

**SHORT COMMUNICATION**

**CHARACTERIZATION OF ITALIAN POPULATIONS OF *XANTHOMONAS*  
*CAMPESTRIS* pv. *CAMPESTRIS* USING PRIMERS BASED  
ON DNA REPETITIVE SEQUENCES**

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**SUMMARY**

One-hundred and forty-one strains of *Xanthomonas campestris* pv. *campestris* (*Xcc*), the causal agent of vascular blackening of crucifers were isolated from different crucifer plants in central and southern Italian regions and characterized for DNA polymorphism by PCR, using primers based on repetitive sequences. By M13-PCR, all strains, except for two, were distributed in three main clusters:  $\alpha$ , with similarity index (SI) 0.78, containing 70 strains grouped in 19 haplotypes;  $\beta$  (SI = 0.80) containing 54 strains in 17 haplotypes;  $\gamma$  (SI = 0.75) containing 15 strains in 6 haplotypes. By BOX-PCR, all strains were distributed in three main clusters:  $\alpha$  (SI = 0.58) containing 102 strains distributed in 9 haplotypes;  $\beta$  (SI = 0.45), including 24 strains distributed in 9 haplotypes;  $\gamma$  (SI = 0.37), including 15 strains distributed in 3 haplotypes. A very large variability was observed in these Italian populations of *Xcc* especially using M13-PCR. No obvious relationships were observed between the strains comprised in each cluster, sub-cluster or haplotype and their geographic origin or host plant.

**Key words:** phyto-bacteria, crucifers, PCR, M13, BOX.

*Xanthomonas campestris* pv. *campestris* (*Xcc*) is a widespread agent of black rot/vascular blackening of crucifers that damages all crucifer crops. Typical disease symptoms consist of V-shaped yellow lesions at the margins of the leaves and blackening of vascular tissues (Mazzucchi, 1968; Schaad and Alvarez, 1993). Molecular characterization of the bacterium by M13-PCR and rep-PCR showed very large variability (Zaccardelli, 2001; Zaccardelli *et al.*, 2002, 2006; Tsygankova *et al.*, 2004).

One-hundred and forty-one *Xcc* strains (Table 1), all of which induced hypersensitive reactions in bean pods and whose identity had been confirmed by a specific PCR protocol amplifying the *brcC* gene of the *hrp/brc*

*Xcc* cluster (Zaccardelli *et al.*, 2007), were collected from several crucifers showing typical disease symptoms (broccoli, cabbage, cauliflower, crambe, kale, kohlrabi, rutabaga, savoy cabbage and a *Brassica* sp.) in different Italian regions (Campania, Emilia Romagna, Latium, Marche, Apulia, Sicily, Tuscany and Umbria). These bacterial isolates were characterized by PCR-based analyses, using the primers M13 and BOX, designed on repetitive DNA sequences. Characterization included the outgroup strains: *X. vesicatoria* LNPV 4-77, *X. arboricola* pv. *pruni* NCPPB 1607, *X. translucens* pv. *cerealis* NCPPB 3212, and *X. axonopodis* pv. *phaseoli* NCPPB 1420. All strains were routinely grown on YD-CA (Stolp and Starr, 1964) at 27°C for 48 h. Total genomic DNA was extracted and purified from bacterial pellets, collected after growth on nutrient glucose agar (NGA) (Civerolo, 1990), using the CTAB-method (Wilson, 1989). DNA concentration was measured spectrophotometrically using a Bio-photometer (Eppendorf, Germany) and DNA was stored at -80°C until used.

