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GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF PLANT PRODUCTION AND TECHNOLOGIES

PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF *POTATO VIRUS Y* (PVY) RESISTANCE ON POTATO VARIETIES AND CROSSES: STUDY OF TRANSMISSION ABILITY OF SOME PVY STRAINS BY APHIDS

MAHMOOD AYYAZ

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PhD THESIS

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PhD Thesis

Supervisor

Professor Dr. Çiğdem ULUBAŞ SERÇE

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Mahmood AYYAZ tarafından **Prof. Dr. Çiğdem ULUBAŞ SERÇE** danışmanlığında hazırlanan "**Phenotypic and Genotypic characterization of *Potato virus Y* (PVY) resistance on potato varieties and crosses; study of transmission ability of some PVY strains by aphids**" adlı bu çalışma jürimiz tarafından Niğde Ömer Halisdemir Üniversitesi Fen Bilimleri Enstitüsü, Bitkisel Üretim ve Teknolojileri Ana Bilim Dalı'nda Doktora tezi olarak kabul edilmiştir.

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MÜDÜR V.

THESIS CERTIFICATION

I certify that the thesis has been written by me and that, to the best of my knowledge and belief. All information presented as part of this thesis is scientific and in accordance with the academic rules. Any help I have received in preparing the thesis, and all sources used, have been acknowledged in the thesis.

Mahmood AYYAZ

SUMMARY

PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF
POTATO VIRUS Y (PVY) RESISTANCE ON POTATO VARIETIES AND
CROSSES; STUDY OF TRANSMISSION ABILITY OF SOME
PVY STRAINS BY APHIDS

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Potato virus Y (PVY), a member in the *Potyviridae* family is a destructive plant virus affecting potato production in Turkey. The present study was conducted to investigate the phenotypic (virus inoculation and testing using DAS-ELISA) and genotypic characterization (using $GP122_{718}$, $GP122_{564}$, STM0003 and RysC3 markers) of *Potato virus Y* (PVY) resistance in six parent potato varieties (Bettina, Hermes, Nectar, Savanna, Slad Blue and Galata) and five crosses (Bettina × Hermes, Bettina × Nectar, Bettina × Savanna, Bettina × Galata and Bettina × Salad Blue). Additionally, transmission capacity of two PVY strains ($PVY^{NTN\ (A)}$ and $PVY^{N:O}$) by *Myzus persicae, Aphis gossypii and Aphis fabae* aphid species were also investigated. The PVY isolates and strains obtained from potato fields of Niğde were used for preparing the inoculum sources for phenotyping and transmission studies. In phenotypic characterization studies among the tested parents, Hermes, Salad Blue, Galata, Savanna and Nectar were evaluated as virus-susceptible. In the analyzed crosses, Bettina× Galata was found as tolerant with minimum tuber susceptibility. The genotypic characterization of PVY revealed that STM0003 marker is compatible with phenotypic data for the tested parents and crosses. In the studies of virus transmission by vectors, *M. persicae* was evaluated as efficient vector for both $PVY^{NTN\ (A)}$ and $PVY^{N:O}$ in the tested plants.

Keywords: $PVY^{NTN\ (A)}$, $PVY^{N:O}$ Marker Assisted Selection, *M. persicae*, Ry_{sto}, Ry_{adg}, STM0003

ÖZET

PATATES ÇEŞİT VE MELEZLERİNDE *PATATES Y VIRÜSÜ* (PVY) DAYANIKLILIĞININ FENOTİPİK VE GENOTIPİK KARAKTERİZASYONU VE BAZI PVY IRKLARININ YAPRAK BİTLERİ İLE TAŞINMA DURUMUNUN ARAŞTIRILMASI

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Potyviridae familyasında yer alan *Patates Y virüsü* (PVY), Türkiye'de patates üretimini etkileyen önemli bir bitki virüsüdür. Bu çalışmada, altı adet ebeveyn (Bettina, Hermes, Nectar, Savanna Slad Blue and Galata) patates çeşidinde ve 5 melez popülasyonda (Bettina × Hermes, Bettina × Nectar, Bettina × Savanna, Bettina × Galata and Bettina × Salad Blue) PVY'ye karşı dayanıklılığın fenotipik (virus inokulasyonu ve DAS-ELISA kullanılarak test etme) ve genotipik olarak karakterizasyonu (GP122718, GP122564, STM0003 ve RysC3 işaretleyicileri kullanılarak) amaçlanmıştır. Ek olarak, *Myzus persicae*, *Aphis gossypii* ve *Aphis fabae* afid türleri tarafından iki PVY ırkının ($PVY^{NTN (A)}$ ve $PVY^{N:O)}$ taşınma kapasitesi araştırılmıştır. Niğde'de patates yetiştirilen alanlardan temin edilen PVY izolatları ve ırkları ile fenotipleme ve genotipleme çalışmalarında gerekli olan inokulum kaynakları hazırlanmıştır. Fenotipik çalışma sonuçlarına göre test edilen ebeveynler arasında Hermes, Salad Blue, Galata, Savanna ve Nectar çeşitleri virüse duyarlı olarak değerlendirilmiştir. Araştırılan melezlerde Bettina × Galata minimum yumru hassasiyeti ile PVY'ye tolrtanslı olarak bulunmuştur. PVY'nin genotipik karakterizasyonu sonucunda, STM0003 markörünün test edilen ebeveynler ve melezlerde fenotipik çalışmalarla uyumlu olduğu ortaya konmuştur. Vektörle taşıma çalışmalarında, *M. persicae* yaprak biti, test edilen bitkilerde $PVY^{NTN(A)}$ ve $PVY^{N:O}$ taşınmasında etkili vektör olarak değerlendirilmiştir.

Anahtar sözcükler: *Patates Y virüsü*, markör yardım ile seleksiyonu, *M. persicae*, Ry_{sto}, Ry_{adg}, STM0003

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SYMBOLS AND ABBREVIATIONS

Simgeler	**Descriptions**
%	Percent
µg/µL	Microgram/microliter
µL	Microliter
µM	Micromolar
bp	Base pair
cM	Centimorgan
Kb	Kilobyte
kDa	Kilodalton
mg/l	Milligram per liter
mg/ml	Milligram/ Milliliter
min	Minutes
ml	Milliliter
mM	Millimolar
ng/l	Nanogram/liter
nm	Nanometer
s	Second
wt/vol	Weight/volume

Abbrevations	**Descriptions**
AD	After Death
AFLP	Amplified Fragment Length Polymorphism
BC	Before Christ
CAPS	Cleaved Amplified Polymorphic Sequence
CP	Coat Protein
DAS-ELISA	Double-Antibody Sandwich Enzyme-Linked immuno Sorbent Assay
ER	Extreme Resistance
FAO	Food and Agriculture Organization
HR	Hypersensitive Resistance

IC	Imminocapture
IgG	Immunoglobulin G
ISSR	Inter-Simple Sequence Repeat
MAS	Marker Assisted Selection
PCR	Polymerase Chain Reaction
PMTV	Potato Mop-top Virus
pNPP	Para-nitrophenyl Phosphate
PVA	Potato Virus A
PVM	Potato Virus M
PVS	Potato Virus S
PVX	Potato Virus X
PVY	Potato Virus Y
RAPD	Randomly Amplified Polymorphic DNA
RJs	Recombination Junction
RLFP	Restriction Fragment Length Polymorphism
RT	Reverse Transcriptase
SCAR	Sequence Characterized Amplified Region
SSR	Simple Sequence Repeats
TRV	Tobacco Rattle Virus
VPg	Virak Genome Linked Protein
OD	Optical Density

CHAPTER I

INTRODUCTION

Potato (*Solanum tuberosum* L.) is an autotetraploid member of Solanaceae family. It is also one of the diverse and largest family including about 90 genera and more than 3000 species (Knapp et al., 2004). Potato belongs to the genus *Solanum*, which is the largest genus within Solanaceae having around 1,500 species. The *Solanum* genus also includes eggplant (*S. melongena*), tomato (*S. lycopersicum*) and woody nightshade (*S. dulcamara*) (Knapp, 2008). *S. tuberosum* is divided into two subspecies: *andigena* and *tuberosum*. The subspecies *tuberosum* is adapted to lower altitudes and longer day length, and is the widely cultivated subspecies of potato in North America and Europe. The subspecies *andigena*, which is cultivated limitedly to Central and South America, adapted to high altitudes and short days for tuberization (Roa et al., 2010; Gopal et al., 2013).

The modern cultivated potato was first recorded in Europe in the Canary Islands in 1567, but its origin has long been in dispute. There are two proposed hypotheses for the origin of the European potato; first proposal believes that it is originated from the Andes in a region from western Venezuela to northern Argentina and second one purpose that it is from lowland south central ‘‘Chilean’’ area (Ovchinnikova et al., 2011). In South America, the use of potato (*S. tuberosum* ssp. *andigena*) as a food source traces back to several thousand years BC when Peruvian colonists started the cultivation of wild potato. Archaeological documentation has reported the cultivation of potato from the Proto-Chimú and Proto-Nazca periods (200 AD) through to the Chimú period (800-1100 AD). The potato has become an important staple crop with more than one hundred varieties during the Inca period (1100- 1500 AD) (Salaman, 1985).

It is the 3rd most important crop after rice and wheat in the world (Bradshaw and Bonierbale, 2010). Potato is a rich source of starch, carbohydrate and also contains significant amount of vitamin, protein and minerals, such as potassium, calcium, magnesium, phosphorus, iron and zinc (Ortiz, 2006). It is grown and consumed as a staple food, vegetable, processed into snacks like chips and french fries (Bradshaw and Bonierbale, 2010). It also has a number of industrial usages like in starch production and preparation of biodegradable plastic (Doane, 1994). Furthermore, in recognition of its significant importance as a staple food of humans, Food and Agriculture Organization (FAO) of United Nations declared the year 2008 as the International year of the potato (Kole, 2007).

Potato is grown in more than 149 countries worlwide. China, India, Russian Federation, Ukraine and United States of America are among the top potato producing countries. Turkey is an important potato producing country with 4 million 750 thousand tonnes production in 2014 (TURKSTAT, 2016). There are contradictory reports without any reliable information from where and how potato was introduced into Turkey. According to Şenol (1971) the Russian scientist Zhukovski, estimated that potato was the first planted in Akova in the Sakarya river valley, near Adapazarı and Bosporus region during 1850s. In contrast, Ilisulu (1957) reported that potato initially arrived in Turkey during the 1870s from the Caucasia and north Russia. Since then, it has been cultivated in the high uplands of Black Sea region and Eastern Anatolia. Additionally, there are records about potato production in Erzurum province in the Eastern Anatolian region during 1870s. During the end of 18th and early 19th century there were several migrations from Caucasia to Anatolia region. It can be estimated that probably the immigrants had brought potato to the region (Çalişkan et al., 2010).

The geographical conditions of Turkey are favorable for potato production and it is grown in 75 of total 81 provinces. However, major potato production comes from the Central Anatolia region, which contributes about 61% of total production of the country. Furthermore, Central Anatolia region of Turkey comprising the provinces of Nevşehir and Niğde, which contributes altogether 23% of the total potato production of the country (Çalişkan et al., 2010).

Potato plant is the host of many fungal and viral diseases (Khurana and Singh, 1986; Kumar et al., 2012; Jeffries, 1998). Potato production is affected by more than thirty-six plant virus, viroid and phytoplasma diseases (Khurana and Singh, 1986; Jeffrie et al., 2006). In case of early and severe infections, the viruses may cause serious losses in terms of tuber yield (Hooker, 1981; Garg, 1987; Khurana and Singh, 1986). Viruses infecting potato are categorized by their mechanisms of transmission e.g. aphid transmitted such as *Potato virus Y* (PVY) and *Potato virus A* (PVA), mechanically transmitted such as *Potato virus X* (PVX), *Potato Virus M* (PVM) and *Potato virus S* (PVS), and soil-borne viruses such as *Tobacco rattle virus* (TRV) and *Potato mop-top virus* (PMTV) (Halterman et al., 2012).

PVY is a single stranded positive-sense RNA virus, having genome size of ~9.7 kb, with a poly (A) tail at the 3′ terminus and a VPg protein covalently linked to the 5′ terminus (Berger et al., 2005). PVY is "a type" species of the genus *Potyvirus*, family *Potyviridae* that has five strains (PVY^{C}, PVY^{O}, PVY^{Z}, PVY^{N} and PVY^{E}) based on reactions with potato indicators carrying the resistance genes *Nc*, *Ny*, and *Nz* or the symptoms produced on tobacco (*Nicotiana tabacum*) respectively (Galvino et al., 2012; Jones, R. A. C. 1990; Kerlan et al., 2011). Furthermore, two types of PVY recombinants have been identified based on genome sequences. PVY^{NTN}, which has two recombination types (A and B): $PVY^{N:O}$ referred as PVY^{N}-Wi (A) or PVY^{N242}; and $PVY^{NTN-NW;}$ and PVY^{N-Wi} may also be referred as PVY^{N}-Wi(B) or PVY^{N}Wi-P (Beczner et al., 1984; Chrzanowska., 1991; Crosslin., 2006; Chikh Ali et al., 2010b). PVY is a major potato pathogen of both commercial and seed potatoes threatening producers causing significant damage by reducing the crop yield from 10 upto 75% (Ottoman et al. 2009).

