

Transfer of *Co-1* Gene Locus for Anthracnose Disease Resistance to Fresh Bean (*Phaseolus vulgaris* L.) Through Hybridization and Molecular Marker-Assisted Selection (MAS)

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Abstract

Anthracnose disease, caused by the fungal pathogen *Colletotricum lindemuthianum* brings about great yield losses in the Blacksea Region of Turkey. The present study is carried out to start the resistance breeding program against anthracnose disease. Five pathogenic strains (2175, 3071, 3303, 3321, 4071) identified previously from Blacksea Region were inoculated on seven foreign anthracnose resistant varieties of fresh bean (MDRK, PM, Kaboon, Widusa, Machinac, Isles and Chinook) as well as on nine breeding lines (T23, TK57, TK1, Ç31, Ç28, T7, T26, TK15 and T21) which were developed as Ayşe kadın type by pure-line selection, a method used for self-pollinated crops. Results indicated that while all the foreign varieties were resistant to these pathogen strains, the pure-lines were all susceptible. Thus foreign varieties and pure-lines were selected as parents to perform 63 combinations of genetic cross with the intention to start anthracnose resistance breeding program, only 18 of which produced seed. Through self fertilization of 360 F₁ plants an F₂ population of 4365 plants were obtained which were phenotyped for resistance both by inoculation with five pathogen strains as well as using resistance gene (*Co-1*) linked molecular marker (SEactMcca). Since Ayşe Kadın type lines are of Andean origin and *Co-1* locus controls resistance against Andean pathogens additional confirmation of *Co-1* presence in the resistant F₂ lines is of great importance. Marker screening results indicated the presence of *Co-1* gene in 6 out of 18 hybrid lines. Confirmation of resistance trait inheritance on 6 F₂ lines both by inoculations as well as molecular marker screening for *Co-1* gene have given us the opportunity to continue with the backcross studies with high confidence. With this study anthracnose resistance breeding has been initiated in Turkey for the first time and molecular marker assisted selection has been integrated into the breeding program.

Keywords: *Colletotrichum lindemuthianum*, hybridization, inoculation, molecular marker, *Phaseolus vulgaris*, strain

1. Introduction

Fresh bean (*Phaseolus vulgaris* L.) is a widely grown crop throughout the world. Although genus *Phaseolus* has approximately 70 species; only 5 bean species, *P. vulgaris*, *P. dumosus*, *P. coccineus*, *P. lunatus* and *P. acutifolius*, have been domesticated to date (Freytag & Debouck, 2002). The gene pool origins and area of domestication of these 5 species have been determined as Mesoamerican and Andean regions (Delgado-Salinas, 1985; Freytag & Debouck, 2002). Among the five species, *P. vulgaris*, is cultivated worldwide and is consumed as a source of protein, mineral (Ca, Cu, Fe, Mg, Mn and Zn) and vitamins (folate) (Singh, Gepts, & DeBouck, 1991).

History of bean in Turkey goes back only 250 years (Şehirali, 1988). About 59% of Turkish bean genotypes are Andean (T and C type phaseolin) and 41% are Mesoamerican (S type phaseolin) (Logozzo, Donnoli, Leonardo, Papa, Knüpffer, & Zeuli, 2007; Erdinç, 2012). Following China and Indonesia Turkey is the third biggest fresh

bean producer with 587.967 t annually (FAO, 2010). Although the production is widely spread in Turkey, the majority of it is concentrated in Central Black Sea Region (Şehirli, 1988). Located in this region, city of Samsun is major contributor with an average annual production of 123.161 t (Anonymous, 2011).

The fungus *Colletotrichum lindemuthianum* causes anthracnose disease, which is a major problem in Central Black Sea Region. Anthracnose results in major economic losses by causing young seedlings to die, withering of green components of mature plants and spotting in pods (Lenne, 1992; CIAT, 1998). In a study by Alam and Rudolph (1993) isolates of *C. lindemuthianum* from various regions of Turkey have been named alpha, beta, gamma and delta. Similarly in a survey conducted between 2005 and 2007 in Central Black Sea Region same races were also detected. Recently those isolates were also evaluated according to CIAT- binary code system to catalogue on race basis. When fresh bean lines and commercial varieties were screened with those strains (2175, 3071, 3303, 3321, and 4071) they all exhibited susceptibility to all the races (Madakbaş, Dolar, Bayraktar, & Ellialtıoğlu, 2006; Madakbaş, 2007).