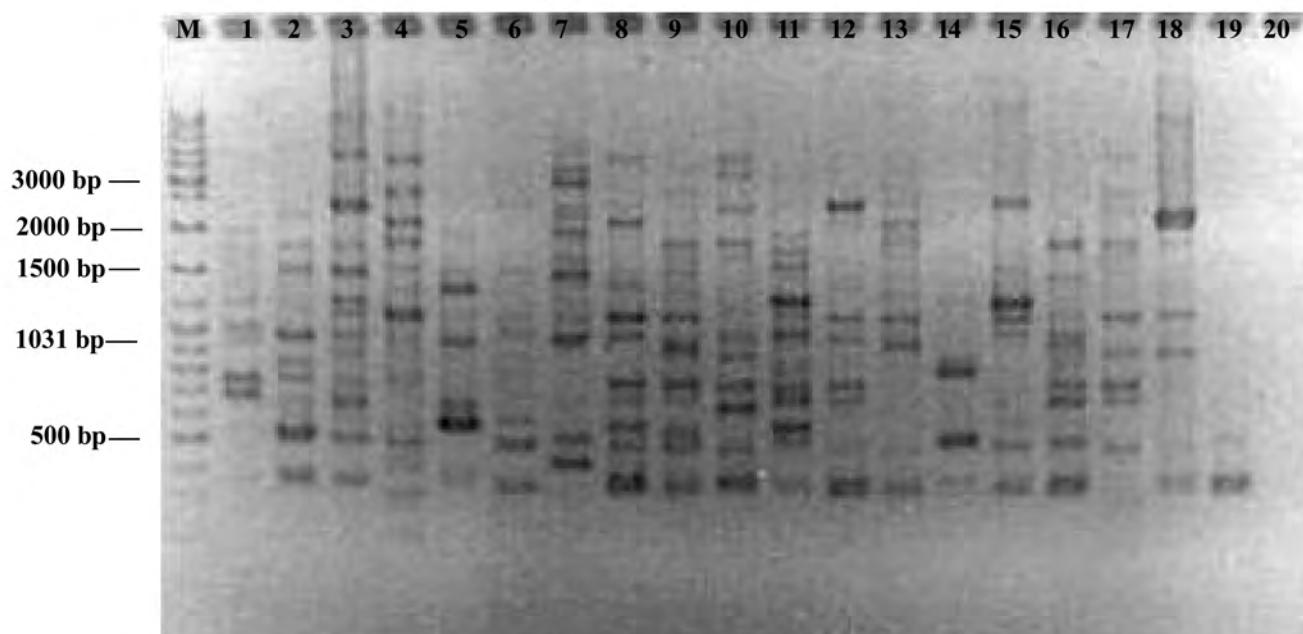
Characterization by M13-PCR was done in 25  $\mu$ l reaction mixture containing 50 ng M13-primer (5'-GAGGGTGGCGGTTCT-3'), 200  $\mu$ M dNTPs, 3 mM MgCl<sub>2</sub>, 1 U *Taq*-DNA polymerase (Sigma, Germany) and, as a template, 25 ng DNA. The amplification program was: 94°C for 30 sec (1 min in the first cycle), 55°C for 1 min, 72°C for 1 min, for a total of 40 cycles and a final extension at 72°C for 6 min.

Characterization by rep-PCR was done according to the protocol by Rademaker and de Bruijn (1997), using the primer BOX (5'-CTACGGCAAGGCGACGCT-GACG-3') and 25 ng of DNA.

Amplification products were stored at 4°C until visualization by standard agarose gel electrophoresis and ethidium bromide staining. Gel concentration was 1.5% and electrophoresis was in TAE buffer. As molecular marker, a DNA Ladder Mix (M-Medical-Genenco, Italy) was used in the gels containing the amplicons of M13-PCR, whereas a Ladder Mix by MBI Fermentas (Canada) was used in the gels containing BOX-PCR. Amplicons.

Two distinct (one for M13-primer and one for BOX-primer) rectangular binary matrixes (1 = presence of band, 0 = absence of band) were constructed based on

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**Fig. 1.** Gel electrophoresis of M13-PCR products from *Xanthomonas campestris* pv. *campestris* strains. Lane M: molecular weight marker DNA Ladder Mix (M-Medical-Genenco, Italy); lane 1: ISPaVe 1032; lane 2: ISPaVe 016; lane 3: ISCI 1; Lane 4: ISCI 12; lane 5: ISCI 31; lane 6: ISCI 48; lane 7: IPV-NA 38; lane 8: IPV-NA 1'; lane 9: IPV-NA 39; lane 10: ISCI 70; lane 11: ISCI 13; lane 12: ISCI 74; lane 13: ISCI 88; lane 14: ISCI 8 EP.; lane 15: IPV-CT 62.4; lane 16: IPV-CT 66.1; lane 17: IPV-CT 67.2; lane 18: DAPP-PG 327; lane 19: DAPP-PG 328; lane 20: negative control (water).

gel patterns. Using the software NTSYS version 2.0, the similarity triangular matrixes were constructed using the band-based Dice similarity coefficient (SD). From each similarity matrix, the unweighted pair group method with arithmetic mean (UPGMA) was used to cluster the patterns.

Amplicons of 300-3000 and 200-2500 bp in size and a total of 38 and 26 markers useful to construct the matrixes were obtained with M13-PCR (Fig. 1) and BOX-PCR (Fig. 2), respectively. Cophenetic values of 0.87 (M13-PCR) and 0.90 (BOX-PCR) were determined for these matrixes, indicating a high goodness-of-fit for the cluster analysis.

