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Evaluation of crucifer germplasm for black rot (*Xanthomonas campestris* pv. *campestris*) resistance

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ABSTRACT

Black rot is a devastating bacterial disease caused by *Xanthomonas campestris* pv*. campestris* (Pam.) Dowson, inflicting 10-50% losses in cauliflower production. Therefore, 41 accessions/ varieties of five crucifer species including *Brassica oleracea* var. *botrytis* L. (C genome), *B. rapa* (A genome), *B. napus* (AC genome), *B. carinata* (BC genome) and *Eruca sativa* were screened in field conditions against artificial inoculation of *Xcc* race 1. Mean disease severity and disease incidence (%) were observed in different accessions/ varieties of cauliflower (7.51 to 8.46, 88 to 94), *B. rapa* (4.81 to 8.8, 66.33 to 98.00), *B. carinata* (0.07 to 6.48, 7.33 to 87), *B. napus* (1.46 to 7.33, 31.00 to 96.66) and *E. sativa* (7.10 to 7.24, 86 to 88.33), respectively. All the varieties/ accessions of cauliflower and *E. sativa* were observed highly susceptible. Three accessions of *B. rapa* were found to be susceptible and remaining were classified into very susceptible types. Out of 16 accessions of *B. carinata* screened, 12 were susceptible, two were very susceptible, one was partially resistant and one was fully resistant. While in *B. napus,* three accessions as partial resistant, seven as susceptible and four were very susceptible against *Xcc* race 1. Newly identified *Xcc* resistant and partially resistant/ tolerant sources can be used for breeding black rot resistant cole crops by introgressing gene(s) of interest into *B. oleracea.*

Key words: Artificial epiphytotic, *Brassica* species, black rot resistance, cauliflower.

INTRODUCTION

Black rot disease caused by the gram negative bacterium *Xanthomonas campestris* pv. *campestris* (*Xcc*) (Pammel) Dowson is the most destructive disease in vegetable *Brassicas* worldwide (Vicente and Holub *et al*., 14). *Xanthomonas* bacterium infect the crop from nursery to seed production stage causing reduction in yield and quality ranging from 10 to 50% under congenial environmental conditions (Singh *et al*., 9). Since chemical control being more expensive, cumbersome, less effective, health hazardous and environment pollutant, therefore emphasis should be laid on search for more potent sources especially host plant resistance. Hence, resistance breeding is rewarding and environmentally safe tool by exploring available useful genetic sources harbouring resistance to this disease.

Screening and validation of germplasm to identify resistance source against disease is an essential step in resistance breeding programme. The presence of at least nine races of the pathogen makes breeding for black rot resistant cultivars a complex approach (Tonu *et al*., 13). Although, races 1 and 4 are thought as the main factors causing disease in cole crops, the extensive screening of *B. oleracea* accessions led to a conclusion that resistance to either of these two races did not exist or was very rare in contrast to common resistance to less important races (2, 3, and 6). The race specific resistance to races 1 and /or 4 is frequently found in species of alien *Brassica* other than *Brassica oleracea* (Taylor *et al*., 11). Resistance to known races of black rot in crucifers caused by *Xanthomonas campestris* pv. *campestris* is either absent or very rare in *B. oleracea* (Soengas *et al*., 10). The aim of this research was to evaluate crucifer species at juvenile stage to quantify the presence of *Xcc* resistance. There is an urgent need to unveil novel resistance sources in *Brassica* species for their utilization in vegetable *Brassica* resistance pre-breeding to develop durable resistant genetic stock (s) /line(s).

MATERIALS AND METHODS

The crucifer germplasm used in present study were maintained at research farm of Division of Vegetable Science, ICAR-IARI, New Delhi. Total 41 accessions/ varieties of five crucifer species including two varieties (Pusa Meghna and Pusa Sharad) of *B. oleracea* var. *botrytis* L. (C genome), 7 accessions (Toria Sangam, IC363774, IC363739, IC399828, Toria PT-303, TL-15, T-9) of *B. rapa* (A genome), 14 accessions (ISN-706, BN-4, BN-4-1, TR-4, ISN-233, ISN-733, PAC-401(Hyoloa), BN-1, BNCC-135, EC-33574, TERI-R-9013, TR-3, BNCC, BNCC-136) of *B. napus* (AC genome), 16 accessions (NPC-9,

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NPC-15, IGC-10, Kiran, NPC-5, NPC-11, NPC-12, NPC-14, NPC-16, HC-2, BCS-3, BCS-4, BCS-2, DLSC-1, NPC-13, Pusa Swarnima) of *B. carinata* (BC genome) and 2 accessions (T27, RTM-314) of *Eruca sativa* were screened on artificial inoculation of *Xcc* race 1 during October, 2010 to March, 2011.

