Araştırma Makalesi/Research Article *Derim, 2016, 33 (1):27-46*
DOI:10.16882/derim.2016.07605

Development of resistant tomato population with bacterial canker resistance genes from interspecific hybrids by the support of embryo rescue

Aylin KABAŞ[1*] Esin ARI[2] Sinan ZENGİN[3] Hülya İLBİ[4]
Yeşim AYSAN[5] Asu OĞUZ[6]

[1] Batı Akdeniz Agricultural Research Institute, Antalya
[2] Akdeniz University Agricultural Faculty Agricultural Biotechnology Department, Antalya
[3] Antalya Tarim, Antalya
[4] Ege University Agricultural Faculty Horticulture Department, İzmir
[5] Cukurova University Agricultural Faculty Plant Protection Department, Adana
[6] Antalya Agricultural Quarantine Directorate, Antalya

Received Date: 07 September 2015 Accepted Date: 03 April 2016

Abstract

Bacterial canker is one of the most important diseases causing economic yield loss in tomato production areas in the world. The best way to control for this disease is to use resistant varieties. However, there are few studies on variety breeding studies of this disease compared with other disease resistant breeding studies. In this study we aimed to improve inbred lines carrying bacterial canker resistance genes to use in the breeding of resistant varieties. Susceptible inbred line AK1 (*S. esculentum*) and resistant LA2157 (*S. peruvianum*) were crossed. Embryo rescue and ovule culture techniques were applied in 30 fruits to get F1 hybrids. Rescued embryos and immature ovules were cultured in petri dishes containing solidified MS medium without hormone. 30 healty embryos were excised and cultured from 30 fruits 27-61 day old (1 embryo fruit^{-1}) in embryo rescue method. The two surviving plants from acclimatization were transferred to the greenhouse to get their BC1 progenies. Resistance tests were performed according to the stem inoculation method in the BC_1 and BC_2 progenies. The mixture of 14 aggressive Turkish *Cmm* strains were used to confirm the resistance. The plants were valued by 0-4 scale. Plants with 0 and 1 scale values were used to obtain next progenies. A total of 80 BC_3 resistant progenies were transferred to our variety breeding programme.

Keywords: Tomato, *Clavibacter*, Embryo rescue, Breeding

* Sorumlu yazar (Corresponding author): demirelliaylin@hotmail.com

Domateste türlerarası melezlemelerde embriyo kurtarma desteğiyle bakteriyel kansere dayanıklı populasyonun geliştirilmesi

Öz

Bakteriyel kanser dünyada domates üretimi yapılan alanlarda verimde önemli ekonomik kayıplara neden olmaktadır. Dayanıklı çeşit kullanımı, hastalığın kontrolünde en etkili yoldur. Hastalığa dayanıklı çeşit ıslah çalışmalarında ise diğer hastalıklarla karşılaştırdığımızda yeterince çalışma bulunmamaktadır. Bu çalışmanın amacı dayanıklı çeşit geliştirme programlarında kullanılabilecek, bakteriyel kansere dayanıklı genleri taşıyan hatları geliştirmektir. Araştırmada hassas hat AK1 (*S. esculentum*) ile dayanıklı LA2157 (*S. peruvianum*) melezlenmiştir. F1 hibrit elde etmek için açılan 30 meyvede embriyo kurtarma ve ovül kültürü tekniği kullanılmıştır. Kurtarılmış embriyolar ve olgunlaşmamış ovuller, hormonsuz MS ortamındaki petri kaplarına alınmıştır. 27-61 günlük 30 meyveden embriyo kurtarma metodu ile 30 sağlıklı embriyo (1 embriyo meyve^{-1}) çıkartılmış ve kültüre alınmıştır. Aklimatizasyon sonucu canlı kalan 2 bitki, BC_1 geriye melez bireylerini elde etmek amacıyla seraya aktarılmıştır. BC_1 ve BC_2 bitkilerinde gövde inokulasyon metodu kullanılarak dayanıklılık testlemeleri yapılmıştır. Dayanıklılığı belirlemede 14 agresif Türk *Cmm* izolatı karışımı kullanılmıştır. Bitkiler 0-4 skala değerine göre değerlendirilmiş, 0 ve 1 skala değerine sahip bitkiler kademe ilerlemesi için kullanılmıştır. Çalışma sonucunda toplam 80 BC_3 dayanıklı bitki, çeşit ıslah çalışmalarına aktarılmıştır.

Anahtar kelimeler: Domates, *Clavibacter*, Embriyo kurtarma, Islah

1. Introduction

Tomato is the most important and widely grown vegetable in the world. However, there are more than 200 diseases and pests restricting the growing of this vegetable. Bacterial canker disease which is caused by *Clavibacter michiganensis* subsp. *michiganensis* (Smith) (*Cmm*) is one of the most important diseases of tomato (Davis et al., 1984). The symptoms of bacterial canker are leaf wilting, open stem cankers and finally plant death (Jones, 1991; Gleason et al., 1993). The evident of diseased fruit symptoms is small brown spots named as “bird eye” (Gleason et al., 1993). Severe infections cause over 84% of yield loss (Jones et al., 1991; Gleason et al., 1993).