By M13-PCR, a total of 43 *Xcc* haplotypes were obtained (Table 1) and all *Xcc* isolates, except for 2 belonging to the haplotype V, were contained in a super-cluster, named  $\delta$ , with a similarity index (SI) of 0.72 (Fig. 3). This super-cluster was subdivided in three main clusters:  $\alpha$  with SI = 0.78, containing 70 isolates grouped in 19 haplotypes;  $\beta$  (SI = 0.80) containing 54 isolates grouped in 17 haplotypes;  $\gamma$  (SI = 0.75) containing 15 isolates grouped in 6 haplotypes and the *Xanthomonas vesicatoria* outgroup strain. The other three outgroup strains (*X. arboricola* pv. *pruni*, *X. translucens* pv. *cerealis* and *X. axonopodis* pv. *phaseoli*) were outside the super-cluster  $\delta$ .

By BOX-PCR, a total of 21 *Xcc* haplotypes were obtained (Table 1) all comprised in one super-cluster,

named  $\delta$ , with SI = 0.26 (Fig. 4). This super-cluster was subdivided in three main clusters:  $\alpha$ , with SI = 0.58, containing the major part of the isolates (102) distributed in 9 haplotypes;  $\beta$  (SI = 0.45), including 24 isolates distributed in 9 haplotypes;  $\gamma$  (SI = 0.37), including 15 isolates distributed in three haplotypes and three *Xanthomonas* spp. outgroup strains. The outgroup strain of *X. arboricola* pv. *pruni* was outside (SI = 0.15) the super-cluster  $\delta$ . No significant correlation ( $P \leq 0.01$ ) was found between the BOX-PCR similarity matrix and the M13-PCR one, as revealed by the low (0.163) Pearson's product-moment correlation coefficient.

These results suggest that primer M13 is superior to primer BOX in discriminating genetically different *Xcc* isolates (M13-PCR yielded twice more haplotypes) and that a very large variability exists in Italian populations of *Xcc*.

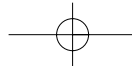
No obvious relations were observed between the strains contained in each cluster, sub-cluster or haplotype and their geographic origin or host plant.

A different frequency of each haplotype was observed in each Italian region. For instance, a higher number of haplotypes was found in Umbria with respect to Campania, probably because of the higher number of Umbrian localities from which infected samples were collected. At least three of four European *Xcc* isolates showed the same fingerprinting profile of some Italian isolates.