Careful selection of genes providing resistance to races producing anthracnose disease is required. The varieties carrying resistance genes provide a short-lasting control over the disease lasting only until new strains of fungus emerge. The only way to maintain permanent resistance is to combine the genes present in resistant varieties in a single variety (Goncalves-Vidigal & Kelly, 2004a). Studies conducted to date have identified 13 gene loci (*Co-1-Co-13*) providing resistance to anthracnose. While twelve of 13 genes (*Co-2-Co-13*) are of Mesoamerican origin, only *Co-1* gene and its five allelic forms are of Andean origin (Kelly & Vallejo, 2004). The major difficulty with the process of compiling the resistance genes is that detection of the resistant plants takes time since it requires systematic inoculation with different strains of the fungus. Although anthracnose disease has significant detrimental effects in Turkey especially in coastal regions where fresh bean cultivation is common, the studies concerning determination of pathogen strains common to Turkey and of varieties resistant to these strains in Turkey have been initiated only recently. A literature review indicates that in other countries where cultivation is common, strains of the common fungi have been identified and the varieties resistant to the identified strains have been developed (Kelly, Hosfield, Varner, Uebersax, Afanador, & Taylor, 1995; Kelly, Hosfield, Varner, Uebersax, Long, & Taylor, 1998; Balardin & Kelly, 1998; Sharma, Kumar, Sharma, Sud, & Yagi, 1999; Kelly, Hosfield, Varner, Uebersax, & Taylor, 2000; Kelly, Hosfield, Varner, Uebersax, & Taylor, 2001; Acosta-Gallegos, Ibarra-Pérez, Rosales-Serna, Cázares-Enríquez, Fernández-Hernandez, Castillo-Rosales, & Kelly, 2001a,b; Miklas, Kelly, & Singh, 2003). In Turkey, where this agent is present and poses risk of spreading, it is crucial to search for sources of resistance against this pathogen, to protect and make use of the present gene sources and to transfer resistance to native varieties by selective breeding. In breeding for resistance, the present genetic stock serves as the source; detection of resistance and transfer of this resistance to other varieties are performed by “back cross breeding” method (Bayraktar, Macit, Vural, & Turhan, 1974; Demir, 1975; Sosyal, 1992; Ersayın, 1995; Kurt, 2001). Research on developing anthracnose resistant strains has gained momentum in recent years (Miklas, Afanador, & Kelly, 1996; Kelly et al., 2001). Aside from studies on inheritance and developing resistant strains by back cross breeding, research on MAS breeding has also been intensively performed to identify resistance genes (Kurt, 2001; Vallejo & Kelly, 2005; Miklas, Kelly, Beebe, & Blair, 2006).

Under scope of GDAR (General Directorate of Agricultural Research and Policy) project “Breeding of Fresh Bean Lines of Middle Black Sea Region Through Selection” between 2003 and 2009, nine dwarf fresh bean lines (T23, TK57, TK1, Ç31, Ç28, T7, T26, TK15 and T21) which were developed as Ayşe kadın type using pure-line selection, a method used in self-pollinated plants, were detected to be resistant against five pathogen strains (2175, 3071, 3303, 3321, and 4071). Transfer of *Co-1* gene to the variety registration candidate lines was attempted by hybridization with resistant MDRK, PM, Kaboon, Widusa, Machinac, Isles and Chinook varieties, that carry *Co-1* gene of Andean origin. Molecular marker assisted selection was performed to control the inheritance of the gene and to confidently proceed with backcross breeding studies in scope of resistance breeding. Furthermore information on genes conferring anthracnose resistance in bean and suggestions on how to make use of these genes in resistance breeding through backcrossing have been discussed.