Delhi isolate of *Xcc* race 1 was multiplied on Yeast Glucose Chalk Agar (YGCA) medium at 25ºC for 3 days. The culture was carefully scrapped from the media with sterilized slide. The scraped bacterial culture was mixed in 100 ml sterilized distilled water and mixed thoroughly by vortex and final concentration of 10^8-10^9 cfu/ ml was made. Three youngest leaves from ten plants of each accessions were inoculated in three replication with Delhi isolates of *Xcc* race 1 at 30 days after sowing in direct seed sown *Brassica* crops and in cauliflower at 45 days after transplanting by using leaf cut and dip technique. The plant leaves were inoculated by clipping 10 points to the secondary veins at the margins with small scissors dipped in the bacterial suspension. To maintain high humidity, sufficient moisture was maintained by frequent irrigation. The disease reaction was recorded twice at 15 and 30 DAI and the final score of disease reaction at 30 DAI.

The inoculated plants were assessed for disease reaction based on disease scores '0 to 9' and percentage of inoculated points in leaves showing symptoms were recorded as per scale given by Vicente *et al.* (15). The severity of symptoms was assessed on a six-point scale of 0 to 9 based on the relative lesion size (0 = no symptoms; 1 = small necrosis or chlorosis surrounding the infection point; 3 = typical small V-shaped lesion with black veins; 5 = typical lesion half way to the middle vein; 7 = typical lesion progressing to the middle vein; and 9 = lesion reaching the middle vein). Generally, plants with a score of 0, 1, or 3 with 0 to 25% of points showing symptoms were considered resistant; plants with a score of 3 with more than 25% of points showing symptoms and with a score of 5 with less than 50% of points showing symptoms were considered partially resistant; plants with a score of 5 with more than 50% of points showing symptoms and with a score of 7 with less than 75% of points showing symptoms were considered susceptible; and plants with a score of 7 with more than 75% of points showing symptoms and with a score of 9 were considered very susceptible. The data generated on disease incidence and disease severity were statistically analysed using randomized block design (Gomez and Gomez, 2).

RESULTS AND DISCUSSION

Utilization of host plant resistance is the most effective method to control *Xcc* and some common resistance sources are widely used in resistant cultivar breeding programme. It is essentially crucial that plant population must be exposed to pathogen in such a way that resistant and susceptible plants can be distinguished without any ambivalence revealing the efficacy of screening. All susceptible accessions exhibited typical V shape symptoms at 7 to 10 days after inoculation (DAI). In case of susceptible genotypes, the yellowish spot enlarged assuming 'V' shape developed from margin to the centre of the leaves. This resulted in collapsing of tissue within chlorotic lesions and darkening of veins extending from the lesion. The increase in severity of disease was due to yellowish growth, which increased towards mid rib of the leaves. As the disease progressed, the yellowing became more pronounced and exhibited black veins symptom at 30 days after inoculation. In case of resistant accessions, small necrosis or chlorosis surrounding the infection point were observed at 15 days after inoculation (DAI), while further growth was checked due to hypersensitive reaction. Typical small V-shaped lesion with black veins with less than 50% incidence were observed in partially resistant accessions, where a little further growth was found at 15 DAI and checked at 30 DAI. Disease severity and disease incidence (%) of crucifer accessions/ varieties against *Xcc* race 1 are given in Table 1 and illustrated in Fig. 1 & 2.