**Table 1.** Bacterial strains of *Xanthomonas campestris* used in this study.

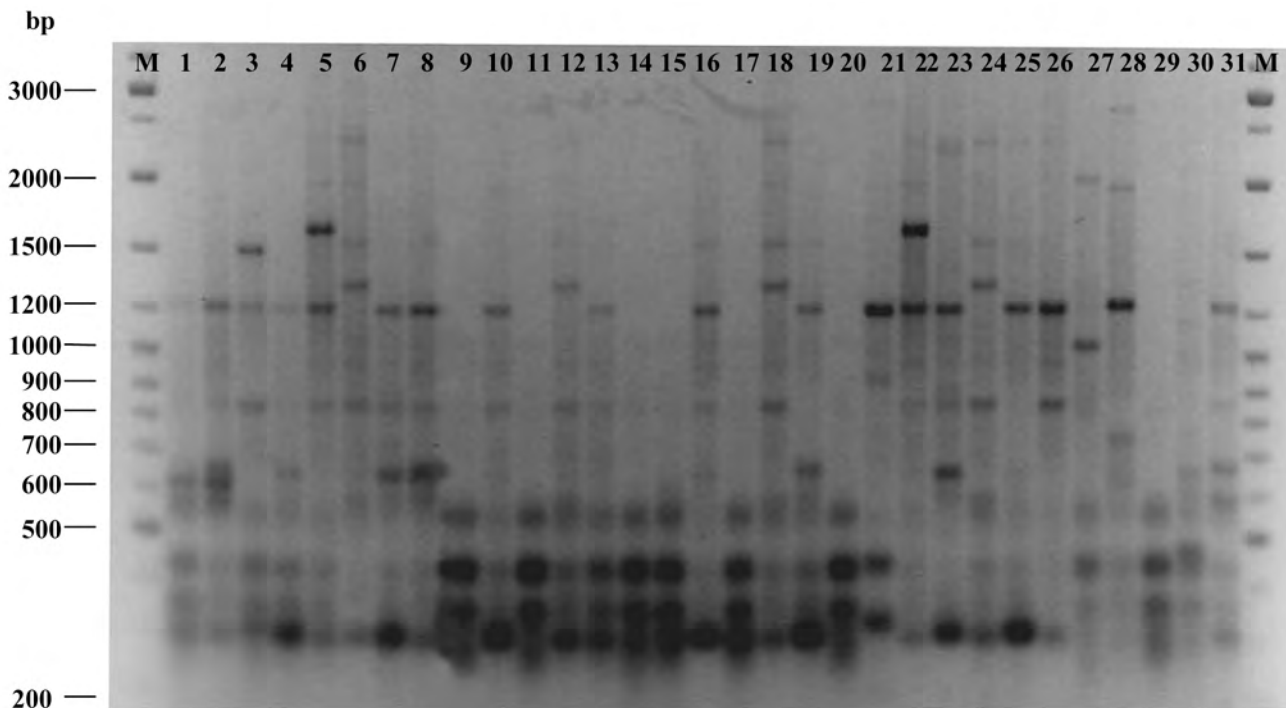
Strains	Plant host	Italian Region / Country (Locality, District)	M13 haplotypes	BOX haplotypes
ISCI 1	Kohlrabi	Campania (Battipaglia, Salerno)	C	B
ISCI 2	Kohlrabi	Campania (Battipaglia, Salerno)	C	A
ISCI 3	Kohlrabi	Campania (Battipaglia, Salerno)	D	A
ISCI 4	Kohlrabi	Campania (Battipaglia, Salerno)	C	A
ISCI 5	Kohlrabi	Campania (Battipaglia, Salerno)	C	C
ISCI 6	Kohlrabi	Campania (Battipaglia, Salerno)	C	A
ISCI 7	Kohlrabi	Campania (Battipaglia, Salerno)	C	A
ISCI 8	Kohlrabi	Campania (Battipaglia, Salerno)	D	A
ISCI 8 EP	Cauliflower	Campania (Battipaglia, Salerno)	S	E
ISCI 12	Cauliflower	Campania (Battipaglia, Salerno)	D	A
ISCI 13	Cauliflower	Campania (Battipaglia, Salerno)	C	E
ISCI 14	Cauliflower	Campania (Battipaglia, Salerno)	A	E
ISCI 15	Cauliflower	Campania (Battipaglia, Salerno)	C	C
ISCI 16	Kohlrabi	Campania (Battipaglia, Salerno)	C	G
ISCI 20	Kohlrabi	Campania (Battipaglia, Salerno)	D	C
ISCI 20 EP	Kale	Campania (Battipaglia, Salerno)	K	B
ISCI 22	Kohlrabi	Campania (Battipaglia, Salerno)	D	A
ISCI 25	Kohlrabi	Campania (Battipaglia, Salerno)	D	A
ISCI 31	Broccoli	Campania (Battipaglia, Salerno)	A	H
ISCI 32	Broccoli	Campania (Battipaglia, Salerno)	A	H
ISCI 38	Kale	Campania (Battipaglia, Salerno)	C	B
ISCI 42	Kale	Campania (Battipaglia, Salerno)	A	B
ISCI 44	Kale	Campania (Battipaglia, Salerno)	A	B
ISCI 45	Savoy cabbage	Campania (Battipaglia, Salerno)	A	B
ISCI 48	Cauliflower	Campania (Battipaglia, Salerno)	A	B
ISCI 50	Cauliflower	Campania (Battipaglia, Salerno)	A	B
ISCI 55	Cauliflower	Campania (Battipaglia, Salerno)	A	B
ISCI 59	Kale	Campania (Battipaglia, Salerno)	A	B