Infected tubers are the main sources of inoculum for the spread of this disease. PVY can also infect tomato, tobacco, pepper and many other plants, including weeds. Weeds may also act as a reservoir for this virus (Jeffries el al., 2006; Khurana, 2004). Environmental conditions, potato cultivar, virus strain, transmission factors and time of infection are the factors that may influence the severity and symptom expression of the disease (Gray et al. 2010). On the other hand, PVY can be transmitted by more than 50 aphid species in a non-persistent manner (Kanavaki et al., 2006), and well as by mechanical and vegetative way (Radcliffe and Ragsdale 2002). In non-persistent mode of transmission, acquirement and inoculation, contact periods are very short (i.e., seconds to minutes) and the aphids remain viruliferous for a very short period of time (minutes to a few hours) (Fereres and Collar, 2001, Pirone and Perry, 2002). *M. persicae* (Sulzer) (Hemiptera: Aphididae) is the most efficient vector for the transmission of PVY. Other vectors, including non-colonizing aphids, have also epidemiological significance together with the presence of aphids along with the region and the production system (Mello et al., 2011).

The usage of certified seeds for planting, elimination of weed reservoirs, application of insecticides to reduce aphid incidence, destruction of PVY sources and adapting boarder cropping systems can be useful to reduce the virus infection level. Beyond all these management practices, PVY is still a serious problem affecting the potato industry. There is a need for continuing work to identify better control of the disease (Gray et al. 2010). The most effective way to control PVY incidence is to use resistant cultivars. The identification and propagation of resistant potato plants can be helpful to control and prevent the spread of PVY infection (Felcher and Douches 2012). The selection of PVY resistant cultivars and cultivation is a cheap, applicable and environmental friendly method (Ottoman et al. 2009).

Solanum species possess two major types of single dominant genes that confers resistance to PVY virus including HR (hypersensitive resistance) conferred by the N gene and ER (extreme resistance) by the *R* gene. Extreme resistance against all strains of PVY is being conferred by the gene *Ry*, thus its presence showed no or very limited symptoms of necrosis after graft inoculation in plants. There are four different known *R* genes: Ry_{adg}, Ry_{sto}, Ry_{hou} and Ry_{chc}, which confer ER to PVY in potato (Cockerham, 1970). Extremely low amounts of virus titers in a resistant genotype, if any, can be detected by sensitive techniques (Vidal et al., 2002). The gene Ry_{adg} was discovered in *S. tuberosum* ssp. *andigena* and is considered as a single dominant gene. Genes Ry_{sto}, Ry_{hou} and Ry_{chc} were identified in *S. stoloniferum*, *S. hougasii* and *S. chacoense* respectively (Cockerham, 1970). Whereas, a generalized hypersensitive response (HR) has been conferred by the gene *Ny* which leads to a highly specific recognition of an elicitor produced by the pathogen. The four *N* genes, viz., Nc_{tbr}, Ny_{chc}, Ny_{adg} and Ny_{dms} that confer HR to PVY have been identified in *S.* tuberosum ssp. *chilotanum*, *S. chacoense*, *S. tuberosum* ssp. *andigena* and *S. demissum*, respectively (Cockerham, 1970; Valkonen et al., 1994). These genes are often strain specific apart form being monogenic in inheritance. Necrosis at the site of inoculation prevents the virus from spreading further (Vidal et al., 2002).

Conventional breeding methods to develop resistant potato cultivars are time consuming (usually takes 7 to 8 years to accomplish) and difficult. Moreover, complex and poorly understood potato genetics contributes towards its primitiveness (Gopal, 2006). There is a need for developing reliable methods to facilitate selection of plants that have the desired traits from among thousands of plants produced during the breeding programs (Witcombe and Virk 2001). In routine selection procedure, the plants are exposed to stress environment for selecting durable or tolerant plants (Mondal et al., 1997). However, marker assisted selection (MAS) methods ensure to screen the desirable trait in thousands of plants in a very short period of time.

The efficiency and precision of selection for a desirable trait during a breeding programme can be increased by MAS that involves the use of DNA markers. This technique can be utilized to detect the allelic variation in the genes related to the traits of interest, characterize genetic resources, and to provide information for assisting parental selection (Collard and Mackill 2008). There is an extensive availability of molecular markers linked to numerous traits in potato (Barone, 2004). MAS can be useful for the introgression of resistance or simultaneous selection of plants with several traits (Solomon-Blackburn and Barker 2001). Gebhardt et al. (2006) reported MAS as an effective tool for selecting major gene of resistance against pathogens and to incorporate those traits in breeding lines for developing cultivars. This technique is being used in potato breeding programs to identify plants that carry genes resistant to PVY more easily (Sorri et al.1999; Solomon-Blackburn and Barker 2001). Tiwari et al. (2012) successfully mapped Ry_{adg}, Ry_{sto} and Ry_{chc} genes conferring ER to PVY and developed molecular markers linked to PVY resistance. Furthermore, Ottoman et al. (2009) applied markers for selecting PVY resistant clones and reported MAS as an effective tool for decreasing the number of clones susceptible to PVY retained for further field evaluations, while at the same time enhance the chances for generating cultivar resistant to PVY.

However, there are various limitations in the use of DNA markers, obtained by mapping resistance genes in a given population, due to the high allelic variation in potato plants. In this respect, validation of markers in breeding material is very important. Therefore, methods and tools are needed to be developed further for the selection of PVY resistant clones obtained by crossing with PVY resistant cultivars. The DNA markers investigated in this study have a potential for selecting PVY resistant cultivars by eliminating the time taking process of phenotypic studies and labor involvement.

The present study was designed with the following objectives;

- To validate several markers linked to PVY resistance by phenotypic and genotypic studies in crosses provided from a breeding program
- To study the transmission ability of selected strains by the aphid species of *M. persicae*, *A. gossypi*, *A. faba*

CHAPTER II

LITERATURE OF REVIEW

The Solanaceae family consists of 90 genera and about 3000-4000 species having a great variation in distribution and habitat (PBI Solanum Project, 2014). The taxonomic position of Solanaceae described by National Resources Inventory, United States Department of Agriculture are given in Table 2.1.This family contains some of the most important plant species, including pepper (*Capsicum* spp), potato (*S. tuberosum* L.), eggplant (*S. melongena*) and tomato (*S. lycopersicum*). Furthermore, world's deadliest plant species including, jimsonweed (*Datura stramonium*), belladonna (*Atropa belladonna*), black henbane (*Hyoscyamus niger*) and, satan's apple (*Mandragora officinarum*) are also the member of this family (Anonymous, 2017b).

The Solanum genus consists of polymorphous and vascular plants having more than 1000 species (Fernald, 1970; Hunziker, 1979). The taxonomy of the genus and its seven subgenera has undergone various revisions. However, overall genus contains shrubs, herbs, trees, and woody or herbaceous vines, having prickles or spines, pubescent or glabrous having simple or stellate hairs (Acevedo-Rodriguez, 1996). The potato (*S. tuberosum*) is placed in *Petota* section which includes the tuber-bearing cultivated and wild potatoes. It is informally classified in the Potato clade, which also includes tomato and its wild relatives in section *Lycopersicon* (Bohs 2005; Weese and Bohs 2007). *S. tuberosum* is a complex species with triploid, diploid and tetraploid members. The tetraploid cultivars are classified into two groups; cv. group Tuberosum and cv. group Andigena (Cribb and Hawkes, 1986). The *S. tuberosum* is divided into two subspecies; *andigena* (*S. tuberosum* subsp. *andigena*) and *tuberosum* (*S. tuberosum* subsp. *tuberosum*). The subspecies *tuberosum* is widely cultivated potato crop in Europe and North America. The subspecies *andigena* is also a cultivated species, but its cultivation is limited to South and Central America (Hawkes, 1990; OECD 1997).

During the previous three centuries, potato is among the main side dishes that accompanied milk and meat, as well as helped millions of people to survive around the world (Thornton et al., 1993). The potato tuber contains high amounts of starch but also enriched in proteins and vitamin C (Storey and Davies, 1992). According to USDA

(2012), consumption of one cup of potato provides 35 percent value for vitamin C, 16 percent of fiber, and 10 percent amount of B6 values of daily diet recommendation.

Table 2.1. Taxonomic position described by National Resources Inventory, United States Department of Agriculture (USDA, NRCS, 2010)

Kingdom	Plantae (plants)
Subkingdom	Tracheobionta (vascular plants)
Superdivision	Spermatophyta (seed plants)
Division	Magnoliophyta (flowering plants)
Class	Magnoliopsida (dicotyledons)
Subclass	Asteridae
Order	Solanales
Family	Solanaceae
Subfamily	Solanoideae
Genus	*Solanum* L.
Section	*Petota*
Subsection	*Potatoe*
Series	*Tuberosa*
Species	*Solanum tuberosum* L.

According to Drewnowski and Rehm (2013), potatoes are the most economical source of potassium among the frequently consumed vegetables, and provides a better nutritional values per penny than any other vegetable. Other studies revealed that potatoes have 75% more food energy per unit area than wheat, and 58% more than rice. Potato provides 78% more protein per production areas than rice, and 54% more than wheat. There is no other crop in comparison to nutritional value, food energy and food value per unit and production, and it is grown world-wide across temperate, tropical and subtropical climatic regions of the world (Camire et al., 2009).

China, India, Russian Federation are the world's largest potato producing countries; whereas Turkey ranked 19th in the world. The advancement in science and technology such as improved fertilizer and irrigation system has enabled growers to increase the yield within limited resources (Thornton and Sieczka, 1993). Potatoes are propagated vegetatively by using tubers, resulting in the establishment and spread of plant pathogens such as virus and virus like diseases (Khurana, 2004). The virus diseases causing systemic infection can decrease the quality and yield of potato (Gray et al., 2010). More than thirty-six plant pathogens including viruses, viroid and phytoplasma have been reported negatively affecting the production of potato (Khurana and Singh, 1986; Jeffrie et al., 2006).

PVY (*Potato virus Y*) is one of the significant viruses causing severe damage to potato and other solanaceous crops upto 10-100% yield losses (Warren et al., 2005). PVY was recognized in potato during 1931, as a member of a virus disease group associated with the degeneration of potato known since the 18th century (Smith, 1931). PVY belongs to genus *Potyvirus*, one of the six genera in the family *Potyviridae* (Shukla and Ward 1989). The virions of PVY are non-enveloped, flexuous rods and filamentous, showing the length of 700-900 nm and the diameter of 11-12 nm (Figure 2.1). Particles have a helical construction with a pitch of 3.3 nm. Virion contains 5.4- 6.4 % ribonucleic acid (Leiser and Richter, 1978) and 93.6-94.6% protein (Robaglia et al., 1989). The genome of potyviruses is approximately 10 kb long in size having single strand linear RNA molecule (Scholthof et al. 2011). The 5' terminus of RNA has a genome linked protein (VPg). Infectivity retained when deproteinized with phenol or detergent. Poly A region present at the 3' end, but not essential for infectivity. Two kinds of proteins, VPg and coat protein (CP), are detected in the viral particles. The calculated molecular weight of CP is 29.95 kDa (Shukla et al., 1986). No lipid or other components have been detected in the particles (Binyam, 2015). The formation of pinwheel and cylindrical shape viral protein inclusion bodies in the cytoplasm of an infected cell is the special character associated with potyviruses and can be identified by using light and electron microscopy (Stevenson et al. 2001).

The disease severity and infection rate of PVY, depends upon involved strains, time of infection, host tolerance and environmental factors. PVY can be transmitted by mechanically, sowing the infected seed tubers and through insect vectors in non-persistent manner by a number of aphid species (Bostan and Dumlupinar, 2006; Kerlan, 2006.).

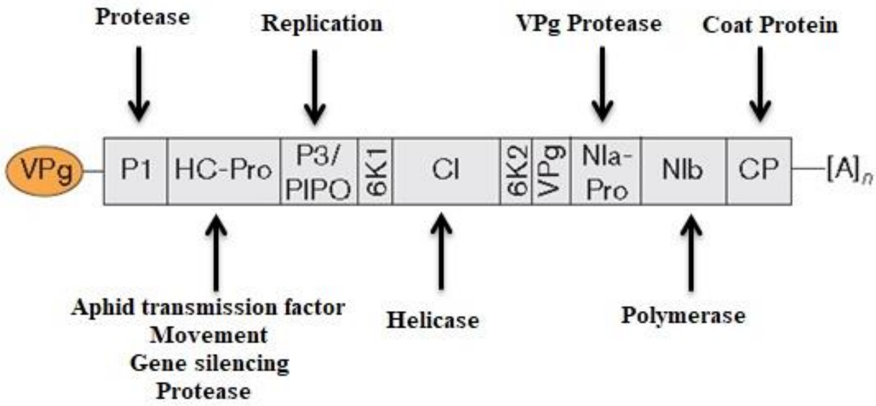

Figure 2.1. The genomic structure of Potato virus Y (PVY) Source: Ivanov et al., 2014

PVY possess a wide range of hosts (including solanaceous, non solanaceous species, weeds and even ornamentals plants) and distributed worldwide (Stevenson et al. 2001; Kerlan 2006a). Milošević et al. (2006) reported PVY infecting about nine families including 14 genera of Solanaceae such as tomato (*Lycopersicon esculentum*), eggplant (*S. melongena*), pepper (*Capsicum* spp.), tobacco (*Nicotiana* spp) and many weeds (*Physalis* spp., *Datura* spp., *S. dulcamara* and *S. nigrum*) (Jeffries, 1998). Hairy nightshade (*S. sarrachoides*) is a common weed, attractive to the potato-colonizing aphids, susceptible to PVY and serves as sources of infection for seed producers (Cervantes and Alvarez 2011). Weeds such as *S. nigrum*, *S. dulcamara* and *Physalis floridana* are commonly known to be susceptible to PVY. Furthermore, PVY strains have been differentiated by using *P. floridana* (Kerlan, 2006). Plants belonging to Leguminoceae and Chenopodiaceae are reported as host of PVY (Stevenson et al. 2001). Moreover, biennial and perennial arable weeds such as Horseweed (*Conyza canadensis*), Chicory (*Cichorium intybus*), Shepherd's purse (Capsella *bursa-pastoris*), Prickly lettuce (*Lactuca serriola*), Dandelion (*Taraxacum officinale*), Buckhorn plantain (*Plantago lanceolata*), Common lambsquarters (*Chenopodium album*) and Black nightshade (*S. nigrum*) can be a natural virus reservoir for transmission by insect vectors (Powell et al. 2006). The experimental reported host range of PVY comprises of 495 species in 72 genera of 31 families. About 287 species in 14 genera of the Solanaceae (among which 141 species from *Solanum* and 70 species from *Nicotiana*). Furthermore, about 28, 25, 20 and 11 species from *Amaranthaceae, Leguminosae*, *Chenopodiaceae*, and *Compositae* have been reported as host for PVY respectively (Binyam, 2015).