In cauliflower, the mean per cent disease incidence (PDI) varied from 88 to 94% after 30 days of inoculation. Maximum PDI value was recorded in Pusa Sharad (94%) followed by Pusa Meghna (88%) and severity ranged from 7.51 (Pusa Meghna) to 8.46 (Pusa Sharad). Typical V shape symptom as exhibited at 7 DAI and the yellowish spot enlarged from margin to the centre of the leaves. Disease severity and incidence progressed rapidly by collapsing of tissue within chlorotic lesions and black darkening of veins and reached up to mid veins. Commercial varieties of cauliflower, namely, Pusa Meghna (early group), Pusa Sharad (mid group) were observed highly susceptible with high disease severity and incidence. Similar screening findings were also reported earlier (Raghvendra *et al*., 5). Cauliflower varieties, namely, Pusa Himjyoti, Pusa Sharad, Pusa Shukti and Palam Uphar were reported susceptible, while BR-161 was resistant during *in vitro* evaluation against *Xcc* race 1 (Saha *et al*., 6). However, only few accessions, *viz*., Leamington and Late Enterprise out of the 61 cauliflower genotypes (Sharma *et al*., 8) and AL-15, BR-1, BR-202-2 (Saha *et al*., 7) were reported as resistant against *Xcc* pathogen. Most of them are not commercially grown by Indian farmers due to poor curd quality.

Table 1. Artificial screening of different accessions/ varieties of crucifer species against black rot disease (*Xcc* race 1) reactions.