Control methods of the disease are to use of disease-free seeds, some physical and chemical seed treatments (Dhanvantari, 1989; Fatmi et al., 1991; Kritzman, 1993), copper hydroxide applications (Hausbeck et al., 2000), soil solarization (Antoniou et al., 1995), and biological control (Planth

Growth Promoting Rhizobacteria= PGPR) (Backman et al., 1997; Weller, 1988; Wei et al., 1996). But, these methods can not efficiently control of the disease. Therefore, the most efficient and environmental friendly method is to use of resistant tomato varieties.

Resistance against the disease is controlled by multiple genes in tomato. Wild tomato species *S. pimpinellifolium*, *S. hirsutum* and *S. peruvianum* are the resistant sources of *Cmm* (Berry, 1989; Sandbrink, 1995). LA2157 line obtained from *S. peruvianum* has several beneficial traits for the cultivated tomatoes such as cold tolerance, nematode and bacterial canker resistance (van Heusden et al., 1999). Also, line LA2157 was found to be more resistant than LA407 line from *S. hirsutum* (Kabaş et al., 2010). However, this species shows some limitations in tomato breeding studies because of the incompatibility. This incompatibility is derived from the source of parents in the crosses. If the *S. esculentum* is used as the male parent, unilateral incompatibility is observed. The opposite of the this situation requires tissue culture procedures to rescue hybrid and backcross embryos or ovules (Lefrancois et al., 1993; Francis et al., 2001).

Embryo rescue technique is an important method for breeding programs. This technique was applied first by Smith (1944) for excision of the hybrid embryos obtained from *S. esculentum* and *S. peruvianum* crossing, and rescue of embryos successfully. Later, this study was followed by other embryo-ovule rescue studies and immature seed cultures (Choudhury, 1955; Imanishi, 1988; Chen and Adachi, 1992; Segeren et al., 1993; Bruggemann et al., 1996; Chen and Adachi, 1996; Doğanlar et al., 1997; Bal and Abak, 2003; Bhattarai et al., 2009). The most focused issues in these studies have been the determination of the most appropriate period of embryo development stage and set up of the suitable nutritional medium.

Although several studies have been performed on hybrid embryos obtained from *S. esculentum* and *S. peruvianum*, there is no a detailed breeding study in literature focused on developing resistant varieties against to *Cmm*, up to now. The major reason can be due to the performing disease resistance test by inoculation method, because some factors such as temperature, plant age, inoculum concentration and incubation period during inoculation method can be effective on occurrence of disease symptoms (Chang et al., 1992). Some chromosomal regions for *Cmm* resistance genes in *S. peruvianum* were defined (van Heusden et al., 1999). However useful molecular markers are not currently avaliable to use in marker assisted selection (MAS) without the classical test methods.

In the present study, we aimed to improve resistant tomato breeding lines carrying bacterial canker resistance genes derived from interspecific F1 progenies of *S. esculentum* x *S. peruvianum* via embryo rescue to be used in tomato breeding studies.

2. Materials and Methods

2.1. Materials

2.1.1. Plant materials

The seeds of *S. peruvianum* LA 2157 were provided by Tomato Genetic Resource Center (TGRC) as the resistant material in the experiment. Susceptible inbred line, *S. esculentum* AK1 was improved in the breeding studies at Batı Akdeniz Agricultural Research Institute (BATEM) in Antalya, Turkey.

2.1.2. Bacterial strains

C. michiganensis subsp. *michiganensis* (*Cmm*) strains were provided from Yesim AYSAN's culture collections at Cukurova University, Agricultural Faculty, Plant Protection Department. The strains were originally isolated from diseased tomato plants at different locations of Turkey between 1996 and 2010 years. Their pathogenicities were determined to be aggressive on tomato plants in our previous work (Horuz et al., 2014).

2.2. Methods

Susceptible line, *S. esculentum* AK1, was used as female parent while resistant line, *S. peruvianum* LA 2157 was used as male parent for the interspecific cross. Disease ressitances of backcross (BC_1, BC_2 and BC_3) progenies were tested on scale of 0 and 1 values.

2.2.1. Embryo rescue and ovule culture

Tomato fruits were harvested at 17 different dates in between the 27^{th} and 61^{th} days after pollination (DAP). The fruits were kept at +4°C. Before opening, fruits were washed under the tap water during 5 minutes and were sterilized for 10 minutes with the 5 % sodium hypochlorite solution and rinsed three times in sterile water. For the last stage of fruit

sterilization, they were burned for 30 seconds after being sprayed with 96% alcohol.

A kind of double selection method was used for choosing of ovules in the present study. This method was based on the classification of the ovules according to their size, shape and colour described by Imanishi (1988) and Chen and Adachi (1992). Two types ovules were cultured; the first type was regular, green, equal sized ovules for the direct culture and the other was immature seed type, the bigger ovules for the embryo rescue. The characteristic of the latter was explained to be "the ovules with a lighter yellowish brown colour and a slightly rounder" by Imanishi (1988).

The fruits were opened individually. After cutting sterile fruits, excised ovules following abrasion of their jelly-like coating were placed in 9 cm petri dishes or the embryos rescued from immature seeds were cultured individually in 40 ml small glass jars for the germination. The medium without hormone (pH= 5.8) in petri dishes or glass jars was constituted from the prepared full strength Murashige and Skoog (MS) (1962) medium (SIGMA), 30 g L^{-1} sucrose (MERCK) and 8 g L^{-1} agar (SIGMA). Cultures were placed to 25°C temperature in darkness during the first week, then moved to 16:8 hr photoperiod conditions with cool white fluorescent lights. Germinated seedlings were transferred to culture tubes for further growth. When the in vitro plantlets had enough shoot and root systems, they were subjected to acclimatization. The survived plantlets were transferred to the greenhouse for selfing to get F_1 seeds.