PVY can be transmitted by vegetatively or mechanically by grafting, wounding, cutting of infected seed (Radcliffe and Ragsdale 2002). During mechanical transmission (also called sap transmission), PVY is spread through infected plants to healthy plants by rubbing, resulting in short distance spread of the virus. This transmission requires mechanical damage by wind or human activity. The transmission capacity of PVY strains may differ according to aphid vectors (Basky and Almasi, 2005; Cervantes and Alvarez, 2008; Fereres et al., 1993). It has been observed that the efficiency of transmission will vary among aphid populations within a species and virus isolates within a strain (Verbeek et al., 2010).

Aphids are the primary source of PVY transmission in the field. PVY may be vectored by 50 different aphid species, majority of them don't have potato as main host, either they may or may not colonize potato (Ragsdale et al., 2001). PVY is a non-persistent, stylet-borne virus. In non-persistent viruses there is no latent period, vector requires the limited period of time (seconds to minutes) for acquiring the virus and the ability to transmit the virus (Bradley, 1954). Once the plant is inoculated by sap feeding aphid, the virus is then translocated to the tubers (Cervantes and Alvarez 2008).

The green peach aphid (*M. persicae*) is considered as the most efficient aphid species for transmitting PVY (Cervantes and Alvarez 2008). The increased usage of fungicides against late blight results in the destruction of biological controlling agents such as entomopathogenic fungi; it is thought to be a factor in raising the populations of *M. persicae*. Furthermore, resistance has been reported against the insecticides used controlling *M. persicae.* The increase in the acreages of host crops of *M. persicae*; such as canola in the potato growing regions, is among the factors that have caused higher incidences of PVY (Radcliffe and Ragsdale 2002). Pea aphid (*Acyrthosiphon pisum*), bird cherry-oat aphid (*Rhopalosiphum padi*) and Potato aphid (*Macrosiphum euphorbiae*) are the other important vectors of PVY. Soybean aphid (*Aphis glycines*) was discovered as novel vector of PVY during the year 2000. Although, individual soybean aphids are inefficient vectors of PVY, *A. glycines* feeds exclusively on soybean, but also probe other plants while searching for a suitable food source. During searching process, *A. glycines* may transfers PVY inoculum to healthy potato plants (Davis et al. 2005). Aphids as insect pest have significance for potato producers primarily because they vector devastating viruses, but higher population of aphid may also cause feeding injury and reduce yield significantly (Radcliffe and Ragsdale 2002).

Bradley and Rideout (1953) reported that the transmission rate of PVY by *M. solani* (Kltb.), *M. solanifolii* (Ashm), *A. abbreviate* and *M. persicae* were 4%, 9%, 55% and 31% respectively. Besides, the infection rate and the infective ability of *M. persicae* was higher and longer than *A. abbreviata*. Van Hoof, (1980) reported 13 aphid species as effective vector for PVY^N transmission. The recorded transmission potential of PVY^N by *R. padi, Capitophorus hippophaes, Metopolophium dirhodum, Aulacorthum solani, A. pomi, M. albidum, A. pisum, A. fabae, Maerosiphum euphorbiae, Phorodon humuli, R. insertum, M. persieae*, and *M. certus* was 2, 3, 3, 5, 9, 11,14, 24, 29, 35, 50, 50 and 71 %, respectively, whereas, found *Brevicoryne brassicae, Sitobion avenae, Brachycaudus helichrysi, C. theobaldi, Cavariella aegopodii, Hyperomyzus lactueae, Hyalopterus pruni, Microlophium carnosum, Lipaphis erysimi, Nasonovia ribisnigri, M. ascalonicus* species were unable to transmit PVY^N. Katis and Gibson, (1985) reported that *R. padi* (alatae and apterae) was capable of transmiting PVY^O and PVY^N. Furthermore, as reported in this publication, *M. dirhodum* was able to transmit PVY infrequently, *S. avenae* was unable to transmit it.

In 1986, Piron (1986) studied 101 aphid species or species groups in order to check their efficiency and ability to transmit potato virus PVY^N among potato species. According to the obtained results, 23 species were found capable and 78 species or species groups were found to be incapable to transmit PVY^N. Furthermore, among the tested species which are known capable of transmitting the virus, *A. nasturtii, Cryptomyzus galeopsidis, B. helichrysi, C. ribis, H. pruni, Hyadaphis foeniculi, H. lactucae, S. fragariae* and *S. avenae* were reported as being potential vector for PVY^N.

PVY is classified into five strain groups (PVY^C, PVY^O, PVY^Z, PVY^N, and PVY^E) and some of its strains are limited to only specific continents (de Bokx and Huttinga, 1981; Harrison, 1984). The strains can be distinguished according to local and systemic symptoms induced on *Nicotiana tabacum* L. ‘Samsun NN,’ *Physalis pubescens* L., and ‘White Burley’ and several potato (*S. tuberosum* L.) indicators carrying the resistance genes *Nc*, *Nz* and *Ny* (Sorri et al., 1999; Galvino-Costa et al., 2012; Jone, 2006; Kerlan et al., 1999; Kerlan et al., 2011).

The strain groups are designated as stipple streak strains (PVY^C), common and ordinary strains (PVY^O) and tobacco venial necrosis strains (PVY^N) (McDonald, et al., 1994; Singh, 1992). PVY^C (stipple streak strain) induces hypersensitive reactions or mosaic on potato and is not transmitted by aphids (Radcliffe and Ragsdale 2002). PVY^C strain group have been reported from Australia, New Zealand, South Africa, Europe and India (de Bokx and Huttinga, 1981; Fribourg and Nakashima, 1984; Gooding and Tolin, 1973). PVY^O is the common and ordinary strain found in the potato producing areas of the world, causing necrosis, yellowing of leaflets, mottling, leaf drop and mild to severe mosaic symptoms, depending on cultivar (Piche et al. 2004). PVY^N is frequently symptomless or produces leaf death in tobacco and mild to severe mosaic symptoms on potato foliage (Piche et al. 2004). During 1970s, a serologically related sub-group of the necrotic strain PVY^N, known as PVY^{NTN} was firstly described in Europe and later dispersed around the world (Piche et al. 2004), PVY^{NTN} causes potato tuber necrotic rings in the tubers (Gray et al. 2010). A new recombinant strain having characteristics of both PVY^O and PVY^N have been reported and named as $PVY^{N:O}$ (Crosslin al., 2006). $PVY^{N:O}$ is transmitted via vegetative propagating of seed and aphids and produces circular, sunken necrotic lesion on the surface of the tuber (Piche et al. 2004). $PVY^{N:O}$ is asymptomatic on leaves or produces mild symptoms on some potato varieties (Gray et al. 2010). According to Hu et al. (2009b), PVY^N and PVY^O are considered as the parents of at least nine recombinant genomes.

Different types of PVY recombinants have been identified based on the differences in genomic sequence, including PVY^{NTN} that consists of two recombination types. Type A having three RJs (recombination junctions) at the VPg-NIa, HC-Pro/P3, and C terminus of the coat protein region (CP) and type B, comprising of four RJs at the HCPro/ P3, P1, VPg/NIa, and C terminus of the CP gene; $PVY^{N\text{-}Wi}$, with two RJs at HC-Pro/P3 and P1 and also referred to as $PVY^{N\text{-}Wi(B)}$ or $PVY^{NWi\text{-}P}$; $PVY^{N:O}$, with one RJ at HC-Pro/P3, and also referred to as $PVY^{N\text{-}Wi(A)}$ or $PVYN^{N242}$; and $PVY^{NTN\text{-}NW}$ (Beczner et al., 1984; Chikh Ali et al., 2010a; Chrzanowska, 1991; Crosslin et al., 2006; Hu et al., 2009a; McDonald and Singh, 1996; Piche et al. 2004.) Mosaic genomes comprising of fragments of parental PVY^N and PVY^O sequences were found to be present in all these recombinants. Moreover, recombinant types in potato isolates of PVY formed a group of 9 main recombination patterns (Hu et al., 2009a, 2009b). The positions of the main recombinant recombination junctions were found to be relatively conserved (Lorenzen et al., 2008).

Currently, the most common PVY recombinant strains in potato are PVY $^{N\text{-}Wi}$, PVY^{NTN} and PVY$^{N:O}$ (Karasev and Gray 2013). Furthermore, the sequencing data releaved that some of the strains once thought to be different are actually the same. For example, PVY$^{N\text{-}Wi}$ has also been known as PVY$^{N\text{-}Wilga}$, PVY$^{N:O}$, PVY$^{N\text{-}Wi\text{-}P}$ and PVY $^{N\text{-}W}$ (Blanco-Urgoiti et al. 1998; Chrzanowska 1991; Glais et al. 2002; Kerlan et al. 1999; Nie and Singh 2003).

The symptom development and severity depend upon the viral protein interaction with molecular mechanism and machinery of host plant, expression level of viral genes while using protein of host plant and translocation of new formed virus particles within the plant (Visser, 2012). DiFonzo et al. (1994) reports that the plants are more susceptible before flowering during vegetative growth and less susceptible during senescing. In case of PVY infection, *S. tuberosum*, shows streaks, leaf drop, mild to severe leaf mosaic, vein necrosis (Beemster and Rozendaal, 1972). The symptoms of PVY strain varies according to plant age, cultivar and environmental conditions (Gray et al., 2016). PVY produces mild leaf mottling symptoms on *Capsicum* spp. In *Nicotiana* spp., symptoms such as severe venial necrosis with necrotic strains and mild mottle with ordinary strains occurred. Symptoms such as mild leaf mottling, turns severe during strains mixed infection are common in tomato (Edwardson, 1974).

The current available measures are limited and expensive to reduce losses caused by viral infections. The spread of the virus can be controlled by several different methods including chemical protection from virus vectors. The use of insecticides is largely ineffective for the control of aphid-borne spread of PVY (de Bokx and Huttinga, 1981). Application of mineral oil before and during the period of peak aphid activity can partially be effective for reducing the spread of PVY (Nolte et al., 2004). The use of sticky insect traps and reflective surfaces can also help to reduce virus spread by the aphid vectors (Loebenstein and Raccah, 1980). The eradication of the virus infected plant sources can be achieved by breeding new varieties possessing extreme resistance (Kang et al., 2005). The incorporation of PVY resistant cultivars in breeding program is considered as an environment friendly and economical way of restricting viral diseases in potato (Solomon-Blackburn and Barker, 2001).

The wild *Solanum* species have been used widely as a source of virus resistance in potato breeding programs since 1930s. But due to the limited utilization of genetic resources and problems faced in the conventional breeding viz., several generations of backcrossing and longer phenotypic selection procedures results in the narrow genetic base of potato cultivars (Gopal and Oyama, 2005). Secondly, conventional methods of potato breeding are time consuming, and considered mostly primitive because of the complex and poorly understood genetics. It is due to the fact that introducing a desirable trait through conventional breeding usually takes around 10 to 15 years to accomplish (Gopal, 2006). Furthermore, gene introduction from wild species to a cultivated potato is considered to be a major challenge due to crossability and sterility problems. Hence, much of the attention has been diverted towards marker-assisted selection (MAS) in order to hasten the utility of diverse genetic resources in a potato breeding program. So, the development of molecular markers has enhanced selection of target genes and introgression (Wenzel, 2006).

A wide range of molecular techniques have been applied in order to ascertain suitable DNA-based markers for MAS in potato. Solomon-Blackburn and Barker (2001) studied the mapped locations of virus resistance genes and molecular approaches to breed virus resistant potatoes. Since then a huge quantity of information has been generated on molecular markers and marker assisted selection for virus resistance in potato breeding programs.