2. Material and Methods

2.1 Plant Material

Dwarf Ayşe Kadın fresh bean lines T23, TK57, TK1, Ç31, Ç28, T7, T26, TK15 and T21 carrying Ayşe Kadın characteristics, which were obtained in the GDAR project “Breeding of Fresh Lines of Middle Black Sea Region Through Selection” in Samsun Black Sea Agricultural Research Institute between 2003 and 2009, were used. In a study conducted previously, these nine lines were screened by molecular markers (SEactMcca) and have been determined to be absent of *Co-1* locus (Madakbaş, Hız, Gültekin, & Sayar, 2009a). Anthracnose resistant varieties

MDRK, PM, Kaboon, Widusa, Machinac, Isles, Chinook, which carry *Co-1* gene and its different allelic forms, had been obtained from USDA in 2005 to be used as donor parents (Table 1).

Table 1. Anthracnose resistant foreign varieties (*Co-1*)

Varieties resistant to <i>Co-1</i> virulence	Contained resistance genes/ allelic forms	Gene pool
MDRK	<i>Co-1</i>	A
Perry Marrow	<i>Co-1</i> ³	A
Kaboon	<i>Co-1</i> ²	A
Widusa	<i>Co-1</i> ⁵	A
Machinac	<i>Co-1</i>	M
Isles	<i>Co-1</i>	M
Chinook	<i>Co-1</i>	M

MDRK: Michigan Dark Red Kidney, Co: *Colletotrichum lindemuthianum*, A: Andean origin, M: MesoAmerican origin.

2.2 Inoculation of Lines and Resistant Varieties with Pathogen Strains

For fungus activation prior to inoculation, pathogen strains were subcultured in petri dishes containing PDA (200 g L⁻¹ potato, 30 g L⁻¹ dextrose and 30 g L⁻¹ agar), spores were collected with assistance of a brush after adding 10 ml distilled water and, concentration was set to 1.2 x 10⁶ by Thomas glass counting (Pastor- Corrales, Okaya, Molina, & Singh, 1995). Under greenhouse conditions, seeding was performed with one seed from each variety planted in a single pot, repeated three times with peat as seed beds and plantlets were grown until the ten day seedling phase after which every set of first true leaves were exposed to spore suspension by spraying (Bigirimana, Rop, Fontain, & Höfte, 2000). Dwarf fresh bean lines T23, TK57, TK1, Ç31, Ç28, T7, T26, TK15 and T21 with Ayşe Kadın properties and anthracnose resistant MDRK, PM, Kaboon, Widusa, Machinac, Isles, Chinook varieties were inoculated with strains, 2175, 4071, 3303, 3321 and 3071 (Madakbaş et al., 2006). Evaluations were performed on the scale of 0 to 9 (0-3 resistant; 4-9 sensitive) (Goncalves-Vidigal, Thomazella, Elias, & Vidigal-Filho, 2004b).

2.3 Hybridization

Fresh bean is one of the most difficult species to hybridize and has the lowest rate of successful hybridization. To hybridize the fresh bean flower, hybridization forceps was immersed in alcohol initially. Alea, residing in the inner side of vexillum was reached after bisecting the vexillum of the closed flower bud with a forceps; one leaf of the alea was detached and carina was reached. Carina was dissected with the forceps and male organs were removed from the inner regions without harming the female organs. Thus, emasculation process was completed in the main parent to be hybridized. Forceps were dipped into alcohol once more and pollen taken from the male parent was besmeared to the stigmatic lip. Following besmearing, carina and vexillum were closed to prevent drying out of the stigmatic lip. Male and female parents were labeled and attached to the flower without harming it (Madakbaş, Ellialtıoğlu, & Ergin, 2009b). Hybridization processes consisting of 63 combinations from 9 lines x 7 foreign varieties in total was conducted. In each combination, 9 sensitive lines were used for hybridization. In each of the 63 combination, 30 hybridizations each were performed and 20 seeds were collected from the combinations that have given seeds (Table 2). The F₁ generation obtained in 2007 was selfed within the same year and F₂ generation was obtained. Hybrids in F₂ generation were re-inoculated with five strains. The ones showing susceptibility were eliminated and molecular marker assisted selection of the resistant ones was initiated.