BC_1 population was generated by using *Cmm* resistant fertile F_1 plants obtained from the interspecific cross of *S. esculentum x S. peruvianum*. BC_2 and BC_3 progenies were produced by backcrossing resistant BC_1 ve BC_2 with susceptible recurrent parent, respectively.

2.2.2. Bacterial inoculations and disease rating

Stem inoculation method described by Mavridis (1982) and Klement et al., (1990) were used in the present study to screen resistant lines. The lines were inoculated by the mixture of 14 aggressive bacterial strains, as seen in Table 1.

The seeds of susceptible and resistant parents, BC_1, BC_2 populations were sown in pots containing sterile peat moss. The seedling were grown until the fourth true leaf stage. Then, the plants were injected by bacterial suspension of 10 µl of 10^7 cfu ml^{-1} as in Figure 1.

Table 1. Pathogenic bacterial strains of *Cmm* used in the study

Strain names	Place of isolation	Year of isolation
Cmm 23 Antalya 1r	Antalya	1996
Cmm 3/1 Tuzla 1r	Tuzla, Karataş, Adana	1996
Çalışkanlar 1c R 2r	Tarsus, Mersin	2002
Astona-Reute Hishtil Tomato2	Antalya	2002
Dikili-İzmir *Cmm* 4 3r	Dikili, İzmir	2003
Muc 1c(2) 3r	Adana	2004
Tarsus 4r	Tarsus, Mersin	2004
Çoruh 1Dom r	Artvin	2004
Eggplant 1 *Cmm*	Aydıncık, Mersin	2005
Erdemli 3/1 A R 1r	Erdemli, Mersin	2007
Cıcık 27 2r	Cıcık, Mersin	2007
ÖY-1c-12A R 1r	Erdemli, Mersin	2010
YY 2	Tokat	2010
SY-13	Tokat	2010

Figure 1. Stem inoculation method for tomato plants

Inoculated seedling were incubated in the greenhouse at 25°C, and 70% relative humidity for 2.5 months (Figure 2). Greenhouse performance of the inoculated crossing lines were evaluated by 0-4 scales (Table 2). The degrees of disease severity were scored 3, 4, 5, 6, 7, 8 and 9 weeks after inoculations. The disease severity was evaluated by Tawsend-Heuberger's formula.

Figure 2. Inoculated plants were incubated in controlled greenhouse conditions

Table 2. Evaluation of disease reaction of crossing lines by 0-4 scales

Scale	Wilting area of plant (%)	Disease susceptibility character
0	No disease symptoms	Resistant
1	1-25% wilting of plant	Low susceptibility
2	26-50% wilting of plant	Medium susceptibility
3	51-75% wilting of plant	High susceptibility
4	76-100% wilting of plant or dead plant	Very high susceptibility

Totally, 25 plants from resistant and susceptible parents, 65 plants from BC_1 and 113 plants from BC_2 segregating populatios were inoculated with the pathogenic bacterium suspension. Disease development continued during 10 weeks. At the end of this period, the degrees of wilting were scored and bacterial disease characters were detected by the scale as in Table 2. Resistant and low susceptible lines were selected as promising breeding lines for further studies.

3. Results

A total of 120 crossings was performed between *S. esculentum* and *S. peruvianum*. 60 fruits were obtained from the crosses with 90% fruit set success.

3.1. Embryo rescue and ovule culture

A total of 30 fruits were opened and checked in terms of the embryo formation. The fruits of 27-61 DAP were used for the embryo rescue and ovule culture. Mostly immature ovules and also some immature seed development were observed in the fruits. No germination took place among 768 immature ovules sown in petri dishes. On the other hand, a total of 30 healty embryos was excised and cultured directly from 52 immature seeds which were opened under microscope for embryo rescue (Table 3). The average of 1.73 immature seeds per fruit were obtained and 1 healty embryo/fruit was rescued. 12 of the cultured embryos were germinated successfully with the ratio of 40%. Different numbers of embryos were rescued from 30 fruits harvested at different DAP. In order to show the embryo results of different DAP better, Table 4 was designed from Table 3. The fruits of 27-61 DAP were used for the embryo rescue in 17 different DAP (Table 4). Five hybrid embryos were rescued from the youngest fruit, at 27 DAP, while 2 embryos from the oldest one, of 61 DAP. As seen from the Table 4, it is not possible to make a generalization for a certain successful fruit DAP for our study. However, the embryos were germinated till 46 DAP. Out of the 12 germinated embryos, 9 embryos generated plant with proper shoot and root system. After these plants reached to enough size, they were acclimatized. But only 2 of them survived after the acclimatization procedure. Then, these two putative interspecific hybrid plants were transferred to a greenhouse for selfing. The steps of obtaining interspecific hybrids derived from embryo rescue procedures were presented in Figure 3.