The major genes responsible and confering resistance against PVY have been identified from pepper, potato, tomato and tobacco and are being used to develop resistance using classical breeding methods (Provvidenti and Hampton, 1992; Solomon-Blackburn and Barker, 2001). In potato, two types of genetic resistance to PVY has been identified; extreme resistance (ER) conferred by *R* gene. The gene *Ry* confer ER in plants against all PVY strains by suppressing virus accumulation in infected cells leading to cell death (Solomon- Blackburn and Barker 2001; Halterman et al. 2012). The presence of this gene confers no symptoms or very limited necrosis after graft inoculation in plants. Extremely low amounts of virus titers in a resistant genotype, if any, can be detected by sensitive techniques (Vidal et al., 2002). ER has been identified in wild potato species, *S. hougasii*, *S. chacoense*, *S. tuberosum*, *S. stoloniferum*, and Chilotanum and Andigenum groups of solanum (Cockerham, 1943). The resistance in other *Solanum* spp. such as the non-tuber-bearing species of *S. tuberosum* has been identified but the physiological basis is not known yet (Novy et al., 2002; Valkonen et al., 1992). Hypersensitive resistance (HR) is conferred by *Ny* gene, which is usually strain-specific, expressed by local necrotic lesions leads towards preventing the spread of viral infection (Solomon-Blackburn and Barker 2001). Necrosis at the site of inoculation prevents the infection from spreading further (Vidal et al., 2002). HR is conferred by genes including *Ny-1* on chromosome IX (Szajko et al. 2008) and Ny_{trb} and Nc_{spl} on chromosome IV (Celebi-Toprak et al. 2002; Moury et al. 2011). HR to PVY ordinary (O) strain group (PVY^{O}) has been reported in *S. demissum, S. chacoense, S. polyadenium, S. megistacrolobum, S. stoloniferum, S. sparsipilum, S. tuberosum,* Chilotanum and Andigenum Groups (Celebi-Toprak et al., 2002; Cockerham, 1970).

There are four different known *R* genes: Ry_{sto}, Ry_{adg}, Ry_{chc} and Ry_{hou}, which confer ER to PVY in potato. The gene Ry_{adg} was identified in *S. tuberosum* Gp. Andigenum and later characterized as a single dominant gene. The genes Ry_{chc}, Ry_{sto}, and Ry_{hou} were identified in *S. chacoense, S. stoloniferu* and *S. hougasii*, respectively (Cockerham, 1970). Whereas, four *N* genes, viz., Ny_{dms}, Ny_{chc}, Ny_{adg} and Nc_{tbr} that confer HR to PVY have been identified in *S. demissum, S. chacoense, S. tuberosum* Gp. Andigenum and *S. tuberosum* Gp. Chilotanum respectively (Cockerham, 1970; Valkonen et al., 1994).

Three known *R* genes, namely, Ry_{chc}, Ry_{adg}, and Ry_{sto} which confer ER to PVY have been mapped successfully, developed for screening potato varieties for resistance to PVY. The gene Ry_{adg} has been mapped to potato chromosome XI by RFLP markers GP125, TG508, CT168 and CD17 (Hämäläinen et al., 1997). The marker TG_{508} was measured as the closest marker from the gene Ry_{adg} at a distance of 2.0 cM. However, the application of RFLP markers for MAS is difficult but these four markers has been tested and found suitable for MAS for Ry_{adg} in diploid and tetraploid potatoes (Tiwari et al., 2013). Sorri et al. (1999) developed PCR-based CAPS (cleaved amplified polymorphic sequence) marker (ADG2/*Bbv*I) by targeting the ADG2 fragment at 310 bp. This was the pioneer example of diagnostic CAPS marker for selection PVY-resistant genotype in potato. The use of CAPS marker ADG2/B*bv*I in MAS for screening varieties for PVY resistance have been proven and well demonstrated. Ottoman et al. (2009) successfully screened a full-sib tetraploid population segregating for Ry_{adg} using the CAPS marker ADG2/B*bv*I.

Afterward, Kasai et al. (2000) developed SCAR (sequence characterized amplified region) marker named RYSC3 by targeting the differences in nucleotides between ADG2 fragments, from resistant and susceptible lines. If a resistant genotype has the Ry_{adg} gene, this marker generates a 321 bp fragment. In potato, RYSC3 marker has been widely used in order to select resistant genotypes which carry the gene Ry_{adg} against PVY. Therefore, the RYSC3 is recommended as potent tool in MAS for the gene Ry_{adg} in potato breeding programs (Bhardwaj et al., 2007; Gebhardt et al., 2006; Heldák et al., 2007; Rizza et al., 2006; Sagredo et al., 2009; Tiwari et al., 2010 ; Whitworth et al., 2009**)**.

Brigneti et al. (1997) mapped the gene Ry_{sto} in potato on chromosome XI using AFLP (Amplified Fragment Length Polymorphism) markers M17 and M6. Furthermore, two reported AFLP markers M45 and M5 cosegregated with Ry_{sto}. Rizza et al. (2006) reported that marker M45 could recognize both Ry_{adg} and Ry_{sto} genes, associated with ER by producing 500 bp fragment in PCR. Later, Song et al. (2005) mapped gene Ry_{sto} obtained from *S. stoloniferum* on chromosome XII using AFLP markers. Heldak et al. (2007) developed a CAPS marker GP122$_{406}$ (*Eco*RV) for the Ry_{sto} gene for MAS in potato. The marker STM0003 has been validated to select genotypes containing Ry_{sto} in potato (Valkonen et al., 2008). A novel CAPS marker GP122$_{406}$, targeting the gene Ry_{sto}, has been developed for MAS in potato (Heldák et al., 2007). Song et al. (2008) developed two Sequence Tagged Sites (STS) markers, YES3-3B and YES3-3A from AFLP marker E+ACC/M+CTC365 for the gene Ry_{sto} has been validated for MAS in European potato cultivars.

The gene Ry_{sto} has been successfully mapped on chromosome XII of potato with the help of RFLP-derived CAPS markers (Flis et al., 2005). An inter-simple sequence repeat (ISSR) marker named UBC 857$_{980}$, one sequence tagged site (STS) marker GP81$_{400}$, three CAPS markers GP204$_{800}$ (*Taq*I), GP122$_{718}$ (*Eco*RV) and GP269$_{650}$ (*Dde*I) linked to the gene Ry_{sto} were developed for MAS in potato (Flis et al., 2005). Witek et al. (2006) used a robust and simple multiplex PCR technique for detecting the $Ry\text{-}f_{sto}$ gene in German and Polish potato cultivars. The marker GP122$_{564}$ showed a product of 564 bp, after digestion with *Eco*RV was found to be connected to the gene $Ry\text{-}f_{sto}$. A successful validation of this marker has been done for selection of potato genotypes with the gene $Ry\text{-}f_{sto}$. Valkonen et al. (2008) developed and validated the markers GP122$_{564}$ (*Eco*RV) and GP122$_{718}$ (*Eco*RV) for the selecting genotypes of potato having the gene $Ry\text{-}f_{sto}$.

Hosaka et al. (2001) discovered the gene *Ry*$_{chc}$ which confers the ER against PVY in monogenic control and Japanese cv. Konafubuki. At the distal end of the chromosome IX this gene has been mapped by using RLFP and RAPD 38-530 (OPC-01) markers (Sato et al., 2006). The RAPD marker 38-530 associated with the *Ry*$_{chc}$ gene has been reported practically useful for MAS in potato.

The gene *Ny*$_{tbr}$ on chromosome IV has been mapped by using RFLP markers TG208 and TG316 in potato (Celebi-Toprak et al., 2002). Recently, a new gene *Ny-1* that controls HR to both necrotic (PVY^{N}) and common (PVY^{O}) strains of PVY has been reported (Szajko et al., 2008). Moreover, this gene has been mapped on the short arm of potato chromosome IX using COSII and SCAR markers. Szajko et al. (2008) reported one COSII marker $C2\_At3g16840_{1100}$ digested with *Taq*I and two SCAR markers $SC895_{1139}$ and $GP41_{443}$ were found to be linked with gene *Ny-1*. Later these markers were also integrated into MAS for screening PVY resistance potato varieties.

CHAPTER III

MATERIALS AND METHODS

3.1 Preparation of PVY Inoculum Source Plants

The *Solanum tuberosum* cv. Pentland Dell and *Nicotiana tabaccum* cv. Xanthi-nc indicator plants were used to prepare inoculum source plants. The preparation of virus free nursery of *N. tabaccum* is shown in Figure 3.1. In order to prepare PVY isolates and strains inoculum sources (confirmed by DAS-ELISA using polyclonal antibody and IC-RT-multiplex PCR as described in section 3.1.1 and 3.1.3, respectively) belonging to Niğde province were provided by Miss. Vildan Bolat (Department of Plant Production and Technologies, Faculty of Agricultural Sciences and Technologies, Nigde Omer Halisdemir University). The test plants were inoculated by mechanical inoculation using the most prevalent strains $PVY^{NTN\ (A)}$ and $PVY^{N:O}$ for aphid transmission studies and with PVY^{N}: PVY^{O+C} for validation of potato genotypes.The plants were kept under dark conditions for 4-5 hours before inoculation. The silicon carbide powder was applied on the leaf surface as an abrasive. Virus inoculum was prepared using 1 g sample /5 ml extraction buffer (0.1 M phosphate buffer pH 7.2, 1% sodium sulphite, 1% Polyvinylpyrrolidone-40) by grinding them in a mortar with pestle under cold conditions. About 500 µl virus containing solution was applied on plant leaves using cotton buds. After 2-3 minutes of inoculation, the plants were washed using distilled water (Lopez-Pardo et al., 2013).

Figure 3.1. The virus free seedlings of *Nicotiana tabacum* cv. Xanthi-nc

The plants after inoculation were maintained under controlled conditions at 23 ± 1 °C and 60 ± 5% RH with a 15:9 h light: dark photoperiod. After 15 days of inoculation, plants were tested by DAS-ELISA using monoclonal antibody, IC-RT-multiplex PCR method (as described in sections 3.2.2.1 and 3.1.3) to confirm the presence of specific PVY strain innoculated. Possible multiple virus infections were checked using RT-PCR analysis (as described in section 3.1.2).

The prepared inoculum source plants (Figure 3.2) were used in phenotypic studies for mechanical inoculation of the virus on *S. tuberosum* plants (progeny and crossess) and in aphid transmission of PVY strains as discussed in sections below.

Figure 3.2. Mosaic symptoms on potato cv. Pentlend Dell developed after PVY inoculation

3.2 Double Antibody Sandwich Enzyme-Linked İmmunosorbent Assay (DAS-ELISA) for the detection of PVY

The collected leaf samples were brought to the laboratory and subjected to DAS-ELISA (Clark and Adams, 1977) using PVY and PLRV polyclonal antiserum kit according to the manufacturer's (Bioreba®) instructions discussed below.

Coating IgG

As a first step of DAS-ELISA tests, 96-well micro titer plate was coated with the provided PVY and PLRV-specific IgGs. After diluting it with coating buffer in 1:1000 ratio, diluted antibody (200 µl) was added to per microplate well and incubating at 30±2 °C for 2-6 hours.

Washing

The IgG coated plates were washed 4 times with washing buffer using Microplate Washer (Microplate Washer Wellwash™).

Adding plant extract

The preparation of plant samples was done by homogenizing the leaf samples in the provided extraction buffer in the ratio 1:20 (w:v) and a volume of 200 µl with two repetitions was added to each IgG coated microtiter well followed by incubation at 4±2 °C overnight. After incubation, plates were washed as described in washing step above.

Adding conjugate

After plant extract incubation and washing, virus specific IgG conjugated with alkalen phosphatase was diluted in 1:1000 ratio in conjugate buffer, added to well and incubated at 30±2 °C for 5 h in a humid box. The plates were washed as described in washing step above.

Adding substrate

Following conjugate incubation step and washing, substrate application was performed by dissolving 1 mg/ml para-nitro phenyl phosphate (pNPP) in substrate buffer followed by the addition of 200 µl to each microplate well. Substrate incubation was done at room temperature under dark conditions.

The development of yellow color was measured spectrophotometrically at 405 nm using ELISA plate reader (Bio-Rad®). The samples having absorbance values two fold greater than those of negative control were considered as virus positive.

3.2.1 Double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) for strain identification of PVY

The PVY infected samples detected by polyclonal antisera using DAS-ELISA (as described in section 3.2.2.1) were subjected to DAS-ELISA for strain identification using monoclonal antibodies according to the manufacturer's (Agdia®) instructions for the detection of PVY^{C}, PVY^{N} and PVY^{O+C} as explained below.

Coating of IgGs

As a first step in ELISA, three 96-well ELISA plates were coated separately with PVY^{C}, PVY^{N} and PVY^{O+C} specific IgGs. The antisera were diluted with coating buffer in 1:200 ratio, 100 µl added to per well which was followed by incubation at 30±2 °C for 4-6 hours.

Washing

The IgGs coated plates were washed 4 times with diluted washing buffer using Microplate Washer (Microplate Washer Wellwash™).

Adding plant extract

The preparation of plant samples was done by homogenizing the leaf samples in provided extraction buffer in a dilution 1:10 followed by adding 100µl in per well and incubated at 4±2 °C overnight. Then the plates were washed as described in washing step above.

Adding conjugate

After plant sample incubation and washing, conjugate was made ready by diluting the provided specific antisera of -PVY^{C}, PVY^{N} and PVY^{O+C} and anti-mouse IgGs conjugated with alkaline phosphates in 1:200 ratio and added in each well. Followed by incubation at 30 °C for 2 h in a humid box and washed as described in washing step above.