ISCI 61	Kale	Campania (Battipaglia, Salerno)	A	G
ISCI 62	Kale	Campania (Battipaglia, Salerno)	A	B
ISCI 64	Kale	Campania (Battipaglia, Salerno)	A	B
ISCI 65	Kale	Campania (Battipaglia, Salerno)	A	B
ISCI 67	Cauliflower	Campania (Battipaglia, Salerno)	A	B
ISCI 70	Crambe	Campania (Battipaglia, Salerno)	E	I
ISCI 72	Crambe	Campania (Battipaglia, Salerno)	E	H
ISCI 73	Crambe	Campania (Battipaglia, Salerno)	M	C
ISCI 74	Crambe	Campania (Battipaglia, Salerno)	E	A
ISCI 75	Crambe	Campania (Battipaglia, Salerno)	D	I
ISCI 76	Crambe	Campania (Battipaglia, Salerno)	E	C
ISCI 88	Cabbage	Campania (Pontecagnano, Salerno)	M	A
ISCI 117	Brassica spp.	Campania (Oliveto Citra, Salerno)	K	C
ISCI 119	Brassica spp.	Campania (Oliveto Citra, Salerno)	ISCI 119	C
ISCI 120	Brassica spp.	Campania (Oliveto Citra, Salerno)	K	H
ISCI 121	Brassica spp.	Campania (Oliveto Citra, Salerno)	K	C
ISCI 122	Kale	Campania (Oliveto Citra, Salerno)	S	C
ISCI 127	Kale	Campania (Battipaglia, Salerno)	I	E
ISCI 129	Kale	Campania (Battipaglia, Salerno)	ISCI 129	ISCI 129
ISCI 130	Kale	Campania (Battipaglia, Salerno)	I	G
DAPP-PG 247	Cabbage	Umbria (Todi, Perugia)	DAPP-PG 247	L
DAPP-PG 248	Cabbage	Umbria (Todi, Perugia)	DAPP-PG 248	D
DAPP-PG 249	Cabbage	Umbria (Todi, Perugia)	J	L
DAPP-PG 305	Cauliflower	Umbria (S. Andrea d'Agliano, Perugia)	O	A
DAPP-PG 306	Cauliflower	Umbria (S. Andrea d'Agliano, Perugia)	Q	A
DAPP-PG 308	Cauliflower	Marche (Monte Rubbiano, Ascoli Piceno)	DAPP-PG 308	A
DAPP-PG 309	Cauliflower	Marche (Monte Rubbiano, Ascoli Piceno)	Q	A
DAPP-PG 310	Cauliflower	Marche (Monte Rubbiano, Ascoli Piceno)	F	B
DAPP-PG 311	Cauliflower	Marche (Monte Rubbiano, Ascoli Piceno)	DAPP-PG 311	B