Table 3. The results of ovule and embryo rescue cultures performed in 30 fruits

Order of opened fruit	Pollination date	Harvest date	DAP	Date of culture	# of rescued embryo*	# of germinated embryo	# of cultured ovules	# of germinated ovule
1	06.05.11	20.06.11	46	20.06.11	2	2	182	
2	06.05.11	20.06.11	46		-	-	117	
3	07.05.11	20.06.11	45	30.06.11	1	1	0	
4	07.05.11	20.06.11	45		5	3	0	
5	09.05.11	20.06.11	43		-	-	0	
6	09.05.11	20.06.11	43		-	-	0	
7	10.05.11	20.06.11	42		1	0	0	
8	11.05.11	20.06.11	41		-	-	0	
9	11.05.11	20.06.11	41		2	0	0	
10	11.05.11	20.06.11	41		-	-	0	
11	13.05.11	20.06.11	39	01.07.11	1	1	0	
12	25.05.11	20.06.11	27		5	2	0	
13	13.05.11	05.07.11	54	05.07.11	-	-	0	
14	20.05.11	05.07.11	47		-	-	0	None of
15	23.05.11	05.07.11	44		1	0	0	cultured
16	06.05.11	05.07.11	61		2	0	0	ovules were
17	07.05.11	05.07.11	60		-	-	0	germinated
18	25.05.11	05.07.11	42		1	0	0	
19	09.05.11	05.07.11	58		1	0	0	
20	10.05.11	05.07.11	57		-	-	0	
21	27.05.11	05.07.11	40		3	3	0	
22	11.05.11	05.07.11	56		1	0	125	
23	11.05.11	05.07.11	56	06.07.11	3	0	93	
24	17.05.11	05.07.11	50		-	-	0	
25	17.05.11	05.07.11	50		-	-	0	
26	17.05.11	05.07.11	50		1	0	0	
27	06.05.11	05.07.11	61		-	-	173	
28	13.05.11	05.07.11	54		-	-	0	
29	06.05.11	05.07.11	61		-	-	0	
30	06.05.11	05.07.11	61		-	-	78	
Total	-	-	-	-	30	12	768	0

*: These embryos were rescued from selected immature seeds which were either quite rare or absent in a fruit.

Table 4. The results of embryo rescue cultures realized at 17 different DAP

Order #	DAP	# of opened fruit in this DAP	# of total embryos rescued in this DAP	# of total germinated embryos in this DAP	Germination rate of rescued embryos (%)
1	27	1	5	2	40.00
2	39	1	1	1	100.00
3	40	1	3	3	100.00
4	41	3	2	0	0.00
5	42	2	2	0	0.00
6	43	2	-	-	-
7	44	1	1	0	0.00
8	45	2	6	4	66.67
9	46	2	2	2	100.00
10	47	1	-	-	-
11	50	3	1	0	0.00
12	54	2	-	-	-
13	56	2	4	0	0.00
14	57	1	-	-	-
15	58	1	1	0	0.00
16	60	1	-	-	-
17	61	4	2	0	0.00
Total	-	30	30	12	40.00

3.2. Bacterial inoculations and disease rating

No symptom was observed up to the end of 7^{th} week after inoculation. Of the 25 resistants parent plants, 3 plants showed 1 disease scalar value in the 8^{th} and 9^{th} week evaluations. While disease severity was 0% in the 7^{th} week, it was only 3% in the 8^{th} and 9^{th} weeks (Table 5).

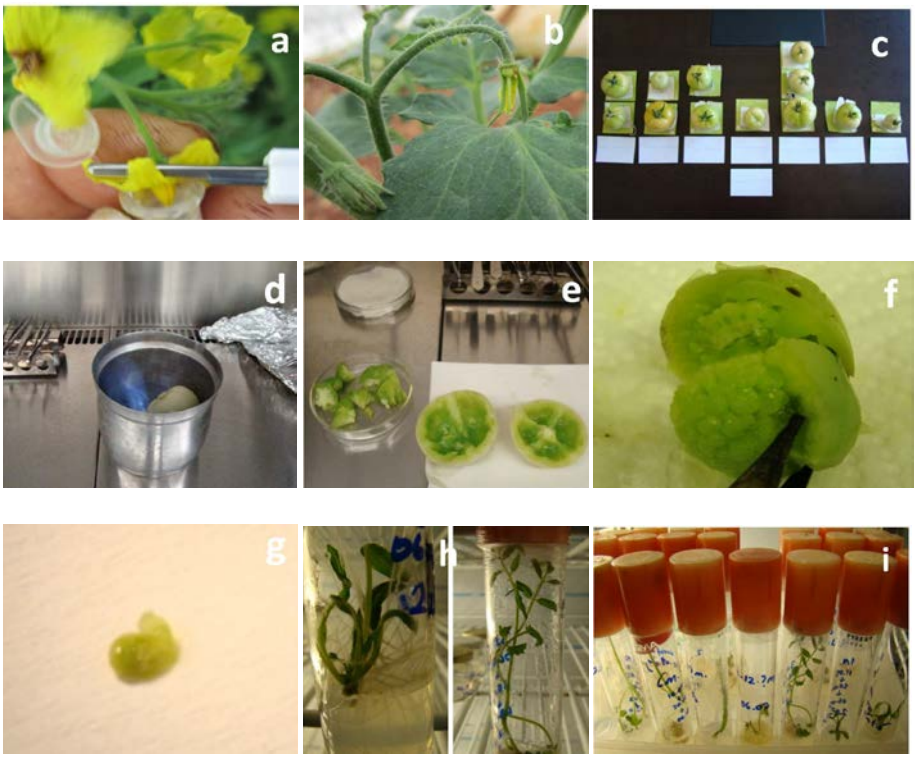

Figure 3. Pollination and embryo culture studies in *S. esculentum* AK1 and *S. peruvianum* LA 2157 crosses; a) collection of pollens, b) 2-3 days-flowers after pollination, c) the harvested fruits, d) sterilization of a fruit with dry burning, e) an opened fruit, f) the ovules to be cultured, g) an appropriate immature seed to be opened and rescued its embryo inside, h) successful embryo growths, i) in vitro plantlets recovered from the hybrid embryos.

