	S	31.80 (30.28)	28.20 (28.35)	24 (29.32)	MR
Gp-13	28.30 (32.13)	25.10 (30.05)	26.70 (31.10)	S	28.30 (28.43)	25.10 (26.64)	21.4 (27.54)	MR
Gp-14	24.70 (29.79)	21.90 (27.89)	23.30 (28.85)	MR	24.70 (26.81)	21.90 (25.13)	19.2 (25.98)	MR
Gp-15	45.90 (42.63)	40.70 (39.63)	43.30 (41.13)	S	45.90 (39.44)	40.70 (36.74)	38.1 (38.10)	S
Gp-16	24.70 (29.79)	21.90 (27.89)	23.30 (28.85)	MR	24.70 (26.88)	21.90 (25.20)	19.3 (26.05)	MR
Gp-17	28.30 (32.13)	25.10 (30.05)	26.70 (31.10)	S	28.30 (29.01)	25.10 (27.17)	22.2 (28.10)	MR
Gp-18	21.20 (27.40)	18.80 (25.69)	20.00 (26.55)	MR	21.20 (24.15)	18.80 (22.66)	15.8 (23.41)	MR
Gp-19	21.20 (27.40)	18.80 (25.69)	20.00 (26.55)	MR	21.20 (24.55)	18.80 (23.03)	16.3 (23.80)	MR
Gp-20	38.90 (38.57)	34.50 (35.96)	36.70 (37.27)	MR	38.90(35.67)	34.50 (33.31)	32.1 (34.50)	S
Gp-21	35.30 (36.44)	31.30 (34.01)	33.30 (35.23)	S	35.30 (33.40)	31.30 (31.22)	2 8.6 (32.32)	S
Gp-22	21.20 (27.40)	18.80 (25.69)	20.00 (26.55)	MR	21.20 (24.31)	18.80 (22.81)	16 (23.57)	MR
CA 960	56.50 (48.71)	50.10 (45.04)	53.30 (46.87)	HS	56.50 (46.82)	50.10 (43.37)	50.2 (45.10)	HS
C.D (p = 0.05)			3.36				3.02	
SE(m)			1.14				1.03	
C.V (%)			4.97				4.90	

*Figures in parentheses represented arc sine transformed values S- Susceptible MR- Moderately resistant HS – Highly susceptible

Table 4: Reaction of red chilli genotypes against *Colletotrichum truncatum* under *in vitro* conditions

Disease ratings	Disease reaction	No of genotypes	List of genotypes
0%	Immune	0	-
>0-5%	resistant	0	-
>5-25%	Moderately resistant	10	GP-5, GP-8, GP-9, GP-11, GP-14, GP-16, GP-18, GP-19, GP-20, GP-22
>25-50%	Susceptible	12	GP-1, GP-2, GP-3, GP-4, GP-6, GP-7, GP-10, GP-12, GP-13, GP-15, GP-17 and GP-21
>50%	Highly susceptible	1	CA 960

Table 5: Reaction of green chilli genotypes against *Colletotrichum truncatum* under *in vitro* conditions.

Disease ratings	Disease reaction	No of genotypes	List of genotypes
0%	Immune	0	-
>0-5%	resistant	0	-
>5-25%	Moderately resistant	13	GP-5, GP-8, GP-9, GP-10, GP-11, GP-12, GP-13, GP-14, GP-16, GP-17, GP-18, GP-19 and GP-22
>25-50%	Susceptible	9	GP-1, GP-2, GP-3, GP-4, GP-6, GP-7, GP-15, GP-20 and GP-21
>50%	Highly susceptible	1	CA 960

Table 6: Screening of chilli genotypes against *Colletotrichum truncatum* under field conditions.

S. No	Field screening			Disease reaction
	PDI (Red fruits)			
	R_1	R_2	Mean	
Gp-1	53.77 (47.14)	21.43 (27.57)	37.60 (37.81)	S
Gp-2	40.04 (39.24)	15.96 (23.54)	28.00 (31.94)	S
Gp-3	15.50 (23.18)	38.90 (38.57)	27.20 (31.42)	S
Gp-4	44.62 (41.89)	17.78 (24.93)	31.20 (33.94)	S
Gp-5	22.88 (28.57)	9.12 (17.57)	16.00 (23.57)	MR
Gp-6	48.05 (43.86)	19.15 (25.94)	33.60 (35.41)	S