DAPP-PG 313	Cauliflower	Umbria (Vernazzano, Perugia)	B	A
DAPP-PG 314	Cauliflower	Umbria (Vernazzano, Perugia)	B	A
DAPP-PG 315	Cauliflower	Umbria (Vernazzano, Perugia)	G	B
DAPP-PG 316	Cauliflower	Umbria (Vernazzano, Perugia)	DAPP-PG 316	A
DAPP-PG 319	Cauliflower	Umbria (Vernazzano, Perugia)	B	A
DAPP-PG 320	Cauliflower	Umbria (Vernazzano, Perugia)	B	G
DAPP-PG 321	Cauliflower	Umbria (Vernazzano, Perugia)	J	A
DAPP-PG 322	Cauliflower	Umbria (Vernazzano, Perugia)	J	A
DAPP-PG 323	Cauliflower	Marche (Monte Rubbiano, Ascoli Piceno)	B	A
DAPP-PG 324	Cauliflower	Umbria (Vernazzano, Perugia)	J	A
DAPP-PG 327	Cauliflower	Umbria (Vernazzano, Perugia)	P	A
DAPP-PG 328	Cauliflower	Umbria (Gioiella, Perugia)	I	DAPP-PG 328
DAPP-PG 329	Cauliflower	Umbria (Gioiella, Perugia)	I	G
DAPP-PG 330	Cauliflower	Umbria (Gioiella, Perugia)	DAPP-PG 330	DAPP-PG 330
DAPP-PG 333	Cauliflower	Umbria (S. Arcangelo, Perugia)	G	B
DAPP-PG 334	Cauliflower	Umbria (Collazzone, Perugia)	L	E
DAPP-PG 335	Cauliflower	Umbria (Collazzone, Perugia)	L	E
DAPP-PG 336	Cauliflower	Umbria (Collazzone, Perugia)	N	A
DAPP-PG 337	Cauliflower	Umbria (Collazzone, Perugia)	L	E
DAPP-PG 338	Cauliflower	Toscana (Sinalunga, Siena)	G	B
DAPP-PG 339	Cauliflower	Toscana (Sinalunga, Siena)	B	A
DAPP-PG 340	Cauliflower	Umbria (Marsciano, Perugia)	B	A
DAPP-PG 341	Cauliflower	Umbria (Marsciano, Perugia)	N	A
DAPP-PG 342	Cauliflower	Umbria (Marsciano, Perugia)	N	DAPP-PG 342
DAPP-PG 343	Cauliflower	Umbria (Collazzone, Perugia)	L	E
DAPP-PG 344	Cauliflower	Umbria (Collazzone, Perugia)	DAPP-PG 344	C
DAPP-PG 345	Cauliflower	Umbria (Collazzone, Perugia)	DAPP-PG 345	C
DAPP-PG 349	Cabbage	Umbria (Marsciano, Perugia)	U	A
DAPP-PG 350	Cabbage	Umbria (Marsciano, Perugia)	U	A
DAPP-PG 351	Cabbage	Umbria (Marsciano, Perugia)	DAPP-PG 351	A
DAPP-PG 352	Cabbage	Umbria (Marsciano, Perugia)	DAPP-PG 352	A
DAPP-PG 353	Cauliflower	Marche (Moresco, Ascoli Piceno)	H	A
DAPP-PG 354	Cauliflower	Marche (Carassai, Ascoli Piceno)	H	F
DAPP-PG 355	Cauliflower	Marche (Carassai, Ascoli Piceno)	H	DAPP-PG 355
DAPP-PG 356	Cauliflower	Marche (Carassai, Ascoli Piceno)	H	A
DAPP-PG 357	Cauliflower	Marche (Moresco, Ascoli Piceno)	H	A
DAPP-PG 358	Cauliflower	Marche (Moresco, Ascoli Piceno)	T	A
DAPP-PG 359	Cauliflower	Marche (Moresco, Ascoli Piceno)	T	A
DAPP-PG 361	Cauliflower	Marche (Moresco, Ascoli Piceno)	DAPP-PG 361	A
DAPP-PG 362	Cauliflower	Marche (Rubbiano, Ascoli Piceno)	F	B
DAPP-PG 363	Cauliflower	Marche (Rubbiano, Ascoli Piceno)	F	B
DAPP-PG 364	Cauliflower	Marche (Rubbiano, Ascoli Piceno)	F	B
DAPP-PG 365	Cauliflower	Umbria (Marsciano, Perugia)	M	C
DAPP-PG 366	Cauliflower	Umbria (Perugia)	F	F
DAPP-PG 367	Cauliflower	Umbria (Perugia)	P	D
DAPP-PG 368	Cauliflower	Umbria (Perugia)	O	A
DAPP-PG 369	Cauliflower	Umbria (Perugia)	DAPP-PG 369	DAPP-PG 369
DAPP-PG 370	Cauliflower	Umbria (Perugia)	B	A
DAPP-PG 371	Cauliflower	Umbria (Perugia)	P	DAPP-PG 371
DAPP-PG 372	Cauliflower	Umbria (Perugia)	Q	A
DAPP-PG 373	Cauliflower	Umbria (Perugia)	DAPP-PG 373	D
DAPP-PG 375	Cauliflower	Marche (Carassai, Ascoli Piceno)	DAPP-PG 375	F
DAPP-PG 376	Cauliflower	Marche (Carassai, Ascoli Piceno)	Z	F
DAPP-PG 377	Cauliflower	Marche (Rubbiano, Ascoli Piceno)	F	B
DAPP-PG 398	Rutabaga	Marche (Monte Rubbiano, Ascoli Piceno)	O	A
DAPP-PG 399	Rutabaga	Marche (Monte Rubbiano, Ascoli Piceno)	V	D
DAPP-PG 400	Rutabaga	Marche (Monte Rubbiano, Ascoli Piceno)	B	A
DAPP-PG 401	Rutabaga	Marche (Monte Rubbiano, Ascoli Piceno)	B	A
DAPP-PG 402	Rutabaga	Marche (Monte Rubbiano, Ascoli Piceno)	B	A
DAPP-PG 403	Rutabaga	Marche (Monte Rubbiano, Ascoli Piceno)	B	A
DAPP-PG 404	Savoy cabbage	Umbria (Marsciano, Perugia)	B	A
DAPP-PG 405	Savoy cabbage	Umbria (Perugia)	B	A