Adding Substrate

Application of substrate for getting color reaction in virus infected sample was done by dissolving para-nitrophenyl phosphate (pNPP) at the concentration of 1mg/ml in substrate buffer and by adding 100 µl of it to per well. Plates were incubated at room temperature under dark conditions. After the reaction, the development of yellow color was photometrically measured at 405 nm using ELISA plate reader (Bio-Rad®). The samples having two-fold O.D values higher than those obtained with negative controls were considered as virus positive.

3.2.2 Reverse transcription and polymerase chain reaction (RT-PCR)

The RT-PCR was performed on the ELISA positive samples to detect any mixed infection of PVX, PLRV, PVS and PMTV.

3.2.2.1 RNA extraction from plant leaves

As initial step, RNA was extracted from the infected leaves using ZR Plant RNA MiniPrep extraction kit according to the manufacturer's (Zymo Research, USA) instructions with minor modifications in the protocol as following:

1. The 200 mg plant sample was ground using liquid nitrogen. The extracted plant samples were immediately transferred into 1.5 µl Eppendorf tube.
2. 800 µl RNA Lysis Buffer were added in the tube.
3. The plant sample was mixed well using vortex mixer and then centrifuged at 10,000-16,000 x g for 1 minute.
4. The 400 µl of the supernatant obtained after step 3 was transferred into Zymo-Spin™ IIIC column having a collection tube and centrifuged at 10,000-16,000 x g for 30 seconds and flow though was collected.
5. Equal volume of ethanol (95-100%) was added to the flow-through in the collection tube and mixed well gently.

6. The mixture was transferred into Zymo-Spin™ IIC column as discussed in step 4 having collection tubes and centrifugation (10,000-16,000 x g) was done for 30 seconds and flow though was discarded.
7. The RNA Prep Buffer (400 µl) was added to the RNA captured Zymo-Spin™ IIC column and centrifuged (10,000-16,000 x g) for 30 seconds and the flow though was discarded.
8. The RNA wash Buffer (700 µl) was added to the RNA captured Zymo-Spin™ IIC column and centrifuged (10,000-16,000 x g) for 30 seconds and the flow though was discarded.
9. The RNA captured Zymo-Spin™ IIC centrifuged (10,000-16,000 x g) again for 2 minutes to ensure complete removal of the wash buffer. The column was transferred into an RNasefree tube.
10. DNase/RNase-Free water (50 µl) was added directly to the column matrix and after 2-3 min incubation, centrifuged for 30 seconds at max. speed.

The eluted RNA was stored at -80°C for conducting the experiment

3.2.2.2 Reverse transcription and polymerase chain reaction (RT-PCR)

Reverse transcription of the viral RNA genome into a complementary DNA (cDNA) was carried out by mixing 1 µL of 50 pmol random primer (6–9 mer), 4 µL of total RNA extracted by Zymo-Spin™ kit, and the total volume was completed to 12.5 µL using RNase free sterile water. The mixture was incubated at 65 °C for 5 min and immediately chilled on ice for 5 min. Later on, 7.5 µL a mixture of 4 µL of 5X reaction buffer (250 mM Tris-HCl with ph 8.3 at 25 ° C) , 0.5 µL RiboLock RNase inhibitor (Thermo Scientific), 2 µL dNTP's (10 mM each), 1 µL Reverse Transcriptase (Thermo Scientific) were added to each reaction tube. The tubes were centrifuged gently and the mixtures were incubated at 25 ºC for 10 min followed by 42 °C for 45 min. Termination of reaction was done by heating the sample at 70 °C for 10 min and cDNA were kept in -20 ºC till use.

PCR was performed with 2 µL cDNA in a final volume of 20 µL containing 2 µL of 10× PCR buffer (200 mM Tris HCl with pH 8.4, 500 mM KCl), 1.5 µL of 25 mM $MgCl_2$, 1 µL of 10 mM dNTPs, 0.2 µL of Taq DNA polymerase (ThermoScientific), and 1 µL of each primer (10 µM) (forward and reverse) (Table 3.1). PCR conditions were 95 °C for 5 min (initial denaturation), 30 cycles of 95°C for 30 s (denaturation), 55 °C for 30 s (primer annealing) and 72 °C for 45 s (extension), followed by final extension for 10 min at 72°C. Finally, 10 µL of the products were analyzed by electrophoresis on a 1.2% agarose gel.The samples negative for all targeted viruses except PVY were selected and used as inoculum source.

Table 3.1. Primers used for the detection of PVY, PLRV and PMTV in RT-PCR

Virus	Sequence (5'–3')		Fragment size(bp)	Reference
PVY	F	ACGTCCAAAATGAGAATGCC	480	Nie and Singh, 2001
	R	TGGTGTTCGTGATGTGACCT		
PLRV	F	CGCGCTAACAGAGTTCAGCC	366	Behjatnia et al., 1996
	R	GCAATGGGGGTCCAACTCAT		
PMTV	F	AGAGCAGCCGTCGAGAATAG	416	Crosslin and Hamline, 2011
	R	TCGTCCACCTCTGCGAGTTG		

3.2.3 Immunocapture-reverse transcription-multiplex polymerase chain reaction (IC-RT-multiplex PCR) for molecular strain identification

PVY positive samples detected by polyclonal DAS-ELISA were subjected to immunocapture-reverse transcription-multiplex polymerase chain reaction (IC-RT-multiplex PCR) analysis for molecular identification of PVY strains using the primers given in Table 3.2 and described as by Chick Ali. (2010a) in Figure 3.3.

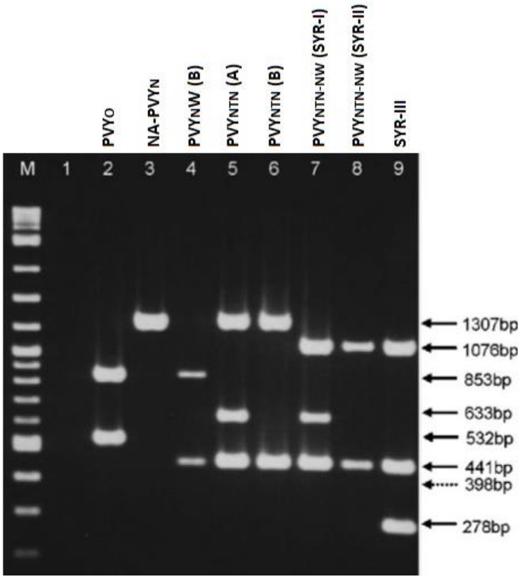

Figure 3.3. Agarose gel profiles of PVY strains according to Chikh Ali et al., 2010a

The immunocapture (IC) step was done by coating PCR tubes (Eppendorf) with 30 µl of the PVY polyclonal antibodies (IgG) (Bioreba) at the ratio of 1:1000 by diluting in coating buffer and incubated at 30°C for 4 h. Afterward, the tubes were washed three times using washing buffer. The leaf samples were ground in extraction buffer (Bioreba) at a 1:10 (wt/vol) ratio. The extracted 30 µl samples were added to the coated each PCR tubes and incubated at 4±2 °C overnight. The tubes were washed three times using washing buffer and then rinsed with one time deionized water including 1% Triton-X 100.

The reverse transcription was performed using Thermo Scientific kit according to Chikh Ali et al. (2013). In order to perform the reverse transcription step for the synthesis of complementary DNA (cDNA), 2 µL of 0.2 µg/µL Random Hexamer Primer (ThermoScientific) and 2 µL of 0.5 µg/µL Oligo $(dT)_{18}$ (Thermo Scientific) were mixed and completed to the total volume of 25 µL using RNase free sterile water,and each immunocaptured tubes were incubated at 65 ºC for 5 min and immediately chilled on ice for 5 min. Later on, a mixture of 8 µL of 5X reaction buffer (250 mM Tris-HCl pH 8.3 at 25°C, 250 mM KCl, 20 mM $MgCl_2$, 50 mM DTT), 1 µL RiboLock RNase inhibitor (Thermo Scientific), 4 µL dNTP's (10 mM each), 1,5 µL M-MuLV Reverse Transcriptase (Thermo Scientific) were added to each reaction tube. The tubes were centrifuged gently and the RT program for first-strand cDNA synthesis started at 25°C, then increased to 42°C in increments of 1°C per 30 s, held at 42°C for 45 min, and then the temperature was raised to 60 in increments of 1°C per 2 min. The transcriptase was inactivated by incubation of the rearion mixture at 70°C for 10 min.

The PCR step was carried out according to Chikh Ali et al. (2010a). Twelve primers given in Table 3.2 were used for PVY strain specific fragment amplification. cDNA solution (3 µl) was added to per PCR tube containing 2 µl of 10× reaction buffer, 0.5 µl of dNTPs (10 mM each), 0.5 µL primers (10 µM each) except S5585m and o6400 primers which were used as 1 µL., 0.2 µl (5 U/µl) of Dream Taq DNA polymerase (Thermo Scinetific). The volume of the mixture was adjusted to 20 µl using RNase free steril water.

PCR amplification was performed in a Thermal Cycler (QIAGEN) using a PCR program consisted of 30 cycles of denaturation at 94°C for 30 s, primer annealing for 30s and primer extension at 72°C for 90 s followed by a final extension at 72°C for 5 min. Annealing temperatures were 64ºC for the first 10 cycles, 62°C for the second 10 cycles, 60°C for the final 10 cycles. PCR product was separated in a 2% agarose gel, stained in ethidium bromide and visualized on UV illumination using gel documentation system (Bio-Rad Laboratories, Inc.). When the PVY strain was identified, the infected potato leaf samples were maintained at -80 °C and used later for the preparation of inoculum source plants for various experiments discussed below.

Table 3.2. The primers used for Immunocapture-reverse transcription multiplex polymerase chain reaction (IC-RT- multiplex PCR)

Primer Name	Polarity	Sequence (5 ′ –3)	Location	Reference	In silico PCR[c]
n156	Sense	GGGCAAACTCTCGTAAATTGCAG	160–179a	Chick Ali et al., 2010a	PVY^{N} and PVY^{NTN}
o514	Sense	GATCCTCCATCAAAGTCTGAGC	515–536b	Chick Ali et al., 2010a	PVY^{O} and PVY^{NW}
n787	Antisense	GTCCACTCTCTTTCGTAAACCTC	770–792a	Chick Ali et al., 2010a	PVY^{N} and PVY^{NTN}
n2258	Sense	GTCGATCACGAAACGCAGACAT	2260–2281a	Lorenzen et al. (2006)	PVY^{N} and PVY^{NTN}
o2172	Sense	CAACTATGATGGATTTGGCGACC	2169–2191b	Lorenzen et al. (2006)	PVY^{O} and PVY^{NW}
n2650c	Antisense	TGATCCACAACTTCACCGCTAACT	2627–2650a	Lorenzen et al. (2006)	PVY^{N} and PVY^{NTN}
o2700	Antisense	CGTAGGGCTAAAGCTGATAGTAG	2678–2700b	Chick Ali et al., 2010a	PVY^{O} and PVY^{NW}
S5585m	Sense	GGATCTCAAGTTGAAGGGGAC	5578–5598b	Lorenzen et al. (2006)	PVY^{O} and PVY^{NW}
o6400	Antisense	GTAACTCCTAAACAAATGGTGGTTCG	6405–6430b	Chick Ali et al., 2010a	PVY^{O} and PVY^{NW}
n7577	Sense	ACTGCTGCACCTTTAGATACTCTA	7582–7605a	Chick Ali et al., 2010a	PVY^{N}, PVY^{NTN} and NA-PVY^{N}
YO3-8648	Antisense	CTTTTCCTTTGTTCGGGTTTGAC	8635–8657b	Schubert et al. (2007)	PVY^{O} and PVY^{NW}
SeroN	Antisense	GTTTCTCCTATGTCGTATGCAAGTT	8864–8888a	Chikh Ali et al. (2008)	PVY^{N}, PVY^{NTN} and NA-PVY^{N}

3.3 Phenotypic and Genotypic Characterization of *Potato virus Y* (PVY) Resistance on Potato Varieties and Crosses

3.3.1 Plant material

A total of six parents' and five crosses belonging to these parents along with a susceptible variety (control) were used to conduct phenotypic and genotypic characterization studies in relation to resistance against *Potato virus Y.* The plant material was kindly provided by the Potato Research Institute, Niğde, Turkey (Table 3.3).

Table 3.3. Potato crosses and varieties used for screening potato virus Y resistance

Varieties and crosses	Resistance to PVY
Bettina	Resistant (ER)[a]
Hermes	Resistant to Y, Susceptible[b] to Y^N
Nectar	Not Known
Salad Blue	Not Known
Savanna	Not Known
Galata	Not Known
Adora	Susceptible
Bettina × Galata	Need to be investigated
Bettina × Nectar	Need to be investigated
Bettina × Salad Blue	Need to be investigated
Bettina × Savanna	Need to be investigated
Bettina × Hermes	Need to be investigated

Source: The resistance classification according to European Cultivated Potato Database (ECPA, 2014), a. ER extreme resistance, b. PVY necrotic strain

3.3.2 Preparation of plant materials for planting

At least 100 seeds belonging to the crosses of potato were sown individually in plastic pot trays having the mixture of 1:1 peat+perlite. The seed nursery was maintained under controlled conditions at 23±1°C and 60 ± 5% RH with a 15:9 h light: dark photoperiod. The emerging seedlings were later transferred into 2.5 l plastic pots having 1:1 peat+perlite mixture. The plants were maintained under insect-free greenhouse condition.