2.4 DNA Isolation and Molecular Marker Analysis

DNA was isolated from 50 mg fresh leaf samples that were collected from the plants grown under greenhouse conditions with Maxwell 16 Tissue DNA isolation kit (Promega) in Maxwell 16 DNA isolation device (Promega). PCR based molecular marker (SEactMcca) primer sequences, PCR conditions and cycles were performed as suggested by Kelly and Vallejo (2004). Since SEactMcca marker is co-dominant and capable of distinguishing 80bp resistant and 79bp sensitive *Co-1* gene alleles, short PCR products were visualized by silver nitrate staining following 6% polyacrylamide gel electrophoresis. The molecular marker assisted detection of the resistance gene presence in plant samples were evaluated by presence of the expected band for each marker.

Table 2. 63 hybrid combinations of nine sensitive lines and seven resistant foreign varieties

	Resistant foreign donor varieties						
	MDRK	PM	Widusa	Kaboon	Machinac	Isles	Chinook
Sensitive	T23 (5)*	T23 (5)	T23 (5)	T23 (5)	T23 (5)	T23 (5)	T23 (5)
parental	TK57 (5)	TK57 (5)	TK57 (5)	TK57 (5)	TK57 (5)	TK57 (5)	TK57 (5)
lines with	TK1 (5)	TK1 (5)	TK1(5)	TK1 (5)	TK1 (5)	TK1 (5)	TK1(5)
Ayşe Kadın	Ç31 (5)	Ç31 (5)	Ç31 (5)	Ç31 (5)	Ç31 (5)	Ç31 (5)	Ç31 (5)
properties	Ç28 (5)	Ç28 (5)	Ç28 (5)	Ç28 (5)	Ç28 (5)	Ç28 (5)	Ç28 (5)
	T7 (5)	T7 (5)	T7 (5)	T7 (5)	T7 (5)	T7 (5)	T7 (5)
	T26 (5)	T26 (5)	T26 (5)	T26 (5)	T26 (5)	T26 (5)	T26 (5)
	TK15 (5)	TK15 (5)	TK15 (5)	TK15 (5)	TK15 (5)	TK15 (5)	TK15 (5)
	T21 (5)	T21 (5)	T21 (5)	T21 (5)	T21 (5)	T21 (5)	T21 (5)

MDRK: Michigan Dark Red Kidney, PM: Perry Marrow, T: Terme, TK: Tekkeköy, Ç: Çarşamba, *: Number of plants from sensitive lines used in hybridization, the bold typed combinations are the ones that gave seeds and 20 seeds were taken from each.

3. Results

When evaluated according to gene for gene relationship explained by Flor (1971), the fact that seven resistant foreign varieties (MDRK, PM, Kaboon, Widusa, Machinac, Isles and Chinook) carrying *Co-1* and its alleles were resistant to local pathogen races while nine dwarf fresh bean lines (T23, TK57, TK1, Ç31, Ç28, T7, T26, TK15 and T21) with Ayşe Kadın properties were susceptible to five strains (2175, 3071, 3303, 3321, and 4071) from Middle Black Sea Region indicates that *Co-1* virulence continues within local lines and the virulence is only broken because of the *Co-1* gene presence in foreign varieties. Therefore, to ensure the transfer of *Co-1* gene region especially to the sensitive lines, the foreign varieties with this gene region were selected as donors and the hybridization of 63 combinations shown in Table 2 was performed. F₂ generation was obtained by selfing of a total of 360 F₁ members following collection of 20 seeds each from the 18 combinations that yielded F₁ hybrid seeds. The inoculation results of F₂ population consisting of 4365 members, which show 3:1 segregation pattern on resistance character (*Co-1* gene), with the five pathogen strains are given in Table 3. Inoculations were performed with five repetitions and the F₂ members with a score of 0-3 were identified as resistant on the scale of 0-9 (Goncalves-Vidigal et al., 2004b).