Gp-7	19.61 (26.27)	49.19 44.52)	34.40 (35.90)	S
Gp-8	27.46 (31.59)	10.94 (19.31)	19.20 (25.98)	MR
Gp-9	32.03 (34.46)	12.77 (20.93)	22.40 (28.24)	MR
Gp-10	33.18 (35.16)	13.22 (21.32)	23.20 (28.78)	MR
Gp-11	13.22 (21.32)	33.18 (35.16)	23.20 (28.78)	MR
Gp-12	15.50 (23.18)	38.90 (38.57)	27.20 (31.42)	S
Gp-13	35.46 (36.54)	14.14 (22.08)	24.80 (29.86)	MR
Gp-14	13.22 (21.32)	33.18 (35.16)	23.20 (28.78)	MR
Gp-15	23.26 (28.82)	58.34 (49.78)	40.80 (39.68)	S
Gp-16	11.86(20.13)	29.74 (33.04)	20.80 (27.12)	MR
Gp-17	34.32 (35.85)	13.68 (21.70)	24.00 (29.32)	MR
Gp-18	32.03 (34.46)	12.77 (20.93)	22.40 (28.24)	MR
Gp-19	30.89 (33.75)	12.31 (20.53)	21.60 (27.68)	MR
Gp-20	46.90 (43.21)	18.70 (25.61)	32.80 (34.93)	S
Gp-21	34.32 (35.85)	13.68 (21.70)	24.00 (29.32)	MR
Gp-22	32.03 (34.46)	12.77 (20.93)	22.40 (28.24)	MR
CA 960	29.07 (32.61)	72.93 (58.63)	51.00 (45.55)	HS
C.D (P=0.05)			4.91	
SE(m)			1.66	
C.V (%)			7.62	

*Figures in parentheses represented arc sine transformed values S- Susceptible MR- Moderately resistant HS -Highly susceptible

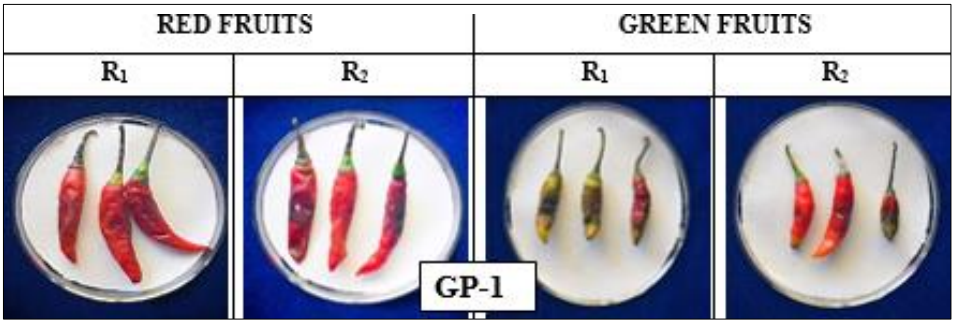
Table 7: Reaction of chilli genotypes against *Colletotrichum truncatum* under Field conditions