Disease symptoms have been observed in the 3rd week after inoculation in susceptible line AK1 (*S. esculentum*) (Figure 4). After 3 weeks of inoculation, severity of the disease increased. Disease severity was determined to be 12%, 46%, 88%, 97%, 97%, 97% and 97% from the 3rd to 9th weeks after *Cmm* inoculation, respectively (Table 6).

Table 5. Bacterial canker disease index and severity on *S. peruvianum* LA 2157 as a resistant material

Scoring times	Inoculated plants	Scales					Disease scale index	Disease severity (%)
		0	1	2	3	4		
3rd week	25	25	0	0	0	0	0	0
4th week	25	25	0	0	0	0	0	0
5th week	25	25	0	0	0	0	0	0
6th week	25	25	0	0	0	0	0	0
7th week	25	25	0	0	0	0	0	0
8th week	25	22	3	0	0	0	0.12	3
9th week	25	22	3	0	0	0	0.12	3

Figure 4. Disease symptoms were observed in the 3rd week after inoculation

Table 6. Bacterial canker disease index and severity on *S. esculentum* AK1 as a susceptible material

Scoring times	Inoculated plants	Scales					Disease scale index	Disease severity (%)
		0	1	2	3	4		
3rd week	25	13	12	0	0	0	0.48	12
4th week	25	2	10	7	2	4	1.84	46
5th week	25	0	4	0	0	21	3.52	88
6th week	25	0	0	0	3	22	3.88	97
7th week	25	0	0	0	3	22	3.88	97
8th week	25	0	0	0	3	22	3.88	97
9th week	25	0	0	0	3	22	3.88	97

Bacterial canker disease course and severity (%) in resistant and susceptible parents was shown in Figure 5. In the BC_1 and BC_2 populations, first disease symptoms occured in the 3rd week on susceptible plants. All the segregating populations were evaluated by 0-4 scales after 2.5 months from inoculation. The plants having 0 scale value were considered as true resistant to *Cmm*. The 8 plants from BC_1, 7 plants from BC_2 were determined to have 0 scale value. These plants and also the plants with 1 scale value were used for generating next segregating populations. Then, the targeted genes of these plants conferring resistance to *Cmm* were transferred to the next progenies.

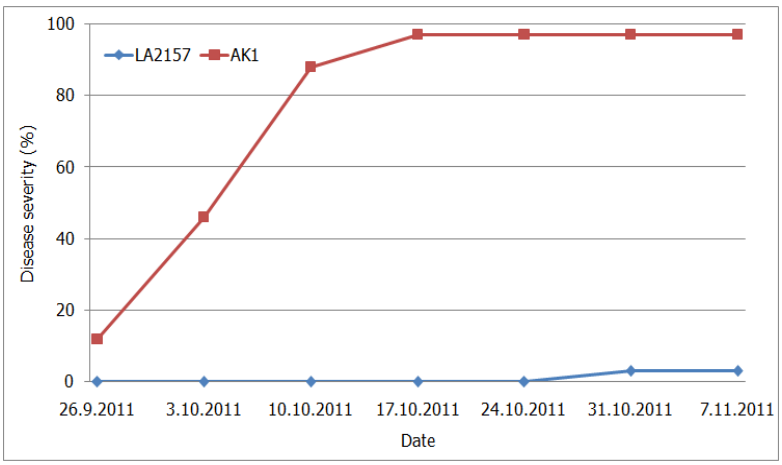

Figure 5. Bacterial canker disease course and severity in resistant LA2157 and AK1

4. Discussion

Bacterial canker in tomato growing area (greenhouse and open field) is a serious problem and spreading rapidly (Louwvs et al., 1998). Several potential sources of resistance to *Cmm* were determined in the scientific studies and the best characterized source of resistance was found *in S. peruvianum* (LA2157) (Francis et al., 2001; Berry 1989, Sandbrink 1995). However, crosses between cultivated tomato and S. peruvianum accession require to embryo rescue (Francis et al., 2001; van Heusden et al., 1999). Therefore the use of LA2157 in breeding programme has been limited.

Growing conditions, genotype, climate, culture type and purpose of embryo rescue are the important factors affecting a successful embryo culture. According to Young et al. (1987), the success rate of embryo rescue is based on the age of embryos. For this reason, fruits are harvested at different times in different studies after the crossings.