Resistant F₂ combination members that show heterozygosity in seed structure and color, T23 X MDRK, T57 X MDRK, T7 X MDRK, TK1 X MDRK, TK57 X PM, TK1 X PM, TK15 X Widusa, T7 X Widusa, Ç31 X Widusa, Ç28 X Widusa, TK1 X Isles, T23 X Machinac, TK1 X Machinac, Ç31 X Machinac, T26 X Kaboon and T21 X Chinook, were selected for the inheritance of *Co-1* gene region by PCR based approach with the use of SEactMcca co-dominant molecular marker with three repetitions. Donor parents were used as positive controls and sensitive parental lines as negative controls in the analysis with the same molecular marker (Figure 1). With SEactMcca co-dominant marker, while resistant/sensitive alleles of 80/79bp length are detected in heterozygotes, only resistant allele of 80 bp or sensitive allele of 79 bp was detected in homozygote F₂ members for this gene region (Figures 1 and 2). In accordance with our inoculation results molecular marker results showed that *Co-1* gene was absent in nine parental lines but present in all seven of the donor foreign varieties. In resistant F₂ population it was observed that only TK1 X Isles, T23 X MDRK, T7 X MDRK, TK1 X MDRK, TK1 X PM and T21 X Chinook combinations carried the molecular marker. Although F₂ individuals of other combinations present resistant phenotype following inoculation, they didn't carry the *Co-1* molecular marker (Figure 1).

4. Discussion

In our previous work strain identification of Central Black Sea Region *C. lindemuthianum* pathogen isolates showed that the strains that pose a threat to the region carry virulence/avirulence of both Andean and Meso-American origin (Madakbaş et al., 2006). It is known from our previous studies that the registration candidate parental lines are sensitive especially to all five strains, and resistance to Meso-American virulence is present in these lines (T23 (*Co-2*, *Co-4*, *Co-4²*, *Co-6*); TK57 (*Co-2*, *Co-4*, *Co-4²*, *Co-6*); TK1 (*Co-2*, *Co-4*, *Co-4²*, *Co-6*); Ç31 (*Co-2*, *Co-4*, *Co-4²*, *Co-6*); Ç28 (*Co-2*, *Co-4*, *Co-4²*, *Co-6*); T7 (*Co-2*, *Co-4*, *Co-4²*, *Co-6*); T21

(*Co-2*, *Co-4*, *Co-4²*, *Co-6*); T26 (*Co-2*, *Co-4*, *Co-4²*, *Co-6*) and TK15 (*Co-2*, *Co-4*, *Co-4²*) respectively) (Madakbaş et al., 2009a); the necessity of immediate breeding of lines against pathogen virulence of especially Andean origin, in order to combat with anthracnose disease has arisen. While the strains that are virulent to fresh bean varieties of Andean origin can infect only Andean germplasm, the strains that are virulent to varieties of Meso-American and Andean origin can infect varieties of both Andean origin and of Meso-American origin (Pastor-Corrales et al., 1995). The transfer of *Co-1* gene, which is the most efficient gene against virulence of Andean origin, and different allelic forms to the lines will produce new resistant patented varieties to be used in Turkish bean production. Balardin and Kelly (1993) have verified that *Co-1²* and *Co-4²* alleles confers extensive resistance. Awale, Falconi, Villatoro, and Kelly (2007) suggests pyramiding of *Co-1²* and *Co-4²* genes obtained from gene pools that are recognized by researchers from all over the world for resistance breeding. The sense in transferring *Co-1* gene to five susceptible varieties as Awale et al. (2007) suggested can be clearly seen considering the majority of the dwarf fresh bean lines we have used in the breeding study have been shown to contain *Co-2*, *Co-4*, *Co-4²* and *Co-6* genes (Madakbaş et al., 2009a; Balardin & Kelly, 1993).