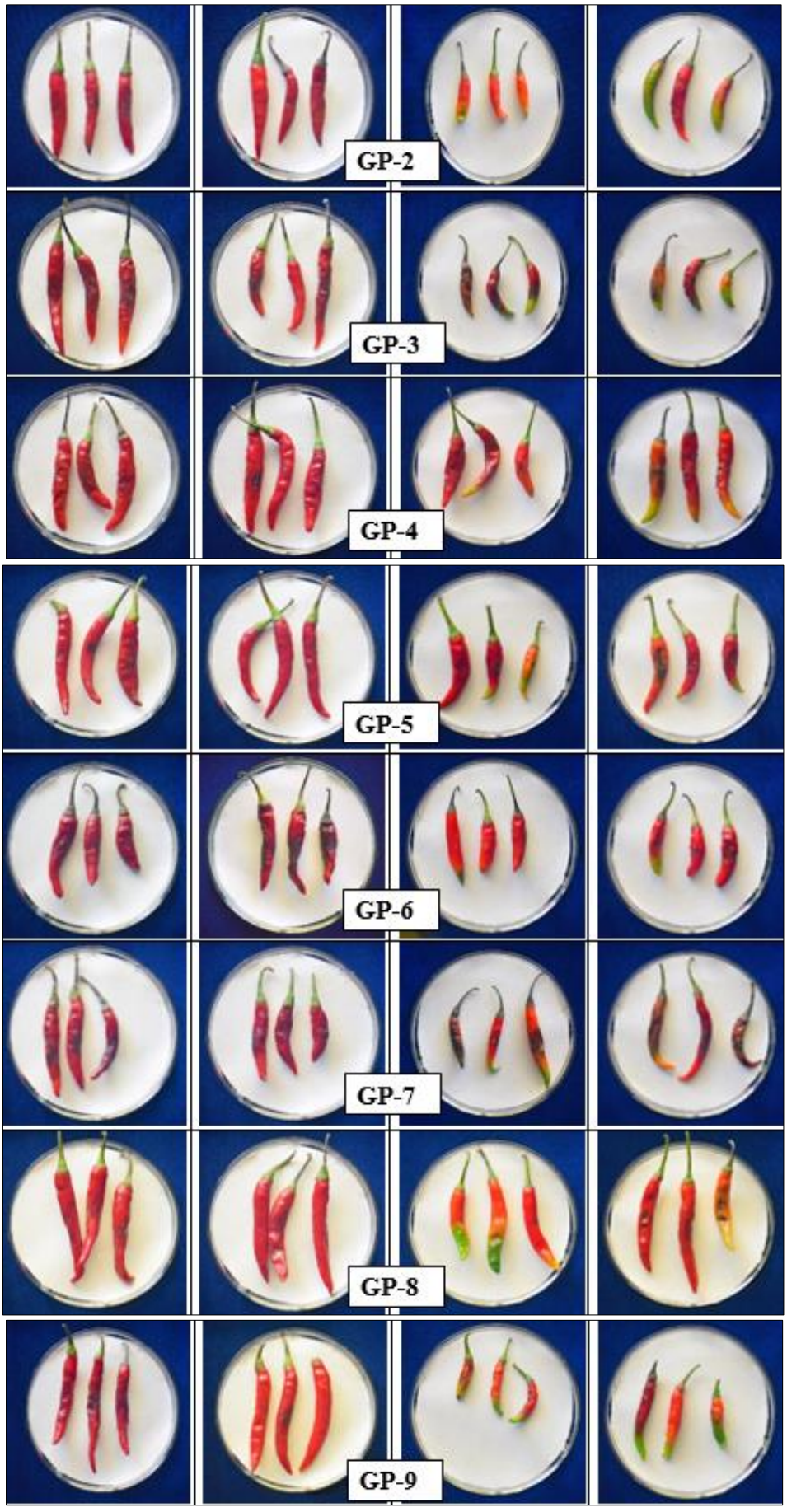
Disease Ratings	Disease Reaction	No of genotypes	List of genotypes
0%	Immune	0	-
>0-5%	resistant	0	-
>5-25%	Moderately resistant	13	GP-5, GP-8, GP-9, GP-10, GP-11, GP-13, GP-14, GP-16, GP-17, GP-18, GP-19, GP-21, GP-22
>25-50%	Susceptible	9	GP-1, GP-2, GP-3, GP-4, GP-6, GP-7, GP-12, GP-15, and GP-20
>50%	Highly susceptible	1	CA 960

Results revealed that red chilli fruits average disease intensity ranged from 13.30 to 53.30% under *in vitro* conditions and 16.00 to 51.00% under field conditions. Among the entries screened, none of them was either immune or resistant. Under *in vitro* conditions, the disease reaction of 10 genotypes *viz.*, GP-5, GP-8, GP-9, GP-11, GP-14, GP-16, GP-18, GP-19, GP-20, GP-22 was moderately resistant, in the remaining 12 genotypes *viz.*, GP-1, GP-2, GP-3, GP-4, GP-6, GP-7, GP-10, GP-12, GP-13, GP-15, GP-17 and GP-21 the disease reaction was susceptible. For green chilli fruits, the average disease intensity ranged from 12 to 50.2% under *in vitro* conditions. Among the entries screened, none of them was either immune or resistant. The disease reaction of 13 genotypes *viz.*, GP-5, GP-8, GP-9, GP-10, GP-11, GP-12, GP-13, GP-14, GP-16, GP-17, GP-18, GP-19 and GP-22 was moderately resistant, in the remaining 9 genotypes *viz.*, GP-1, GP-2, GP-3, GP-4, GP-6, GP-7, GP-15, GP-20 and GP-21 the disease reaction was susceptible.

Under field conditions also none of the genotypes were either immune or resistant. Among the genotypes screened, 13 genotypes *viz.*, GP-5, GP-8, GP-9, GP-10, GP-11, GP-13, GP-14, GP-16, GP-17, GP-18, GP-19, GP-21, GP-22 were moderately resistant in the disease reaction, while the remaining 9 genotypes *viz.*, GP-1, GP-2, GP-3, GP-4, GP-6, GP-7, GP-12, GP-15, and GP-20 were susceptible in the disease reaction.