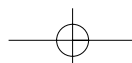


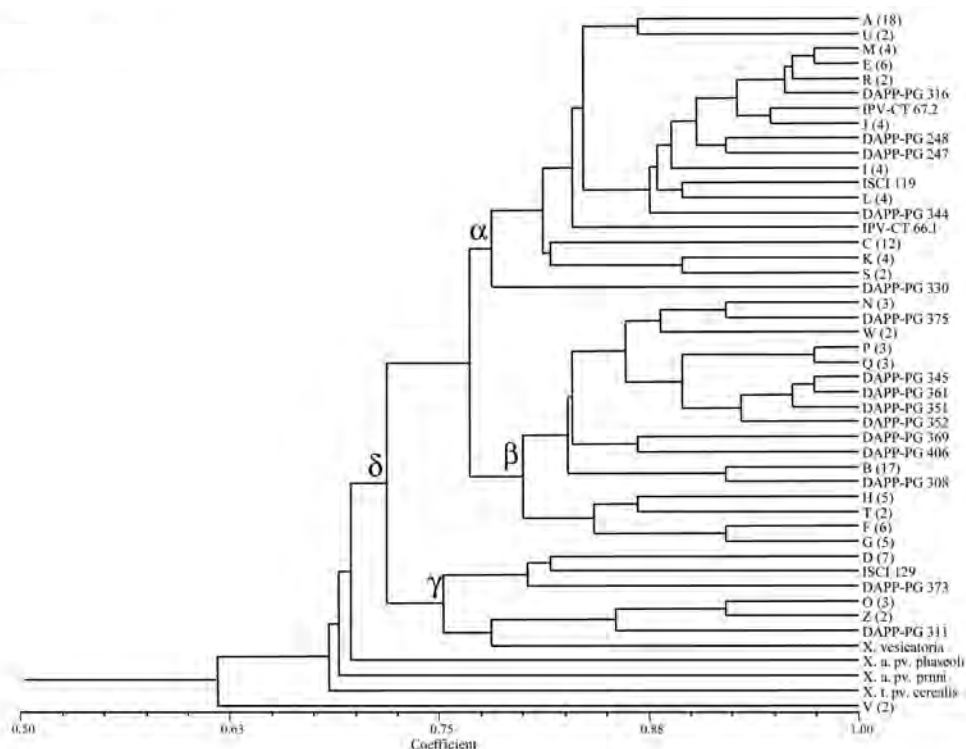
DAPP-PG 406	Savoy cabbage	Umbria (Perugia)	DAPP-PG 406	DAPP-PG 406
DAPP-PG 410	Cabbage	Umbria (Perugia)	V	A
DAPP-PG 411	Cabbage	Umbria (Perugia)	G	B
DAPP-PG 412	Cabbage	Umbria (Perugia)	G	B
DAPP-PG 413	Cabbage	Umbria (Perugia)	W	D
DAPP-PG 414	Cabbage	Umbria (Perugia)	W	D
IPV-CT 62.4	Kohlrabi	Sicilia	R	F
IPV-CT 63.1	Kohlrabi	Sicilia	M	F
IPV-CT 65.3	Broccoli	Sicilia	R	D
IPV-CT 66.1	Broccoli	Sicilia	IPV-CT 66.1	D
IPV-CT 67.2	Kohlrabi	Sicilia	IPV-CT 67.2	C
IPV-NA 38	Cauliflower	Lazio (SabotinoTerracina, Latina)	A	E
IPV-NA 1'	Cauliflower	Campania	E	A
IPV-NA 39	Kohlrabi	Puglia (Foggia)	E	C
ISPaVe 352	Cauliflower	Lazio (Roma)	C	D
ISPaVe 1032	Cabbage	Lazio (Roma)	C	ISPaVe 1032
ISPaVe 016	Kohlrabi	Lazio (Latina)	A	ISPaVe 016
OMP-BO 588/90	Cabbage	Emilia Romagna	A	C
LMG 568	Brussels sprout	United Kingdom	B	LMG 568
LMG 8001	Cabbage	United Kingdom	B	A
LMG 8030	Cauliflower	France	B	A
LMG 8058	Cauliflower	Holland	Z	F

ISCI = CRA- Centro di Ricerca per l'Orticoltura, Operative Station of Battipaglia (Italy); DAPP-PG = Dipartimento di Scienze Agrarie e Ambientali, sez. Arboricoltura e Protezione delle Piante, University of Perugia (Italy); IPV-CT = Istituto di Patologia Vegetale, University of Catania; IPV-NA= Istituto di Patologia Vegetale, University of Naples; ISPaVe = CRA-Istituto Sperimentale per la Patologia Vegetale, Rome (Italy); OMP-BO = Osservatorio per le Malattie delle Piante, Bologna (Italy); LMG = Culture Collection Laboratorium voor Microbiologie, University of Gent (Belgium).