Genotype	Disease severity (0-9)				Disease incidence (%)				Disease reaction
	15 DAI		30 DAI		15 DAI		30 DAI		
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	
B. rapa									
IC363739	3.16	1.53 - 5.36	4.85	2.27 - 6.60	55.66	36.66 - 70.00	69.33	40.00 - 80.00	S
IC 363774	2.98	1.23 - 3.86	4.81	2.10 - 6.03	55.00	33.33 - 66.66	66.33	43.33 - 83.33	S
IC399828	4.51	3.06 - 5.60	7.24	5.17 - 9.00	78.66	50.00 - 100	89.00	60.00 - 100	VS
Toria PT-303	5.70	5.30 - 6.36	8.80	8.10 - 9.00	80.00	70.00 - 90.00	98.00	90.00 - 100	VS
Toria (Sangam)	2.90	2.40 - 3.86	5.94	4.47 - 9.00	65.00	50.00 - 80.00	72.33	60.00 - 86.66	S
Toria TL-15	4.26	3.50 - 5.60	8.77	8.10 - 9.00	78.66	70.00 - 93.33	98.00	90.00 - 100	VS
Toria T-9	3.90	2.23 - 5.60	6.05	3.50 - 9.00	70.33	50.00 - 90.00	79.67	50.00 - 100	VS
B. carinata									
IGC10	3.06	2.30 - 4.90	5.60	3.50 - 8.10	65.33	43.33 - 73.33	74.66	50.00 - 86.66	S
NPC-9	0.06	0.00 - 0.19	0.07	0.07 - 0.24	6.33	0.00 - 8.33	7.33	6.67 - 15.00	R
Pusa Swarnima	4.43	2.86 - 4.09	6.48	3.70 - 9.00	77.33	60.00 - 100	87.00	70.00 - 100	VS
Kiran	3.43	2.30 - 5.36	5.52	4.26 - 6.60	56.66	43.33 - 70.00	69.66	50.00 - 80.00	S
NPC-5	2.61	2.07 - 3.66	3.93	3.27 - 5.37	56.00	43.33 - 73.33	64.00	46.66 - 83.33	S
NPC-11	3.14	1.53 - 5.36	4.18	2.27 - 6.60	55.00	36.66 - 70.00	69.00	40.00 - 80.00	S
NPC-12	3.44	2.30 - 5.36	5.52	4.26 - 6.60	57.33	43.33 - 70.00	67.00	50.00 - 80.00	S
NPC-13	2.60	1.50 - 3.83	6.28	4.16 - 9.00	64.33	50.00 - 76.66	82.33	70.00 - 100	VS
NPC-14	2.98	1.23 - 3.86	4.98	2.10 - 6.03	55.00	33.33 - 66.66	65.66	43.33 - 83.33	S
NPC-15	1.99	0.70 - 3.00	3.10	1.36 - 5.43	46.00	30.00 - 66.66	47.33	30.00 - 76.66	PR
NPC-16	3.30	2.07 - 4.90	5.21	3.27 - 8.10	65.00	50.00 - 73.33	71.00	60.00 - 90.00	S
HC-2	2.67	1.23 - 3.50	4.40	2.10 - 5.70	53.33	33.33 - 66.66	65.00	43.33 - 76.66	S
BCS-2	3.23	2.30 - 3.86	5.78	4.26 -7.80	58.33	43.33 - 66.66	72.66	50.00 - 86.66	S
BCS-3	2.55	1.83 - 4.13	5.34	3.00 - 9.00	60.00	40.00 - 80.00	72.33	50.00 - 100	S
BCS-4	3.29	2.30 - 3.86	5.17	4.26 - 5.93	60.33	50.00 - 83.33	68.00	50.00 - 83.33	S
DLSC-1	3.90	2.30 - 5.36	6.03	4.30 - 7.80	62.33	43.33 - 70.00	71.00	50.00 - 86.66	S
Eruca sativa									
RTM-314	4.51	3.06 - 5.60	7.24	5.17 - 9.00	79.00	50.00 - 100	88.33	60.00 - 100	VS
T-27	4.34	2.86 - 5.77	7.10	4.30 - 9.00	68.66	56.66 - 90.00	86.00	60.00 - 100	VS
B. napus									
BN-1	1.81	0.51 - 2.66	3.96	1.70 - 4.81	45.33	26.66 - 53.33	53.00	33.33 - 63.33	S
BN-4	1.70	1.00 - 2.93	2.72	1.87 - 4.47	37.67	30.00 - 53.33	45.33	40.00 - 58.66	PR
BN-4-1	1.53	0.9 - 1.90	2.23	1.57 - 2.83	36.00	30.00 - 43.33	43.00	40.00 - 50.00	PR
BNCC	3.63	1.40 - 5.66	6.09	2.8 - 9.00	68.33	40.00 - 100	84.66	40.00 - 100	VS
BNCC-136	2.11	1.30 - 2.53	5.82	3.53 - 8.20	49.33	43.33 - 56.67	90.00	46.67 - 100	VS
BNCC-135	2.20	1.80 - 3.55	4.01	3.27 - 5.85	49.00	43.00 - 65.00	62.66	53.33 - 85.00	S
EC-33574	1.63	1.10 - 2.37	4.40	2.84 - 7.00	39.00	33.33 - 43.33	73.33	46.66 - 100	S
ISN-233	1.81	1.05 - 2.03	3.45	2.33 - 5.00	44.00	36.00 - 60.00	60.00	33.33 - 100	S
ISN-706	0.69	0.40 - 1.30	1.46	1.16 - 1.67	22.33	16.66 - 30.00	31.00	30.00 - 40.00	PR
ISN-733	2.06	1.26 - 3.55	4.00	3.27 - 5.85	50.00	43.33 - 65.00	61.66	53.33 - 85.00	S
PAC401 (Hyola)	2.19	1.43 - 3.40	4.22	3.03 - 8.00	40.67	36.66 - 43.33	58.66	43.33 - 100	S
TERI-R-9013	3.90	1.73 - 5.66	7.10	5.67 - 8.33	86.00	53.33 - 100	96.66	90.00 - 100	VS
TR-3	3.99	1.20 - 7.00	7.33	5.00 - 9.00	75.66	40.00 - 100	96.66	90.00 - 100	VS
TR-4	2.71	1.83 - 5.20	5.27	4.03 - 7.67	57.33	43.33 - 93.33	71.67	50.00 - 100	S
B. oleracea var. *botrytis*									
Pusa Meghna	3.98	2.67 - 6.05	7.51	4.19 - 9.00	79.00	55.00 - 100	88.00	60.00 - 100	VS
Pusa Sharad	5.09	3.73 - 6.45	8.46	7.00 - 9.00	82.00	55.00 - 95.00	94.00	80.00 - 95.00	VS
CD at 5%	0.39		0.52		4.80		4.61		