In the interspecific cross of *S. esculentum* x *S. peruvianum*, the first in vitro hybrids were obtained by Smith (1944), via embryo culture at 40 DAP. Imanishi et al. (1985) and Chen and Imanishi (1991) obtained their hybrids at 35-40 DAP via ovule culture. On the other hand, the fruits at 15-32 DAP were used in embryo rescue study by Chen and Adachi (1992) while Segeren et al. (1993) used immature seeds at 15-25 DAP for indirect embryo rescue via callus culture. Doganlar et al. (1997) used 10-40 DAP fruits for ovule cultures. In the Aragao et al. (2002)'s study, the fruits of 25-30-35-40 and 45 DAP were used. They got the best result from 30 DAP and emphasized that the ideal embryo age was between 26 and 35 DAP for embryo rescue. Geboloğlu et al. (2011) obtained the highest success in 28-32 DAP. Apart from the others, Bhattarai et al. (2009) used embryo culture technique for different purpose, to accelerate the normal *S. esculentum* breeding programme, not to rescue the hybrids of an interspecific cross. They cultured the excised immature seeds from 5, 10, 20, 30, 40, 50, 60 and 70 DAP. Among them, the 5-day old embryos were not germinated whereas 60-70 day old matured embryos provided the maximum germination rate of 90 % and also 10-day old embryos germinated at 61 % rate.

As seen from the results, harvesting time has different effects in different embryo culture studies including embryo rescue, ovule or immature seed cultures. However, the successful results were obtained generally from 3-4 weeks old fruits for the direct embryo rescue studies as in the cross of *S. esculentum* x *S. peruvianum*. On the other hand, Chen and Adachi (1992)

proved to rescue hybrid embryos of *S. esculentum* x *S. peruvianum* as early as 13 DAP. In our study, we attempted to rescue the hybrid embryos as late as 61 DAP. The fruits of 27-61 DAP were used for the embryo rescue and ovule culture in this work. Among 30 fruits opened for the cultures, 5 hybrid embryos were rescued from the youngest fruit 27 DAP while 2 embryos from the oldest one at 61 DAP. But the embryos germinated till 46 DAP. The most related literatures to our study, namely the hybrids of interspecific cross of *S. esculentum* x *S. peruvianum* obtained from the oldest fruits, are Smith (1944)'s embryo rescue study at 40 DAP and Imanishi et al. (1985)'s and Chen and Imanishi (1991)'s ovule culture studies at 35-40 DAP. The reason for obtaining quite healty hybrids from so old fruits, till 61 DAP, can be attributed to the notification expressed in Imanishi (1988)'s and Chen and Adachi (1996)'s publications. In both papers, the ovules used in the studies had been classified according to their some characteristics like size, shape and colour. Among them, "the ovules with a lighter yellowish brown colour and a slightly rounder" and bigger shape had shown a very high frequency of germination than the others. We also used this sized ovules for the rescue of their embryos and general sized ovules for the ovule culture. When we opened and cultured 30 ovules, the embryos of them were in the stages from heart to cotyledonary shape. 12 of them were germinated, then 9 of them turned to the whole plant while none of 768 ovules germinated.

Shortly, embryo rescue technique has been a powerful method to secure embryos directly from the immature seeds in earlier or later stages derived from the interspecific hybrids between *S. esculentum* x *S. peruvianum* even though its efficiency was generally low in most studies. However, sometimes only one interspecific hybrid can be enough to get very valuable results. In case of van Heusden et al. (1999)'s study, they studied *Cmm* and carried out a research on a QTL mapping in order to identify QTLs for bacterial canker resistance in an F_2 populations derived from the interspecific F_1 between *S. esculentum* cv. Solentos and *S. peruvianum* LA2157. Embryo rescue technique was applied to secure F_1 progenies of the interspecific cross, but only one single fertile F_1 plant was obtained. However, this single plant yielded hundreds of selfed seeds, resulting a segregating F_2 population of 324 plants. Thanks to embryo rescue, they identified 3 QTLs and their chromosomal regions in S. peruvianum LA2157 conferring a high level of resistance to *C. michiganensis* ssp. *michiganensis*. Additionally, they converted some RFLP markers into SCAR markers for the efficient marker-assisted selection of plants with high resistance to bacterial canker. In the similar manner, we obtained 9 F_1 hybrid plants from *S.*

esculentum x *S. peruvianum* via embryo rescue technique, but only 2 of them survived at the end of acclimatization. However, we had 80 BC_3 plants derived from these 2 unique F_1 hybrid plants.

For screening of parent lines, BC_1 and BC_2 populations, we used stem inoculation method according to Mavridis (1982) and Klement et al. (1990). The plants having 0 scale value based on a 0-4 rating scale were considered as true resistant to *Cmm*. In addition to them, the low susceptible plants with 1 scale were also assessed to be resistant. The resistance of *S. peruvianum* LA2157 which was reported previously in other studies (Sen et al., 2012) was confirmed one more time in our study. In the 9-weeks-disease severity evaluation period, the plants of this line showed 0 disease scale index value during 7 weeks. The first symptoms of *Cmm* in *S. peruvianum* LA2157 plants were observed at the 8^{th} week, and also the mentioned value was determined to be only 0.12 in the 8^{th} and 9^{th} week. However, the first symptoms of *Cmm* in BC_1 and BC_2 populations occured in the 3rd week. On the other hand, 8 plants of BC_1 and 7 plants from BC_2 were determined to be true resistant to *Cmm* with 0 scale value among 65 plants from BC_1 and 113 plants from BC_2 segregating populatios.

In conclusion, we obtained 80 BC_3 tomato plants derived from these resistant and also low susceptible plants to *Cmm* in this study. Bacterial canker is the most serious threat spreading rapidly in tomato growing areas. The best solution for this threat is to improve and use resistant varieties. Our study might contibute to the literature on the resistance breeding works for *Cmm* in tomato. As the further study, the resistant tomato population detected with the classic disease test must be confirmed by using MAS.