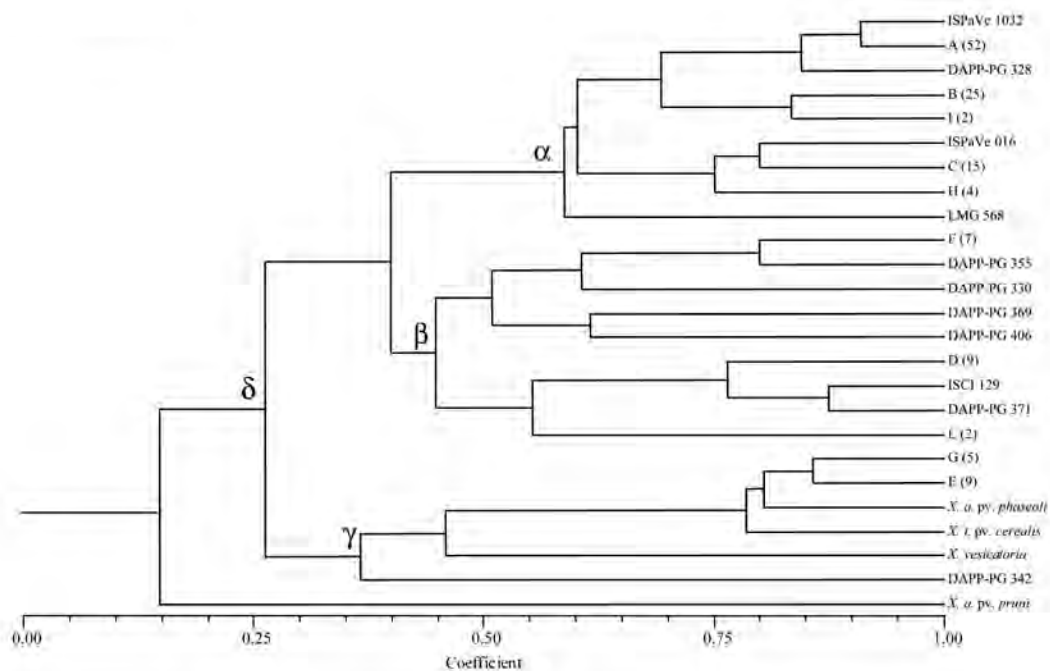


**Fig. 2.** Gel electrophoresis of BOX-PCR products from *Xanthomonas campestris* pv. *campestris* strains. Lane M: molecular weight marker Ladder Mix (MBI Fermentas, Canada); lane 1: ISPaVe 1032; lane 2: ISPaVe 016; lane 3: ISCI 38; lane 4: ISCI 2; lane 5: IPV-CT 62.4; lane 6: IPV-CT 65.3; lane 7: OMP-BO 588/90; lane 8: ISCI 5; lane 9: ISCI 16; lane 10: ISCI 31; lane 11: ISCI 61; lane 12: DAPP-PG 247; lane 13: ISCI 75; lane 14: ISCI 8 EP; lane 15: IPV-NA 38; lane 16: ISCI 120; lane 17: ISCI 127; lane 18: ISCI 129; lane 19: DAPP-PG 328; lane 20: DAPP-PG 329; lane 21: DAPP-PG 342; lane 22: DAPP-PG 355; lane 23: DAPP-PG 369; lane 24: DAPP-PG 371; lane 25: DAPP-PG 330; lane 26: DAPP-PG 406; lane 27: LNPV 4-77 (*Xanthomonas vesicatoria*); lane 28: NCPPB 1607 (*Xanthomonas arboricola* pv. *prunii*); lane 29: NCPPB 3212 (*Xanthomonas translucens* pv. *cerealis*); lane 30: NCPPB 1420 (*Xanthomonas axonopodis* pv. *phaseoli*); lane 31: LMG 568 (*Xanthomonas campestris* pv. *campestris*).





**Fig. 3.** Dendrogram constructed with PCR products obtained with M13 primer, from 141 Italian *Xanthomonas campestris* pv. *campestris* strains and four *Xanthomonas* spp. strains used as outgroups. Each letter indicates the haplotypes listed in Table 1, including the number of *Xcc* strains in brackets. Haplotypes, including only one strain, bear the strain denomination of Table 1. Strains used as outgroups are *X. vesicatoria* LNPV 4-77, *X. arboricola* pv. *pruni* NCPPB 1607, *X. translucens* pv. *cerealis* NCPPB 3212 and *X. axonopodis* pv. *phaseoli* NCPPB 1420. Symbols  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  indicate cluster denomination; the horizontal axis expresses the similarity index values.



**Fig. 4.** Dendrogram constructed with PCR products obtained with BOX primer, from 141 Italian *Xanthomonas campestris* pv. *campestris* strains and four *Xanthomonas* spp. strains used as outgroups. Each letter indicates the haplotypes listed in Table 1, including the number of *Xcc* strains in brackets. Haplotypes, including only one isolate, bear the strain denomination of Table 1. Strains used as outgroups were *X. vesicatoria* LNPV 4-77, *X. arboricola* pv. *pruni* NCPPB 1607, *X. translucens* pv. *cerealis* NCPPB 3212 and *X. axonopodis* pv. *phaseoli* NCPPB 1420. Symbols  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  indicate cluster denomination; the horizontal axis expresses the similarity index values.

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