R = resistant, PR = partially resistant, S = susceptible, VS = very susceptible

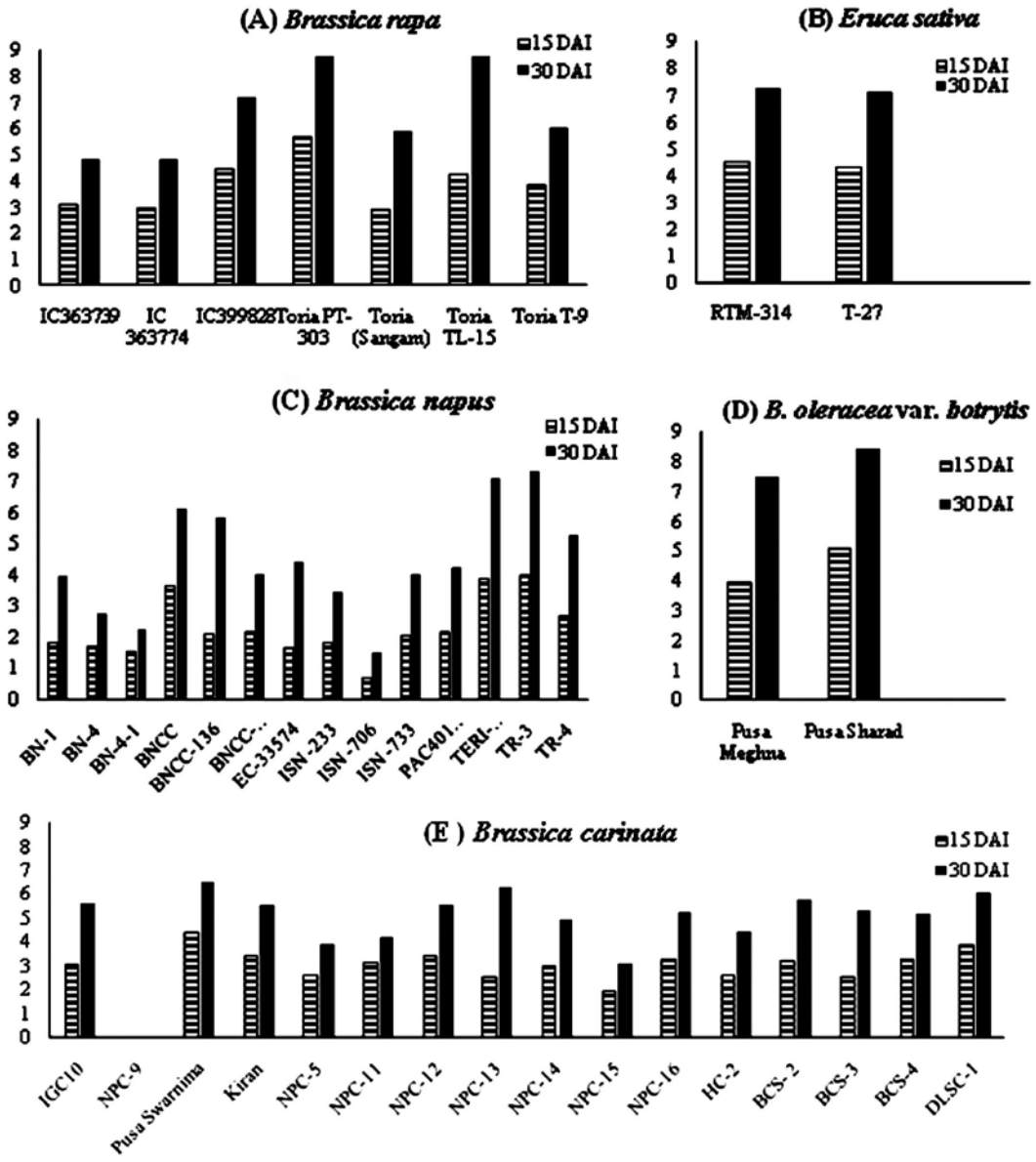

Fig. 1. (A-E) Black rot disease severity (0-9) (y-axis) against *Xcc* race 1 of different accessions/ varieties (x-axis) of crucifer species.