The experiments were performed during the early potato growing season in Niğde from May to September of 2015 and 2016.

Parent variety tuber sprouts were subjected to DAS-ELISA using PVY and PLRV polyclonal antibody kits according to manufacturers (Bioreba®) instruction (as described in section 3.1.1.). Virus negative tubers were sown in plastic pots having 1:1 peat + perlite mixture and used for phenotypic studies.

3.3.3 Mechanical inoculation of PVY

The progeny plants at 3-5 leaf stage were mechanically inoculated using PVY^N: PVY^{O+C} strains infected leaves using phosphate buffer as described in 3.1. The mechanical inoculation of PVY applied under greenhouse conditions is shown in Figure 3.4.

Figure 3.4. Mechanical inoculation of PVY under greenhouse conditions

3.3.4 Phenotypic characterization of potato genotypes for *Potato virus Y* resistance

In order to collect phenotypic data, the leaf samples were collected and subjected to DAS-ELISA to check the presence of PVY in the tested parents varieties and crosses as discussed above in section 3.2.2.1 after 4 weeks of inoculation (Figure 3.5).

Figure 3.5. Development of potato plants inoculated with PVY in insect-proof greenhouse

3.3.5 Second year planting of tubers obtained from ELISA negative progenies

The PVY negative potato tubers of the progenies obtained from the first year experiment involving mechanical inoculation of PVY were preserved under cold storage conditions at Faculty of Agricultural Sciences and Technologies during winter time. Two to three tubers from each progenies were sown in plastic pots having 1:1 peat + perlite mixture in the next potato growing season of Niğde. The emerging plants were maintained under insect-proof greenhouse conditions (Figure 3.6). After 3 weeks of plant emergence, leaf samples from each plant were collected and subjected to DAS-ELISA using PVY polyclonal antiserum kit according to manufacturers (Bioreba®) instructions as discussed above in section 3.2.2.1.

Figure 3.6. Growing of potato tubers obtained from ELISA negative progeny plants of the first year experiment

3.4 Genotypic Characterization of Potato Genotypes for *Potato Virus Y* Resistance

PCR based markers were used to study genotypic characterization of PVY resistance in potato varieties and crosses (Table 3.4). After four weeks of PVY mechanical inoculation, leaf samples were collected from each progeny plants. DNAs were extracted using DNeasy Plant Mini Kit according to the manufacturer's (Qiagen) protocol.

3.4.1 DNA extraction from potato leaves

The collected leaf sample was ground till fine powder with liquid nitrogen using mortar and pestle and transferred to 2 ml eppendorf tubes without letting the samples thaw. Then, 400 µl of Buffer AP1 and 4 µl of RNase A stock solution (100 mg/ml) to a 100 mg of ground sample were added immediately, and vortexed gently to mix the solution with sample.

1. The samples were incubated at 65°C for 10 min.
2. 130 µl of Buffer AP2 for each sample was added to the lysate, mixed and followed by the incubation of sample on ice for 5 min.

3. The lysate was transferred to QIAshredder spin column having 2 ml of collection tube (provided with kit) and centrifuged at maximum speed for 2 mins.
4. The flow-through was transferred into new tubes and absolute ethanol was added to each lysate.
5. The 650 µl mixture including ethanol was transferred to DNeasy mini spin column (provided with kit) and centrifuged for 1 min at ≥6000 x g and flow through was discarded.
6. The DNeasy column was placed in a new 2 ml collection tube (supplied), and 500 µl Buffer AW was added to DNeasy column and centrifuged for 1 min at ≥6000 x g. The flow-through was discarded. This step was repeated twice. The last centrifuge performed at maximum speed.

The DNeasy column was transferred to a new 1.5 ml or 2 ml microcentrifuge tube, and 50 µl amount of Buffer AE (preheated at 65°C) was added to DNeasy membrane. The sample was incubated at room temperature for 5 min and then sample was centrifuged for 1 min at ≥6000 x g to get the selute. The step was repeated twice to increase overall DNA yield.

3.4.2 PCR conditions and protocol

The PCR step was carried out using the primers given in Table 3.4. The reactions were used to amplify the fragment of a specific allel responsible for PVY resistance. DNA solution (2 µl) was added to per PCR tube containing 3 µl of 10× reaction buffer (Dream), 1 µl of dNTPs (10 mM each), 1 µl forward primer (10 µM each) and 1 µl reverse primer (10 µM each), 0.2 µl (5 U/µl) of Dream Taq DNA polymerase (Thermo Scientific). The volume of the mixture was adjusted to 30 µl using RNase free sterile water.

PCR amplification was performed in a Thermal Cycler (QIAGEN) using a PCR program consisted of initial denaturation at 94 ºC, followed by 35 cycles of denaturation at 94 ºC for 30 s. Primer annealing was adjusted according to the primer used (52 °C for $GP122_{718}$, $GP122_{564}$ and RySC3, whereas 50 °C for STM0003) for 30 s and primer extension at 72 °C for 60 s followed by a final extension at 72 ºC for 5 min. PCR product was separated in a 2% agarose gel, stained with ethidium bromide and visualized under UV illumination using gel documentation machine (Bio-Rad Laboratories, Inc.). The evaluations were made according to specific size of DNA amplifications with the tested markers.

The PCR products of markers $GP122_{564}$ and $GP122_{718}$ were subjected to *EcoR*V restriction endonuclease enzyme digestion to provide specific cutting profiles. Thirteen microliter of the PCR product in 10% enzyme buffer was digested with *EcoR*V in a total volume of 15ul. Digestion reaction was completed in 3 h at 37°C. In susceptible genotypes, the PCR product was completely digested generating a 564-bp fragment, while in resistant genotypes; the resistant allele(s) are not digested, generally leading to double band 718 and 564 bp.

Table 3.4. Markers used for Marker Assisted Selection of the tested crossess

Marker name	Resistant Gene	Amplicon size	Forward and Reverse sequence (5'–3')	Reference
$GP122_{718}$ (*Eco*RV)*	*Ry sto*	718	TATTTTAGGGGTACTTCTTTCTTA GATACTTCCAACCGCTTCAC	Flis et al., 2005
$GP122_{564}$ (*Eco*RV)*	*Ry sto*	564	CAATTGGCTCCCGACTATCTACAG ACAATTGCACCACCTTCTCTTCAG	Witek et al., 2006
STM0003	*Ry sto*	110	GGAGAATCATAACAACCAG AATTGTAACTCTGTGTGTGTG	Song et al., 2005
RysC3	*Ry adg*	321	ATACACTCATCTAAATTTGATGG AGGATATACGGCATCATTTTTCCGA	Kasai et al., 2000

3.4.3 Analysis of association between MAS and phenotypic data

Phenotypic data were categorized as infected and non-infected according to DAS-ELISA results and genotypic data categorized to be resistant and susceptible according to the amplification status of the markers in PCR analysis and *Eco*RV cutting profiles. The obtained data were analysed according to Chi-Square test using formula as follow;

Obtained data X2 = Σ ((Observed-Expected) 2) / Observed)

The relationship between the results of phenotypic and genotypic analysess was investigated by correlation analysis using SAS program (version 9.1).

3.5 Studies on Transmission Ability of Some PVY Strains by Aphids

3.5.1 Preparing inoculum sources of PVY strains

In order to prepare inoculum sources of $PVY^{NTN(A)}$ and $PVY^{N:O}$ strains, infected leaf samples which were provided by Miss. Vildan Bolat (Department of Plant Production and

Technologies, Faculty of Agricultural Sciences and Technologies, Nigde Omer Halisdemir University) were tested by DAS-ELISA and IC-RT-multiplex PCR as described in sections 3.2.2.1 and 3.1.3. The strain identified samples were used to inoculate tabacco plants, that served as inoculum source in aphid transmission studies.

3.5.2 Host plant and insect culture

Three aphid species; *M. persicae*, *A. gossypii* and *A. fabae* were used for conducting virus transmission studies. The cultures of three aphid species were received on request from Prof. Dr. Serdar SATAR (Department of Entomology, Faculty of Agriculture, Çukurova University, Adana). The PVY virus free nursery from seeds of Brinjal (*Solanum melongena*) for *M. persicae*, from Cotton (*Gossypium hirsutum*) for *A. gossypii* and from broad beans (*Vicia faba*) for *A. fabae* were raised, and the insect cultures were regularly maintained by taking a single aphid with the help of fine camel hair brush. The developed insect colonies were maintained separately in cages (having net measuring 60 × 30 × 30 cm) at 24±2 ° C temperature, 15 hours daylight / 9 hours darkness and 70% ±2 humidity controlled conditions in a growth chamber.

3.5.3 Transmission experiments of PVY strains by aphid vectors

In order to conduct transmission experiments, *Nicotiana tabacum* cv. Xanthi-nc and *S. tuberosum* cv. Desire test plants were grown and used at 2-3 leaf stage. The each aphid species were collected from the relevant host plants reserving the aphid culture with the help of fine camel hair brush and kept for a pre-acquisition starvation period of 3 h in a petri dish under white cool light. Afterwards, fifty individual aphids were allowed to feed on tobacco leaves infected with each PVY strain (Figure 3.7a) for the acquisition access period of 2-3 minutes and immediately transferred to test plants (tobacco and potato). The plants were were covered by a plastic air box and maintained under controlled conditions at 23 ± 1 ∘C and 60 ± 5% RH with a 15:9 h light: dark photoperiod in the laboratory for inoculation (Figure 3.7b). The aphids were killed using systemic insecticide Thiamethoxam (Actara 25 wg, Syngenta Turkey®) on the following day (Figure 3.7c). Each PVY strain/aphid species was tested in three replication each consisting of five plants of *N. tabacum* and *S. tuberosum* (Figure 3.7d). After twenty days the test plants were subjected to DAS-ELISA for the presence of PVY following the methodology as described in section 3.2.2.1. The confirmation of transferred PVY strains were checked by IC-RT multiplex PCR as described in section 3.1.3.

a b c d

Figure 3.7. Different steps of PVY transmission studies by aphids
Feeding of aphids on the infected plant leaf for 2-3 minutes (a), Transferring aphids to test plants and covering them with plastic air box (b), Application of Thiamethoxam (insecticide) on test plants (c), *Nicotiana tabacum* cv. Xanthi-nc plants after inoculation

CHAPTER IV

RESULTS

4.1 Preparing Inoculum Sources of PVY Strains

4.1.1 Detection of PVY strains by ELISA

A total of 63 ELISA positive samples provided by Miss. Bolat were subjected to serological analysis for the detection of PVY^{C}, PVY^{N} and PVY^{O+C} strains using monoclonal antisera. The results of DAS-ELISA revealed that 54 samples were infected with PVY^{N}, 6 with PVY^{O+C} and 3 with PVY^{N}:PVY^{O+C} as mixed infection, whereas, none of the samples were found to be infected with PVY^{C}. The DAS-ELISA optical density (OD) values are given in Appendix A.

4.1.2 Confirmation of the presence of PVY strains by IC-RT-multiplex PCR

The selected PVY isolates, which were subjected reconfirmation of molecular strain typing for the reconfirmation of $PVY^{NTN (A)}$ and $PVY^{N:O}$ using IC-RT-multiplex PCR specific for the detection of PVY strains. The isolates A11, A12, 1, 2, 5, 6, 7, 11,13,14,19, 25, 28, 38 and 70 showed the band profiles of 1307+633+441 bp indicating the presence of $PVY^{NTN(A)}$ (Figure 4.1). Whereas, the isolate 50 had confirmed $PVY^{N:O}$ specific amplification products of 835+633+441 bp when analyzed according to (Chick Ali et al., 2010a). (Figure 4.2).

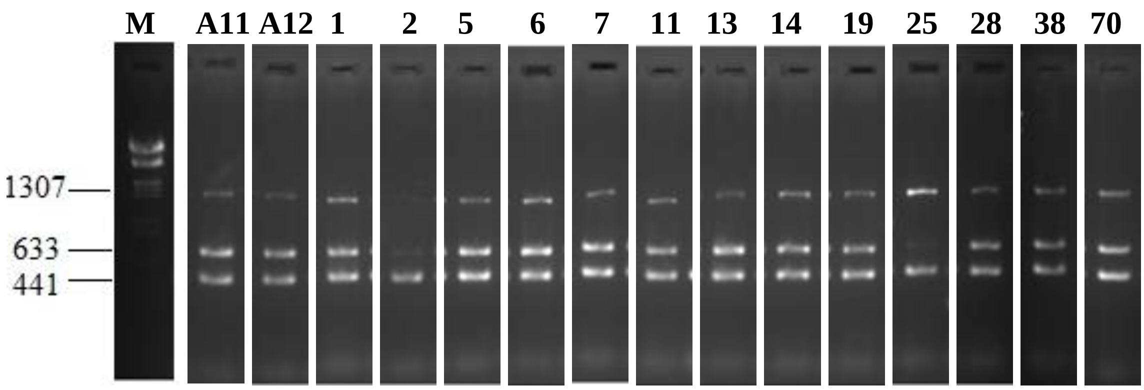

Figure 4.1. Molecular strain typing of $PVY^{NTN(A)}$ isolates using IC-RT- multiplex PCR analysis. M: *Eco*RI+HindIII DNA Ladder (Thermo Scientific), the numbers on column indicates isolate number.