Acknowledgements

We thank J.W. Scott for providing wild genotype LA 2157, also Yusuf Yanar and Sabriye Yazıcı for TOKAT strains of *Cmm*.

References

Antoniou, P.P., Tjamos, E.J., & Panagopoulos, S.G. (1995). The use of soil solarization for controlling bacterial canker of tomato in plastic houses in Greece. *Plant Pathology*, 44(3):438-447.

Aragao, F.A.S., Ribeiro, C.S.C., Casali, V.W.D., & Giordano, L.B. (2002). Tomato embryo culture for introgression of genes of *Lycopersicon peruvianum* in *L. esculentum*. *Horticultura Brasileira*, 20(4):605-610.

Backman, A.C., Bengtsson, M., & Witzgall, P. (1997). Pheromone release by individual females of codling moth, Cydia pomonella. *Journal of Chemical Ecology*, 23(3):807-815.

Bal, U., & Abak, K. (2003). Attempts of haploidy ınduction in tomato (*Lycopersicon esculentum* mill), via gynogenesis I: Pollination with *Solanum sisymbriifolium* lam. polen. *Pakistan Journal of Biological Sciences*, 6(8):745-749.

Berry, S.Z., Madumadu, G.G., Uddin, M.R., & Copplin, D.L. (1989). Virulence studies and resistance to *Clavibacter michiganensis subsp. michiganensis* in tomato germplasm. *Hortscience*, 24(2):362-365.

Bhattarai, S.P., Pena, R.C., Midmore, D.J., & Palchamy, K. (2009). In vitro culture of immature seed for rapid generation advancement in tomato. *Euphytica*, 167(1):23-30.

Brüggemann, W., Linger, P., Wenner, A., & Koornneef, M. (1996). Improvement of post-chilling phtosynthesis in tomato by sexual hybridisation with a *Lycopersicon peruvianum* line from elevated altitude. *Advances in Horticultural Science*, 10(4):215–218.

Chang, R.J., Ries, S.M., & Pataky, J.K. (1992). Effects of temperature, plant age, inoculum concentration, and cultivar on the incubation period and severity of bacterial canker of tomato. *Plant Disease*, 76(11):1150-1155.

Chen, L., & Adachi, T. (2006). Efficient hybridization between *Lycopersicon esculentum* and *L. peruvianum* via 'embryo rescue' and in vitro propagation. *Plant Breeding*, 115(4):251-256.

Chen, L.Z., & Adachi, T. (1992). Embryo abortion and efficient rescue in ınterspecific hybrids, *Lycopersicon esculentum* and the 'peruvianum-complex'. *Japanese Journal of Breeding*, 42(1):65-77.

Chen, L.Z., & Imanishi, S. (1991). Cross-compatibility between the cultivated tomato *Lycopersicon esculentum* and the wild species *L. peruvianum*, *L. chilense* assessed by ovule culture in vitro. *Japanese Journal of Breeding*, 41(2):223-230.

Choudhury, B. (1955). Embryo culture technique-III. Growth of hybrid embryos (*Lycopersicon esculentum x Lycopersicon peruvianum*) in culture medium. *Indian Journal of Horticulture*, 12(4):155-156.

Davis, M.J., Gillaspie, A.G. Jr, Vidaver, A.K., & Harris, R.W. (1984). Clavibacter: a newgenus containing some phytopathogenic coryneform bacteria, including *Clavibacter xyli* subsp. xyli sp. nov., subsp. nov. and *Clavibacter xyli* subsp. cynodontis subsp. nov.,pathogens that cause ratoon stunting disease of sugarcane

andbermudagrass stunting disease. *International Journal of Systematic Bacteriology*, 34(2):107-117.

Dhanvantarı, B.N. (1989). Effect of seed extraction methods and seed treatments on control of tomato bacterial canker. *Canadian Journal of Plant Pathology*, 11(4):400-408.

Doganlar, S., Frary, A., & Tanksley, S.D. (1997). Production of interspecific hybrids between *Lycopersicon esculentum* and two accessions of *Lycopersicon peruvianum* carrying new root-knot nematode resistance genes. *Euphytica*, 95(2):203-207.

Fatmi, M., Schaad, N.W., & Bolkan, H.A. (1991). Seed treatments for eradicating *Clavibacter michiganensis subsp. michiganensis* from naturally infected tomato seeds. *Plant Disease*, 75(4):383-385.

Francis, D.M., Kabelka, E., Bell, J., Franchino, B., & St. Clair, D. (2001). Resistance to bacterial canker in tomato (*Lycopersicon hirsutum* LA407) and its progeny derived from crosses to *L. esculentum*. *Plant Disease*, 85(11):1171-1176.

Geboloğlu, N., Bozmaz, S., Aydin, M., & Çakmak, P. (2011). The role of growth regulators, embryo age and genotypes on immature embryo germination and rapid generation advancement in tomato (*Lycopersicon esculentum* Mill.). *African Journal of Biotechnology*, 10(24):4895-4900.

Gleason, M.L., Gitaitis, R.D., & Ricker, M.D. (1993). Recent progress in understanding and controlling bacterial canker of tomato in eastern North America. *Plant Disease*, 77(11):1069-1076.

Hausbeck, M.K., Bell, J., Medina, Mora C.M., Podolsky, R., & Fulbright, D.W. (2000). Effect of bactericides on population sizes and spread of *Clavibacter michiganensis subsp. michiganensis* on tomatoes in the greenhause and on disease development and crop yield in the field. *Phytopathology*, 90(1):38-44.