Huge variation in black rot disease reaction against *Xcc* race 1 between and within accessions of the *B. carinata* was observed, ranging from complete resistance to full susceptibility. In *B. carinata*, mean disease severity and disease incidence ranged from 0.07 to 6.48, 7.33-96.66%, respectively at 30 day after inoculation against *Xcc* race 1. The NPC-9 was observed with lowest disease severity (0.07) and disease incidence (7.33%) followed by NPC-15, while Pusa Swarnima had the maximum disease severity (6.48) and disease incidence (87%). Both extreme accessions could be used for searching novel gene(s) controlling resistance loci/ locus of black rot and development of mapping population to locate the gene(s). The disease reaction of different accessions of *B. carinata* were found to be resistant (NPC-9), partial resistant (NPC-15), susceptible (IGC-10, Kiran, NPC-5, NPC-11, NPC-12, NPC-14, NPC-16, HC-2, BCS-3, BCS-4, BCS-2, DLSC-1) and very susceptible (NPC-13, Pusa Swarnima). Alien *Brassica* species, namely, *B. nigra* (B genome), *B. carinata* (BC genome), *B. juncea* (AB genome) unveiled resistance against *Xcc* races 1, 3, 4 hypothesizing probable B genome origin (Taylor *et al*., 11). Griffiths *et al*. (3) reported resistance