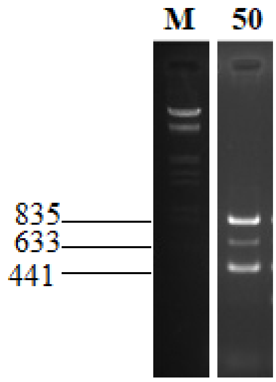

Figure 4.2. Molecular strain typing of PVY$^{N:O}$ isolate using IC-RT- multiplex PCR analysis. M: *EcoR*I+HindIII DNA Ladder (Thermo Scientific), the numbers on column indicates isolate number

4.1.3 Confirmation of the presence of PVY strains in plants used as inoculum source for vector transmission studies

Leaf samples infected with PVY$^{NTN (A)}$ and PVY$^{N:O}$ strains were taken for sap-inoculation of these strains to the test plants which were used as inoculum source. The mechanically inoculated plants were then subjected to IC-RT- multiplex PCR after 2 weeks of inoculation to confirm successful sap transmission of these strains (Figure 4.3). The gel electrophoresis analysis of the amplified products showed different band profiles as obtained in section 4.1.2, which made differentiation of both strains possible.

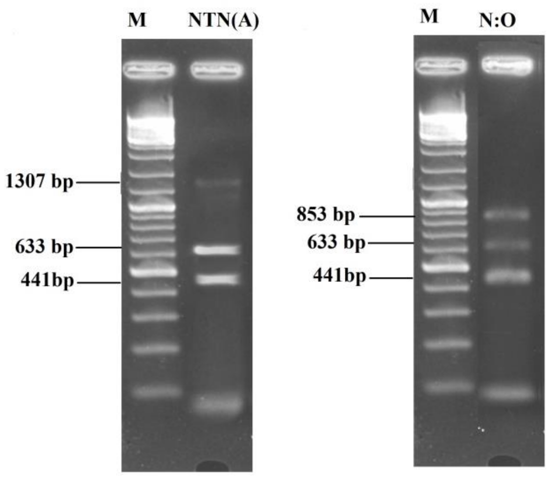

Figure 4.3. Detection of PVY$^{NTN (A)}$ and PVY$^{N:O}$ strains in inoculum sources by using IC-RT- multiplex PCR. M: *EcoR*I+HindIII DNA Ladder (Thermo Scientific), the numbers on column indicates isolate number

4.2 Phenotypic and Genotypic Characterization of Potato Varieties and Crosses for Resistance to PVY

4.2.1 Phenotypic characterization of potato genotypes for PVY resistance

A total of 479 progenies including 90 from Bettina×Galata croseses, 115 from Bettina×Salad Blue, 89 from Bettina×Hermes, 112 Bettina×Savanna and 73 from Bettina×Nectar were inoculated with PVY^{N}: PVY^{O+C} strains (Table 3.5). The DAS-ELISA OD values of the tested crosses are given in Appendix B.

Table 4.1. The results of DAS-ELISA carried out on the progenies of potato crosses

Breeding Material	Number of genotypes	Phenotyping	
		PVY^{r} (negative)	PVY^{s} (positive)
Bettina×Galata	90	38	52
Bettina×Salad Blue	115	63	52
Bettina×Hermes	89	49	40
Bettina×Savanna	112	58	54
Bettina×Nectar	73	40	33

r: resistant, s: susceptible

It was considered not necessary to give second inoculation; because the progenies belonging to the crosses exhibited about 1:1 resistance segregation.The progenies of Bettina×Galata gave minimum, whereas, the progenies of Bettina×Salad Blue gave maximum number of virus negative samples when subjected to DAS-ELISA.The all parents i.e., Hermes, Salad Blue, Galata, Savanna and Nectar, were determined as virus-susceptible, except Bettina. Furthermore, Savanna and Nectar varieties were evaluated as the most susceptible varieties against PVY infection. The DAS-ELISA OD values of the tested crosses are given in Appendix C.

In the second year of the study, plants grown from 482 PVY negative tubers belonging to 236 crosses plant populations were subjected to DAS-ELISA. The tuber susceptibility to PVY are shown in Table 3.6. There was no tuber obtained from the plants of Bettina×Savanna crosses during the first year. For this reason, Bettina×Savanna was not included in the second year experiment. The genotype Bettina × Nectar was evaluated as the most susceptible genotype with 5.5 % tuber susceptibility, whereas, Bettina × Salad Blue and Bettina × Hermes showed similar tuber susceptibility values of 3.22 % and 3.41%, respectively. Furthermore, one out of three tubers from the same genotype in Bettina×Galata had the lowest tuber susceptibility (0.79 %) among the tested cultivars (Table 3.6). With minimum tuber susceptibility, Bettina×Galata is evaluated as PVY tolerant. The DAS-ELISA OD values of the tested crosses are given in Appendix D.

Table 4.2. DAS-ELISA results of the genotypes planted during the second year

Breeding material*	Number of genotypes	Number of tubers	Infected with PVY	The percentage of tuber susceptibility
Bettina × Galata	50	126	1(One tuber from the selected 3 tubers from the same genotype)	0.79 %
Bettina × Salad Blue	62	123	1 (1 tuber from 1 genotype) 2 (2 tubers from 1 genotype)	3.22 %
Bettina × Hermes	88	176	2 (2 tubers from 1 genotype) 2 (2 tubers from 1 genotype) 1 (1 tuber from 1 genotype)	3.41 %
Bettina × Nectar	36	57	2 (2 tubers from 1 genotype) 1 (1 tuber from 1 genotype)	5.55 %

*Since tubers from Bettina×Savanna crosses could not be obtained during the first year of the study, so tuber susceptibility studies were not conducted during the second year.

4.2.2 Symptom development on virus inoculated plants

Symptoms such as vein clearing, chlorosis, mosaic patterns, stunted growth, leaf mottling, crinkling, necrosis and necrotic ring spots were observed on the PVY inoculated plants. The type of symptoms observed on same plants belongning to progenies of the tested crosses before and after inoculation are given in Figure 4.4.

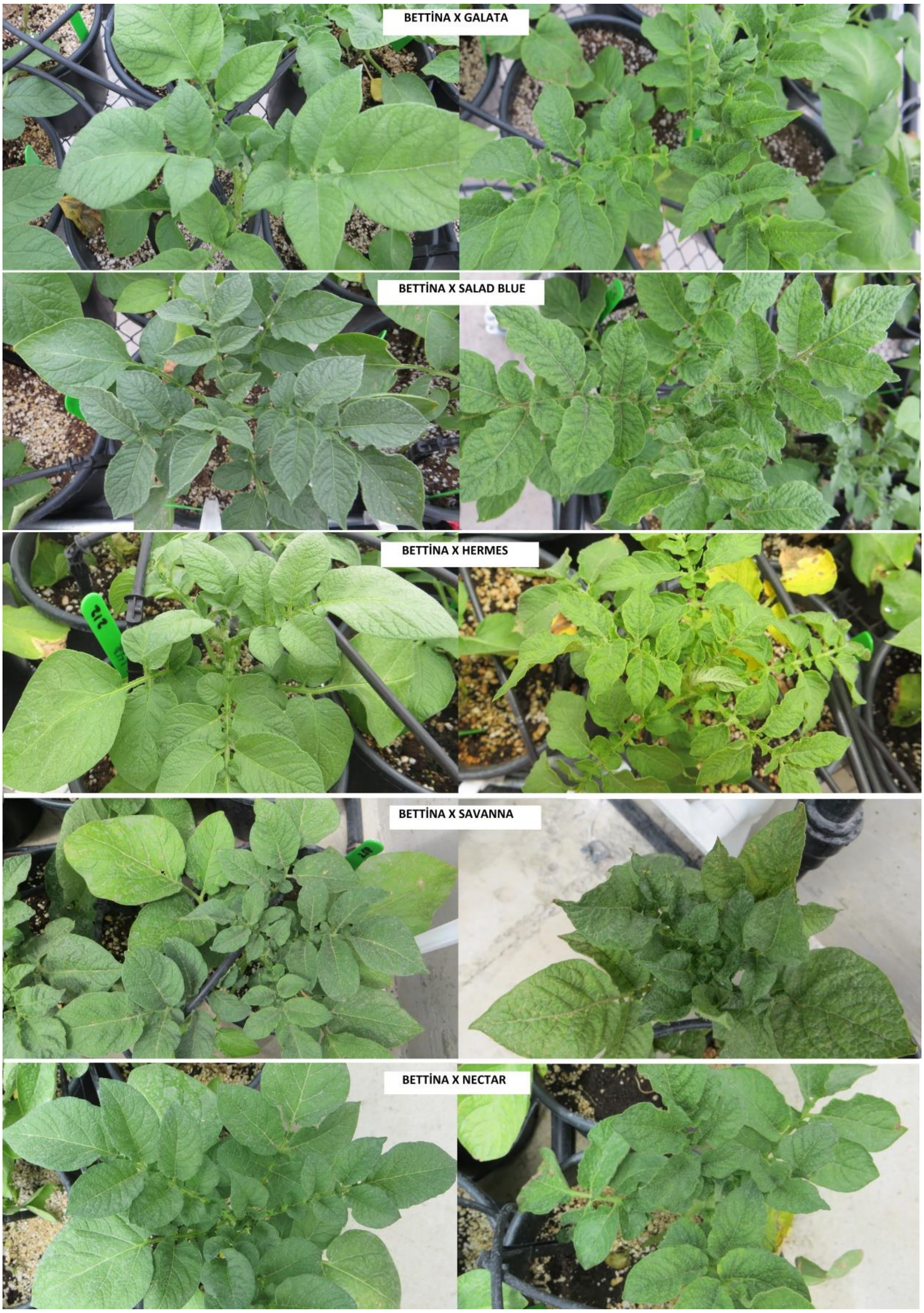

Table 4.3. Symptoms of healthy (left) and PVY-infected (right) plant crosses after 4 weeks of inoculation

4.2.3 Genotypic characterization of potato genotypes for PVY resistance

4.2.3.1 Parent varieties analyzed for PVY resistance by molecular markers

In this part of the study CAPS, SSR and SCAR markers were used to detect Ry_{sto} and Ry_{adg} genes responsible for PVY resistance. The result of MAS carried out on parent varieties are given in Figure 4.5. It was examined that when parents DNAs were analyzed with STM0003 marker, fragments between 100 - 200 bp were determined. However, an amplification product of of 110 bp, which is specific for PVY resistance allele, was observed only in Bettina cultivar. When the same parents were analysed with RysC3 marker, an amplification product of 321 bp specific for PVY resistance allele was observed only in Galata cultivar. The $GP122_{718}$ marker resulted in 728 bp amplification product in all analyzed parents. After the cutting 718 bp fragment with *Eco*RV enzyme, three restriction fragments were obtained around the sizes of 310, 470 and 718 bp in all analyzed parents. Furthermore, when the $GP122_{564}$ marker used for analyzing the parents, it produced an amplification fragment of 564 bp. After the cutting the product of 564 bp with *Eco*RV enzyme, two fragments around the sizes of 400 and 564 were obtained in all analyzed parents.

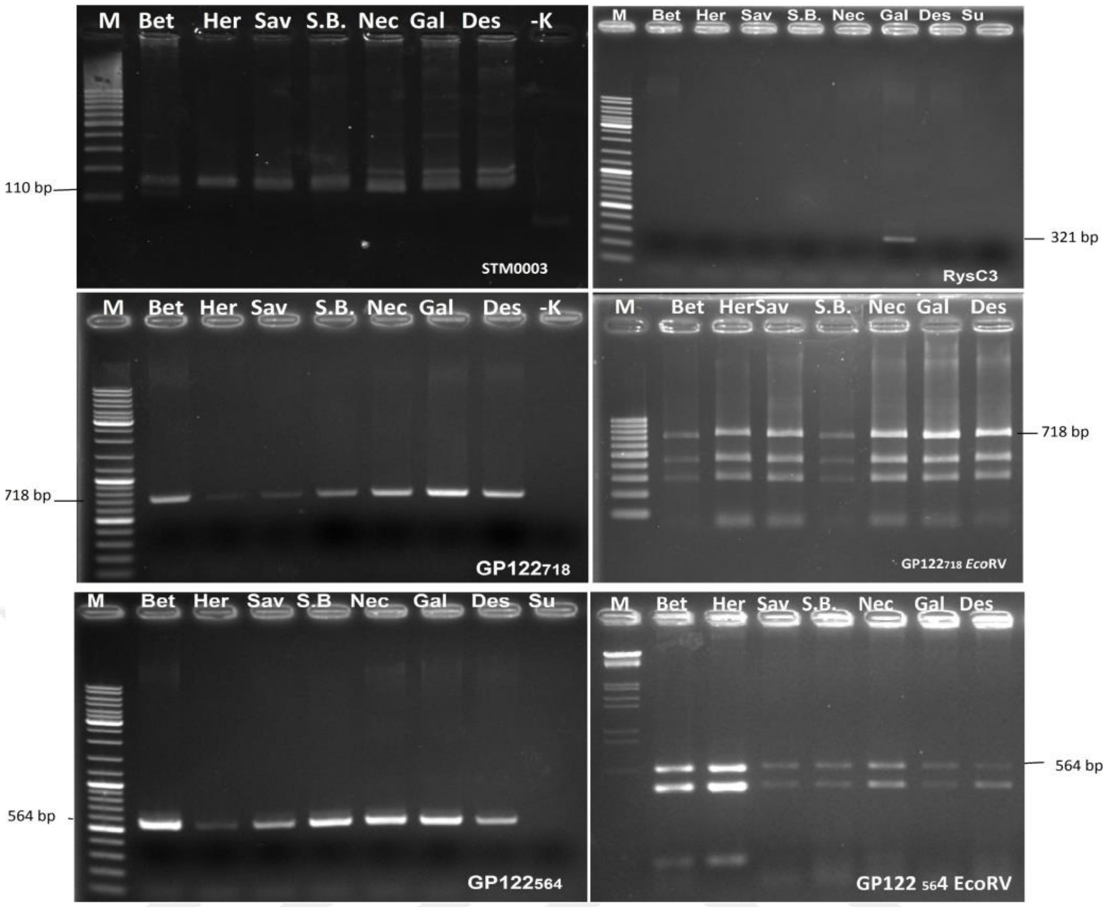

Figure 4.4. Marker assisted analysis of parent cultivars
Bet: Bettina, Her: Hermes, Sav: Savanna, S.B .: Salad Blue, Nec: Nectar, Gal: Galata, Des: Desire, -K: Water used as control

4.2.3.2 Progenies analysed for PVY resistance by molecular markers

4.2.3.2.1. The analysis of the tested crosses with GP122$_{718}$ marker

All progenies produced a 718 bp fragment, when tested with GP122$_{718}$ marker. *Eco*RV enzyme cutting of the same PCR product provided three fragments 310, 470 and 718 bp in all analyzed progenies as shown in Figure 4.6. The detailed gel data about the tested crosses using GP122$_{718}$ marker can be found in Appendix E.