Horuz, S., Kabaş, A., Aysan, Y., Çakır, B., Eroğul, D. & İlbi, H. (2014). Domateste bakteriyel kanser ve solgunluk hastalığına karşı dayanıklılık mekanizmasında etkili olan genlerin ifadelerinin belirlenmesinde kullanılacak *Clavibacter michiganensis subsp. michiganensis* populasyonunun seçimi. *V. Bitki Koruma Kongresi*, 3-5 Şubat 2014, Antalya, s:217.

Imanishi, S. (1988). Efficient ovule culture for the hybridization of *Lycopersicon esculentum* and *L. peruvianum*, *L. glandulosum*. *Japanese Journal of Breeding*, 38(1):1-9.

Imanishi, S., Watanabe, Y., & Hiura, I. (1985). A simple and efficient method for interspecific hybridization between *L. esculentum* and *L. peruvianum*. *Journal of the Yamagata Agriculture Forestry Society*, 42:13-15.

Jones, J.B., Stall, R.E., & Zitter, T.A. (1991). Compendium of Tomato Disease. American Phytopathological Society, St. Paul, MN.

Kabaş, A., Ünlü, A., İlbi, H., Oğuz, A., & Zengin, S. (2010). BATEM domates hatlarının bakteriyel kanser ve solgunluk (*Clavibacter michiganensis subsp. michiganensis*)'a dayanıklılık durumlarının belirlenmesi. *VIII. Sebze Tarımı Sempozyumu*, 23-26 Haziran 2010, Van, 1:485-489 (In Turkish).

Klement, Z., Rudolph, K., & Sands, D.C. (1990). Methods in Phytobacteriology. Akademiai Kiado, Budapest.

Kritzman, G. (1993). A Chemi-thermal treatment for control of seedborne bacterial pathogens of tomato. *Phytoparasitica*, 21(1):101-109.

Lefrancois, C., Chupeau, Y. & Bourgin, J.B. (1993). Sexual and somatic hybridization in the genus Lycopersicon. *Theoretical Applied Genetics*, 86(5):533-546.

Louws, F.J., Bell, J., Medina-Mora, C.M., Smart, C.D., Opgenorth, D., Ishimaru, C. A., Hausbeck, M. K., de Bruijn, F. J., & Fulbright, D.W. (1998). Rep-PCR–mediated genomic fingerprinting: A rapid and effective method to identify *Clavibacter michiganensis*. *Phytopathology*, 88(8):862-868.

Mavridis, A. (1982). Untersuchungen zum Vorkomen bakterieller Krankheitserreger (*C. michiganensis* pv. *michiganense* (E. F. (Smith) Jesen und *Pseudomonas syringae* pv. Tomato (Okabe) Young, Dye & Wilkey) an Tomaten (*Lycopersicon esculentum* Mill.) in Griechenland. zur Erkennung der Resistenz und zum von P. tomato gebildeten Chlorose-ausloesenden Toxin. Diss. Univ. Goettingen.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiological Plant Pathology*, 15(3):473-497.

Sandbrink, J.M., Ooijen, J.W.V., Purimahua, C.C., Vrielink, M., Verkerk, P., Zabel, P., & Lindhout, P. (1995). Localization of genes for bacterial canker resistance in *Lycopersicon peruvianum* using RFLPs. *Theoretical and Applied Genetics*, 90(3-4):444-450.

Segeren, M.I., Sondahl, M.R., Siqueira, W.J., Medina Filho H.P., Nagai, H., & Lourencao, A.L. (1993). Tomato breeding: 1. Embryo rescue of

ınterspecific hybrids between *Lycopersicon esculentum* Mill. and *L. peruvianum* (L.) Mill. *Revista Brasileira De Genetica*, 16(2):367-380.

Sen, Y., Zhu, F., Vandenbroucke, H., van der Wolf, J., Visser, R., & van Heusden, A. 2012. Screening for new sources of resistance to *C. michiganensis* subsp. *michiganensis* (*Cmm*) in tomato. *Euphytica*, 190(2):309-317.

Smith, P.G. (1944). Embryo culture of a tomato species hybrid. *Proceeding of The American Society for Horticultural Science*, 44:413-416.

Thomas, B.R., & Pratt, D. (1981). Efficient hybridization between *Lycopersicon esculentum* and *L. peruvianum* via embryo callus. *Theoretical and Applied Genetics*, 59(4):215-219.

Van Heusden, A.W., Koornneef, M., Voorrips, R.E., Brüggemann, W.G., Vrielink-van Ginkel, P., X Chen, R., & Lindhout, P. (1999). Three qtls from *Lycopersicon peruvianum* confer a high level of resistance to *Clavibacter michiganensis* ssp. *michiganensis*. *Theoretical and Applied Genetics*, 99(6):1068-1074.

Wei, G., Kloepper, J.W., & Tuzun, S. (1996). Induction of systemic resistance to cucumber diseases and increases plant growth by plant 167 growth-promoting rhizobacteria under field conditions. *Phytopathology*, 86(2):221-224.

Weller, D.M. (1988). Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Annual Review of Phytopathology*, 26(1):379-407.

Young, R., Kaul, V., & Williams, A.G. (1987). Clonal propagation in vitro from immature embryos and flower buds of *Lycopersicon peruvianum* and *L. esculentum*. *Plant Science*, 52(3):237-242.