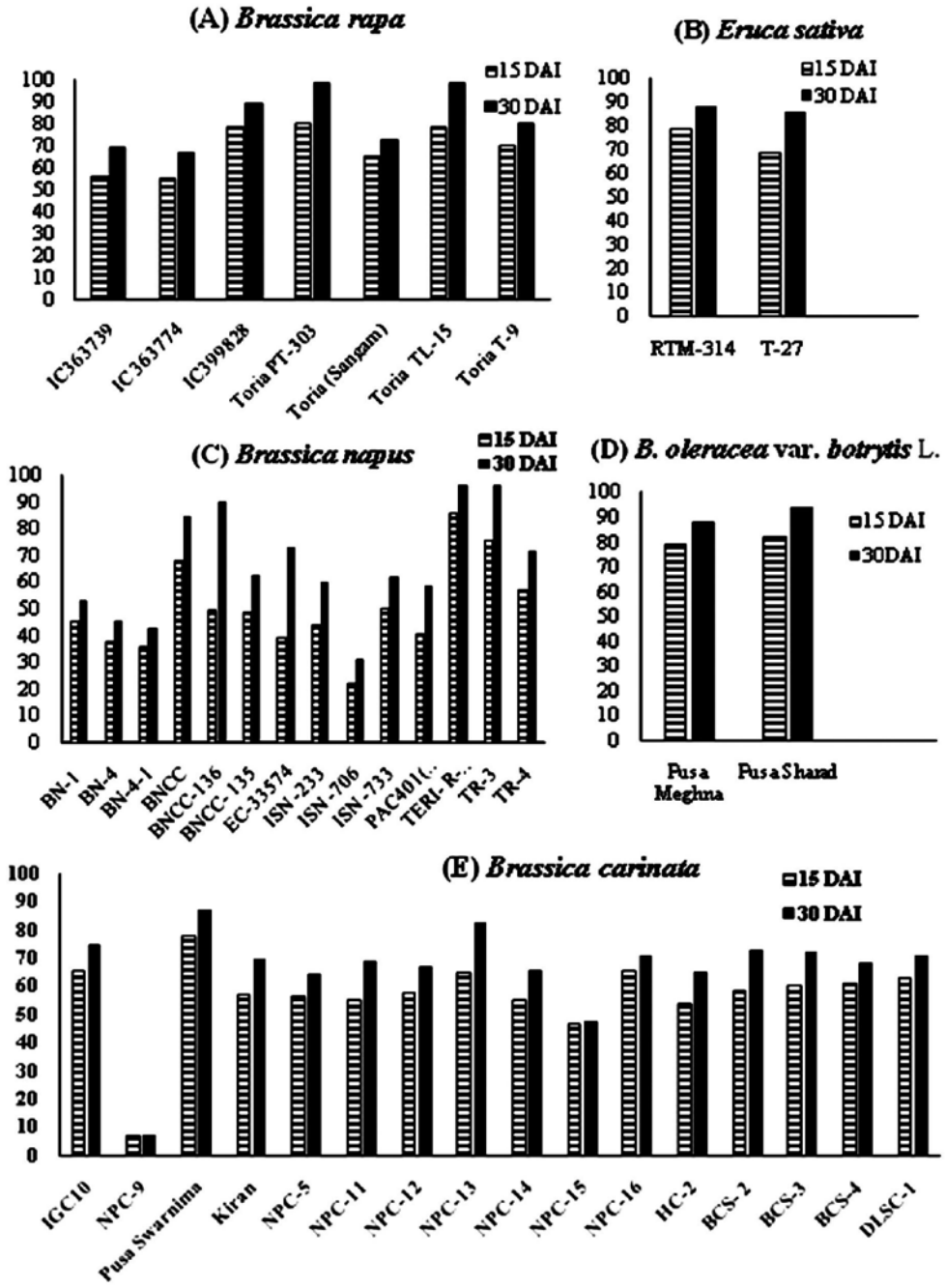

Fig. 2. (A-E) Black rot disease incidence (%) (y-axis) against *Xcc* race 1 of different accessions/ varieties (x-axis) of crucifer species.

in five accessions of *B. carinata* out of 63 evaluated (PI193460, PI193959, PI194254, PI280230, PI633077) determined by repeated symptomless responses after inoculation. Earlier, Tonguc and Griffiths (12) evaluated 54 accessions of *B. carinata* (BBCC) for black rot resistance and only two accessions A19182 and A19183 were found free from any disease symptoms when inoculated with *Xcc* for all the plants tested.

In *B. napus*, the mean per cent disease incidence (PDI) varied from 31.00 to 96.66 after 30 days of inoculation. Maximum PDI (96.66) value was recorded in TR-3, TERI- R-9013 followed by BNCC136, while ISN-706 had minimum value of PDI (31%). Black rot severity was found lowest (1.46) in ISN-706 and highest (7.33) in TR-3 followed by TERI-R-9013. However, no accessions were found resistant to *Xcc*

race 1. Three accessions, namely, BN-4, BN-4-1 and ISN-706 were observed with partial resistance. Seven genotypes, namely, TR-4, ISN-233, ISN-733, PAC-401 (Hyoloa), BN-1, BNCC-135 and EC-33574 were categorized as susceptible, whereas TERI-R-9013, TR-3, BNCC, BNCC-136 were rated as very susceptible against *Xcc* race 1.

No race-specific resistance was found to *Xcc* race 1 in *B. napus*. However, previous findings demonstrated that resistance to *Xcc* race 4 (but not *Xcc* race 1) was most common in *B. rapa* (A genome) and *B. napus (*AC genome), which suggested its A genome origin (Taylor *et al*., 11). The most of the accessions of *B. napus* were susceptible and highly susceptible except BN-4, BN-4-1 and ISN-706, which had partial resistance against *Xcc* race 1. These findings are also in line with those of Lema *et al*. (4) who also reported the race 1 was more virulent than race 4 after screening 76 accessions belonging to four *B. napus* groups

In *B. rapa*, mean PDI varied from 66.33 to 98.00, while mean disease severity ranged between 4.81 to 8.8. Toria PT-303 was observed with maximum disease incidence and severity for *Xcc* race 1. No single accession was found resistant or partial resistant. On the basis of disease severity and incidence (%), the accessions, namely, Toria (Sangam), IC363774 and IC363739 were categorized as susceptible, whereas IC399828, Toria PT-303, TL-15 and T-9 as highly susceptible. However, these findings were contradictory to those of Dias *et al*. (1). They reported huge variations in black rot disease reactions against *Xcc* races 1 and 4 between and within accessions of the *B. rapa*, ranging from complete resistance to full susceptibility. Two accessions of *Eruca sativa* RTM-314 and T-27 were observed very susceptible with more than 75% incidence, while, disease incidence (%) and disease severity ranged from 86 to 88.33, 7.10 to 7.24, respectively. These findings were contradictory to those of Griffith *et al.* (3) as they identified incomplete resistance in all the accessions of *Eruca sativa*.

In conclusion, the newly identified resistant accession NPC-9 of *B. carinata*, partially resistant accessions, namely, NPC-15 of *B. carinata* and BN-4, BN-4-1, ISN-706 of *B. napus* can be used for breeding black rot resistant cole vegetable varieties by introgressing gene(s) of interest into *B. oleracea* employing protoplast fusion and embryo rescue techniques. This effort will go a long way in resistance breeding against black rot in cole crops, especially cauliflower and cabbage for developing durable resistance to minimize dependency on chemical protectants. The broad spectrum resistance from *Brassica* species on its own or in combination with strong race-specific resistance can contribute to the long-term control of the disease.

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