Similar results were reported by Patil *et al.* (2002) [7] after evaluation of 20 cultivars of chilli against *C. capsici* and found none of the cultivar immune. The screening of chilli cultivars was also undertaken by Kasyap *et al.* (2008) [5], Naik *et al.* (2008) [1], Haq *et al.* (2013) [2]. Singh *et al.* (1993) [1] evaluated red ripe fruits of 19 varieties in laboratory and found lowest lesion size in varieties BGI and Lorai (5.75 and 6.00 mm, respectively) and graded them as resistant to fruit rot. Jeyalakshmi and Seetharaman (1998 b) [4] screened 40 genotypes of chilli against *C. capsici* in pot culture and reported that only one CA87-4 was highly resistant. Hegde and Anahosur (2002) [3] screened fifty chilli genotypes under natural conditions and found LCA-301, LCA-324, K-1 and Byadgi Kaddi as resistant. Parey *et al.* (2013) observed only DC-4, Arka Lohith, LCA-301, LCA-235 and LCA-333 exhibited moderately resistant reaction under both conditions.

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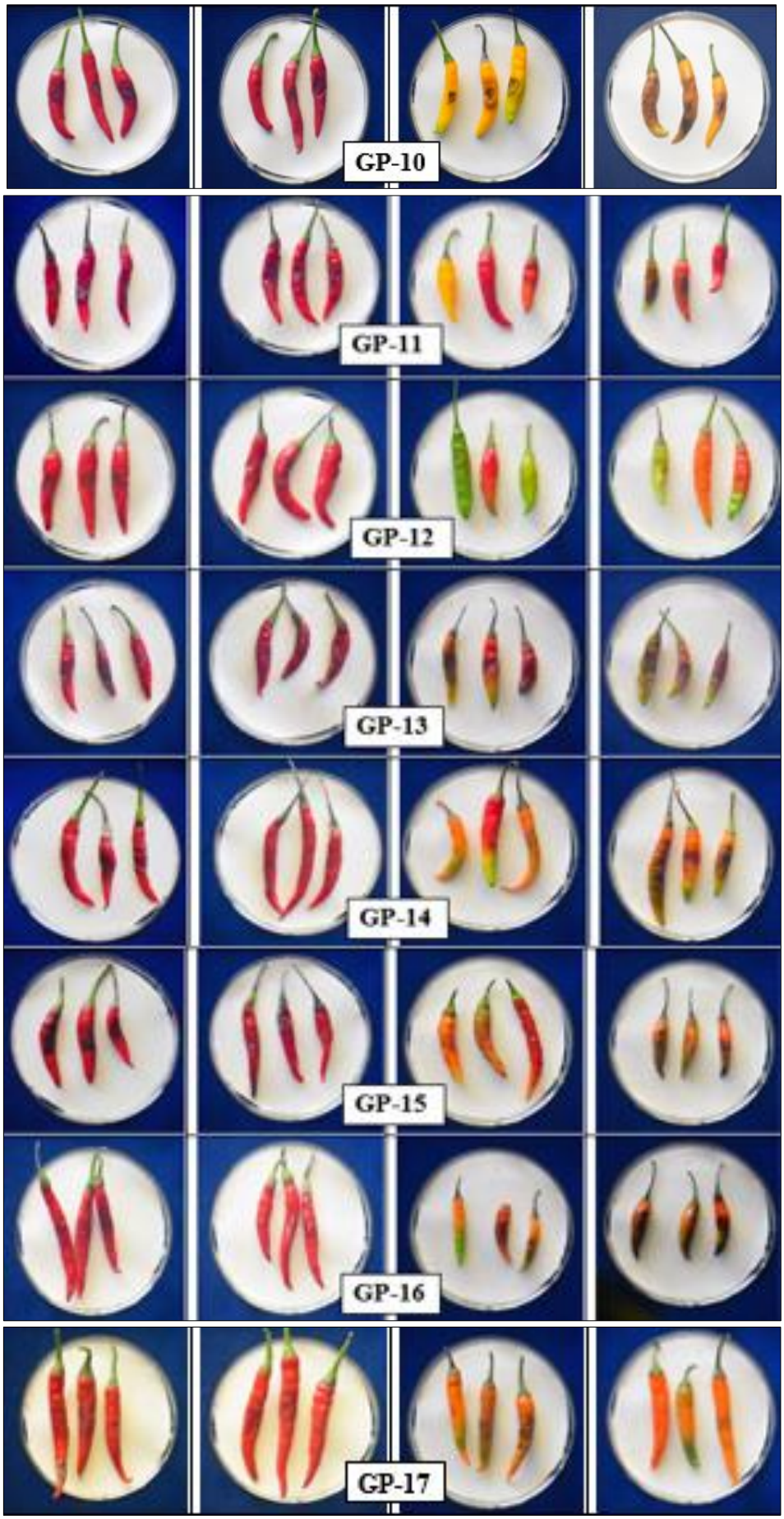
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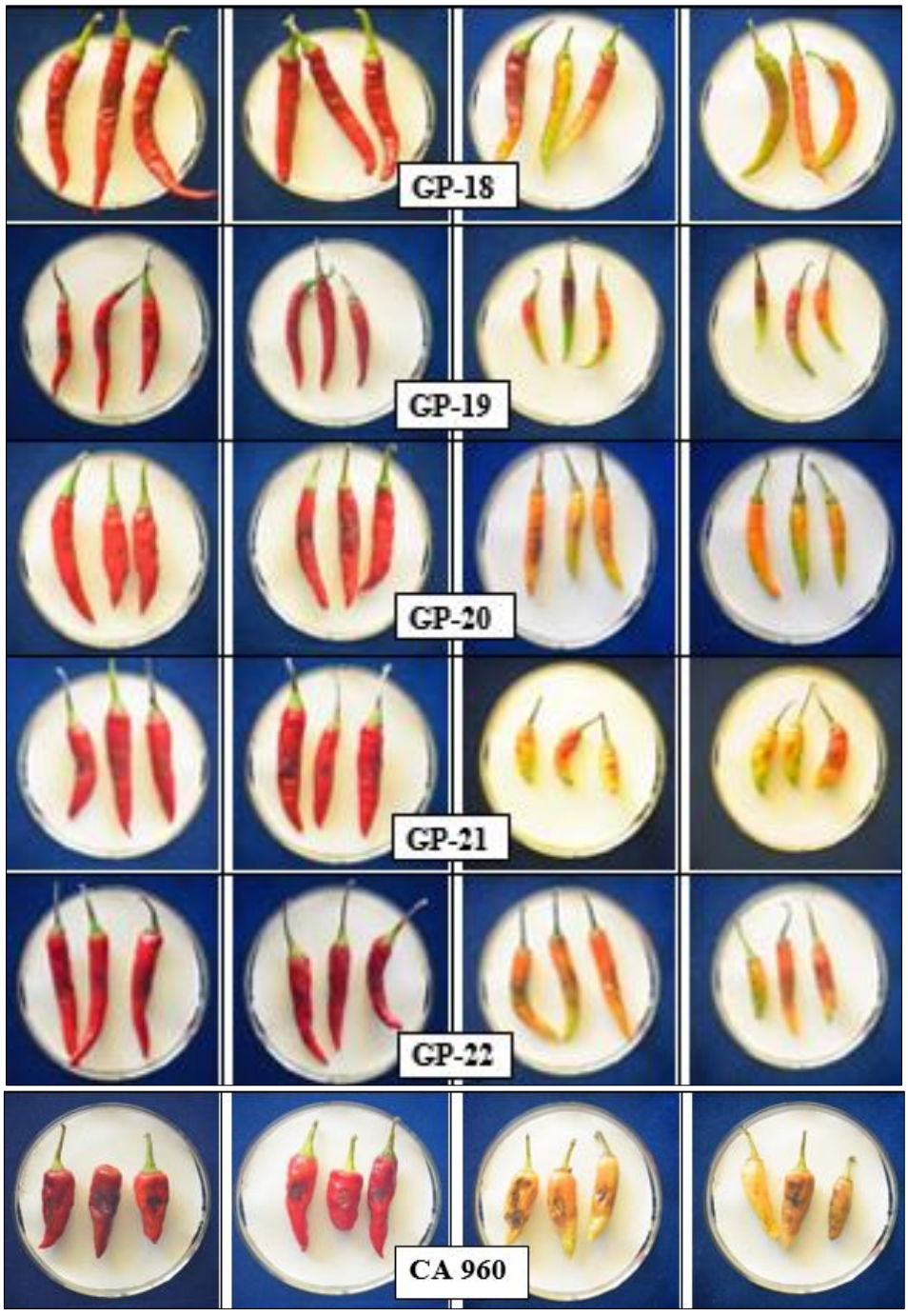

Fig 1: Screening of red and green chilli genotypes against *Colletotrichum truncatum* under *in vitro* conditions

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