In the present study, only 18 combinations were yielded seed after hybridizations containing 63 combinations of seven resistant foreign varieties (MDRK, PM, Kaboon, Widusa, Machinac, Isles and Chinook) and nine sensitive fresh bean lines (T23, TK57, TK1, Ç31, Ç28, T7, T26, TK15 and T21). Low rate of success with hybridizations can be due to the fact that fresh bean is a self-pollinated crop and has a flower morphology that makes it one of the hardest plants to hybridize. Especially in budding phase, probability of cleistogamy is so high that plants self-fertilize themselves before cross-pollination can be performed (Madakbaş, 2007; Madakbaş et al., 2009b). Due to this condition, a high rate of self-fertilization may occur in hybridization despite the best breeding experience and hand skills. The breeding program that we initiated with the present study has progressed to F₂ generation phase showing anthracnose resistance segregation. Following inoculation of the F₂ pathogen strains sensitive individuals (homozygous recessive) were separated; molecular marker screening with samples of resistant individuals (homozygous dominant and heterozygous) of each combination revealed the presence of *Co-1* gene only in individuals of six combinations (TK1XIsles, T23XMDRK, T7XMDRK, TK1XMDRK, TK1XPM and T21XChinook) out of 18 combinations. The reasons for the absence of the molecular marker in 12 hybrid combinations according to inoculation results can be explained in two ways. First is that the determination of resistant phenotypes by inoculations is a relative assessment, and the values that are given on the scale of 0-9 vary upon environmental conditions, the amount of spore inoculum contacting the leaf, dispersion area of spores in spraying and personal judgment of the evaluator. Therefore, resistant assessment may not be reliable especially for plants that scored 3-4, which defines the resistant/sensitive border. Similar problems have been experienced also by other researchers, so the importance of using molecular markers that enables genotype testing in selection is emphasized (Pastor-Corrales 1991, 1992; Sartorato, del Peloso, Rava, de Costa, Fara, & Melo, 2004). Second is that the resistance character in foreign varieties is not controlled only by the presence of *Co-1* gene. Therefore when the hybrid combination F₂ individuals that inherit resistance genes other than *Co-1* in hybridization are analyzed with molecular markers specific to only *Co-1* they give negative results. Kelly and Vallejo (2004) stated that SEactMcca molecular marker is successful in detection of germplasms carrying *Co-1* gene and *Co-1²*, *Co-1³* alleles; however it is incapable of detecting the other alleles of *Co-1* gene. It has been emphasized several times in the literature that markers of resistance genes of especially Andean origin are inadequate and it is a necessity to develop new molecular markers for other resistance genes of Andean origin (Kelly & Vallejo, 2004; Vallejo & Kelly, 2005; Beaver & Osorno, 2009). Since the aim of our study was to transfer the *Co-1* gene to registration candidate lines (T23, TK57, TK1, Ç31, Ç28, T7, T26, TK15 and T21) that are known not to carry *Co-1* gene of especially Andean origin from our previous studies (Madakbaş et al., 2009a), the six combinations of hybrid F₂ population were chosen confidently for use in the backcross studies because they have the resistant phenotype and they carry molecular *Co-1* marker. This study has been the first attempt to start anthracnose resistance breeding program Turkey with integration of marker-assisted selection approach to breeding.

In conclusion besides utilizing classical breeding methods including backcrossing and inoculation, our study reveals presence of anthracnose resistance gene have been with MAS method. Thus hybrids obtained in this study will be backcrossed for five generations with parental lines and each generation will be tested by inoculation and marker analysis as anthracnose resistant variety candidates.

Table 3. Inoculation results of 4365 membered F₂ population, which show segregation in *Co-I* inheritance, with pathogen strains

Hybrid combinations that give seeds	F ₂ population counts and results from inoculation with pathogen strains			Resistant F ₂ %
	Resistant F ₂	Sensitive F ₂	Total F ₂	Resistant F ₂ %
T23 X MDRK	203	47	250	81.2
TK57 X MDRK	200	50	250	80.0
TK1 X MDRK	180	70	250	72.0
T7 X MDRK	193	57	250	77.2
TK7 X PM	167	83	250	66.8
TK1 X PM	205	45	250	82.0
T23 X Widusa	225	75	300	90.0
Ç31 X Widusa	213	87	300	71.0
Ç28 X Widusa	252	98	350	72.0
T7 X Widusa	221	79	300	73.6
TK15 X Widusa	170	80	250	68.0
TK1 X Kaboon	125	45	170	73.5
T26 X Kaboon	132	63	195	67.6
T23 X Machinac	119	61	180	66.1
TK1 X Machinac	102	48	150	68.0
Ç31 X Machinac	140	30	170	82.3
TK1 X Isles	205	45	250	82.0
T21 X Chinook	205	45	250	82.0

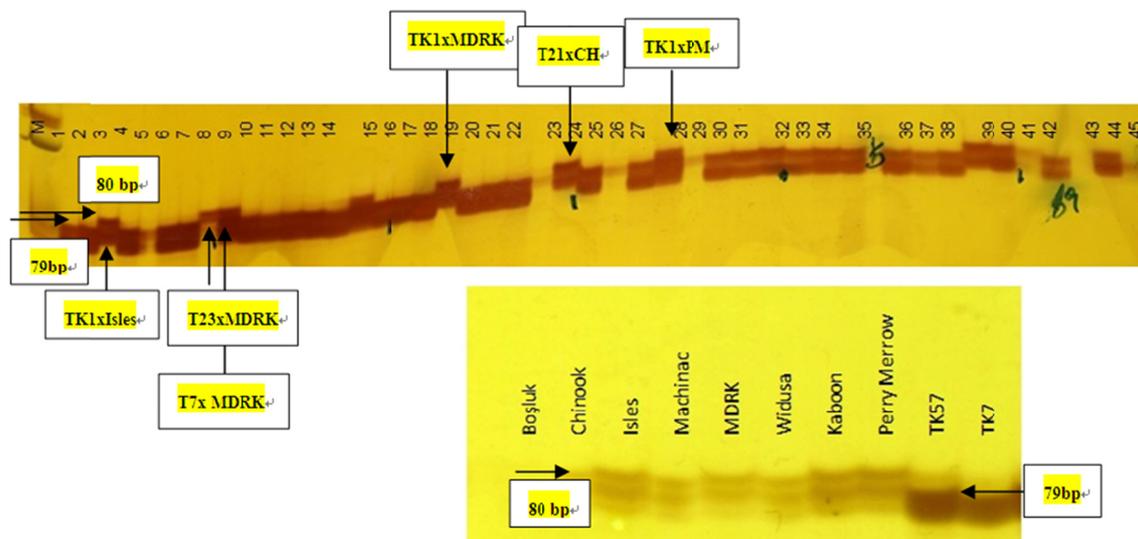


Figure 1 and Figure 2. PCR based SEactMcca *Co-I* molecular marker assisted selection of F₂ resistant individuals obtained from hybridization between dwarf fresh bean lines (sensitive) with Ayşe Kadın properties (sensitive) and varieties resistant to anthracnose, M: 50bp DNA molecular weight marker, (1-29): T23 X MC, TK57 X PM, TK1 X IS, Ç31 X MC, TK1 X MC, Ç28 X W, T7 X W, T23 X MDRK, T7 X MDRK, T26 X K, T23 X MDRK, TK15 X W, Ç31 X W, T7 X MDRK, TK1 X PM, T23 X W, TK1 X K, T7 X W, TK1 X MDRK, T7 X W, Ç31 X W, Ç31 X W, TK15 X W, T21 X CH, TK57 X MDRK, Ç31 X W, TK1 X MDRK, TK1 X PM, TK15 X W.; (30-38): TK15, T21, Ç31, T26, TK1, T23, T7, TK57, Ç28.; (39-45): CH (Chinook), IS (Isles), MC (Machinac), MDRK (Michigan Dark Red Kidney), W (Widusa), K (Kaboon), PM (Perry Merrow). The experiment was repeated for foreign resistant samples between 39-45 since they have not properly propagated in the polyacrylamide gel; the second gel image below belongs to those samples. The samples that carry the *Co-I* gene marker are the ones that have 80bp DNA fragment

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