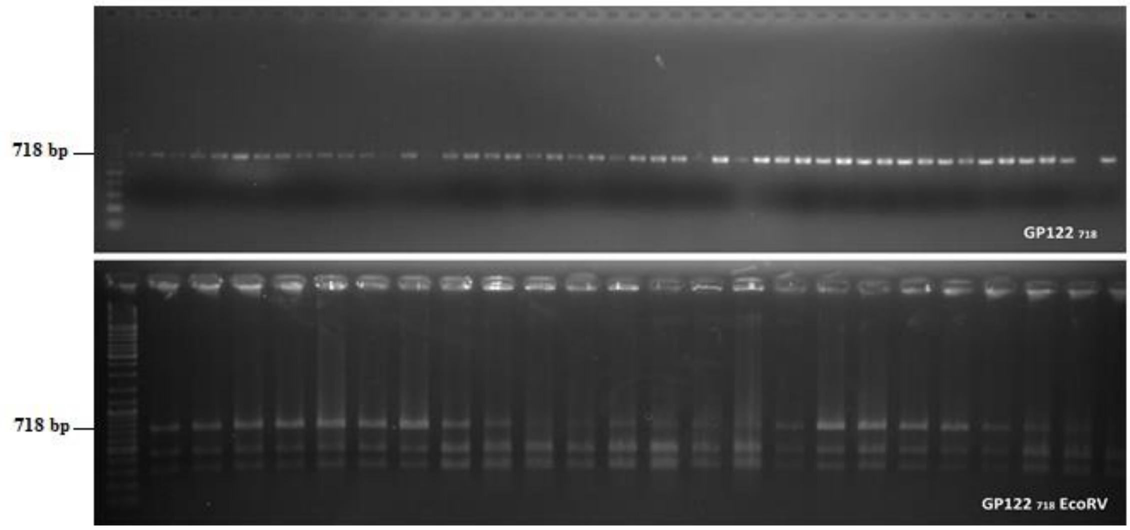

Figure 4.5. Marker assisted analysis of cross progenies using GP122$_{718}$

4.2.3.2.2. Analysis of the tested crosses with GP122$_{564}$ marker

All progenies gave an amplification product of 564 bp, when tested with GP122$_{564}$ marker. *Eco*RV enzyme cutting of the same PCR product provided two restriction fragments of 470 and 564 bp in all analyzed progenies as shown in Figure 4.7. The gel data about the tested crossess using GP122$_{564}$ marker can be found in Appendix F.

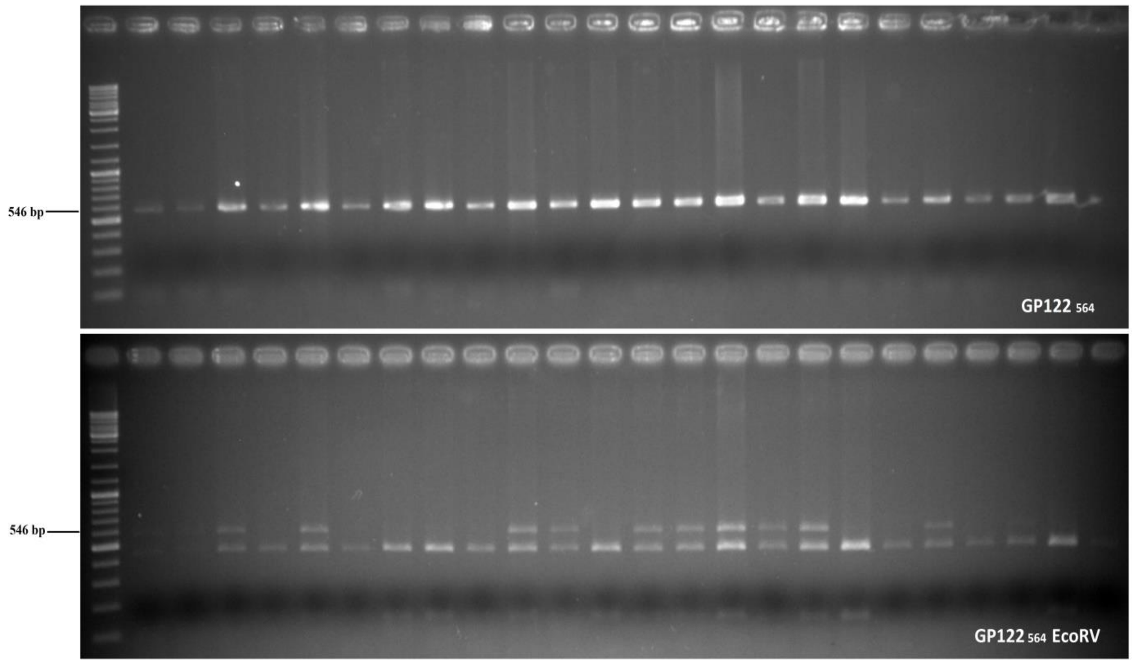

Figure 4.6. Marker assisted analysis of cross progenies using GP122$_{546}$

4.2.3.2.3. Analysis of the tested crosses with RysC3 marker

When progenies analyzed using RysC3 marker, an amplification product of 321 bp was observed only in some Bettina × Galata crosses, whereas, no amplification was observed in all other studied crosses as shown in Figure 4.8. The detailed gel data on the tested crosses using RysC3 marker can be found in Appendix G.

321 bp–

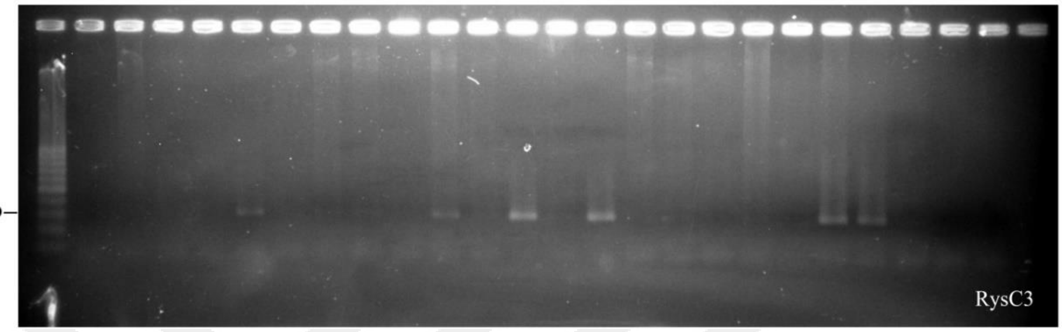

Figure 4.7. Marker assisted analysis of cross progenies using RysC3

4.2.3.2.4. Analysis of the tested crosses with STM0003 marker

The analysis with the marker STM0003 resulted in 110 bp fragment, indicating that the progenies have resistance alleles (Figure 4.9). A total of 36 tested progenies, out of 90 in Bettina × Galata; 53 out of 115 Bettina × Salad Blue; 46 out of 89 Bettina×Hermes; 57 out of 112 Bettina×Savanna and 32 out of 73 in Bettina × Nectar were found to have Ry_{sto} allele (Table 3.7). The detailed gel data about the tested crossess using STM0003 marker can be found in Appendix H.

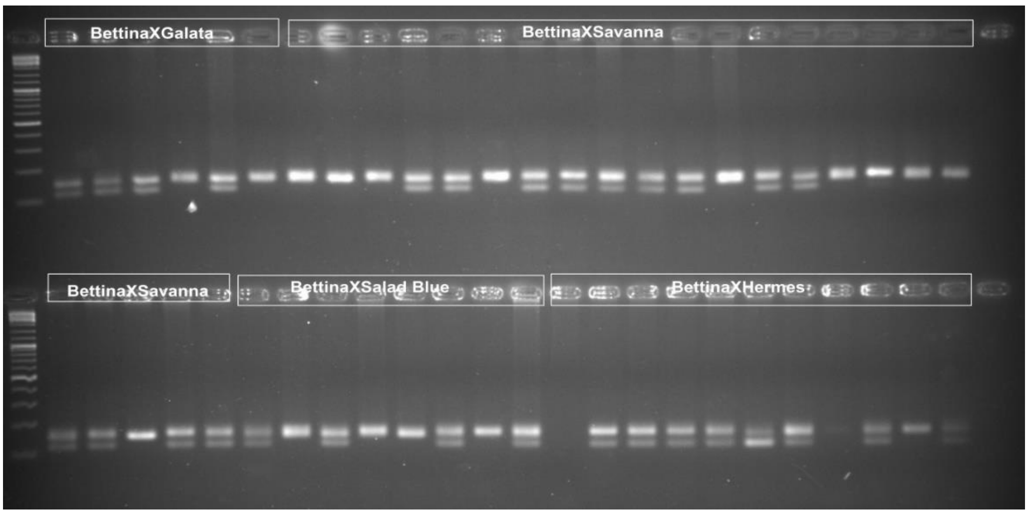

Figure 4.8. Analysis of PVY resistance using STM0003 markers against tested crosses

Table 4.4. Marker assisted analysis of the genotypes tested for the presence of of *Ry*$_{sto}$ allele using STM0003 Marker

Breeding materials	Number of genotypes tested	STM0003 Marker	
		PVY^{r} (Number of individuals with resistance allele)	PVY^{s} (Number of individuals without resistance allele)
Bettina×Galata	90	36	54
Bettina×Salad Blue	115	53	62
Bettina×Hermes	89	46	43
Bettina×Savanna	112	57	55
Bettina×Nectar	73	32	41

r: resistant, s: susceptible

4.3 Rate of MAS and Phenotyping Response (%)

Based on the results obtained from the phenotypic and molecular analyses using STM0003. STM0003 was proven as a feasible for screening the tested crosses against PVY. The maximum percentage rate of MAS and phenotypic response varied from 80 to 98.28 in Bettina× Savana followed by Bettina×Galata, Bettina×Hermes, Bettina×Salad Blue and Bettina×Nectar respectively (Table 3.8).

Table 4.5. Percent response of MAS using STM0003 marker and phenotyping on the tested genotypes

Breeding materials	Number of genotypes	Phenotyping		MAS		Rate of MAS and phenotyping response (%)
		PVY^{+r} (negative)	PVY^{-r} (infected)	PVY^{+r} (presence of resistance allele)	PVY^{-r} (absence of resistance allele)	
Bettina×Galata	90	38	52	36	54	94.73
Bettina×Salad Blue	115	63	52	53	62	84.13
Bettina×Hermes	89	49	40	46	43	87.75
Bettina×Savanna	112	58	54	57	55	98.28
Bettina×Nectar	73	40	33	32	41	80.00

4.3.1 Correlation between phenotypic and genotypic analyses of the tested genotypes of all crosses

As a result of statistical analysis, significant correlation was found between phenotypic and genotypic results of each cross. The calculated r values ranged from -0.87 to -0.37, when analysed with STM0003 (P <0.0001). Similarly, the correlation between the phenotypic and genotypic results of all crosses were almost statistically significant with the r value of -0,80 (P <0.0001). In case of $GP122_{564}$, the r value ranged from -0.02 to 0.11, while the r value ranged from -0.16 to -0.06 in case of $GP122_{718}$ and -0.83 in case of RysC3 (P <0.0001) (Table 3.9).

Table 4.6. Correlation between the phenotypic and genotypic analyses of the tesed genotypes of all crosses

1,,	Breeding Material	Number of genotypes testing in Phenotyping	Number of genotypes tested in MAS	Coefficient of correlation, *r*	*P* value
STM0003	Bettina×Galata	92	90	-0,82	<0,0001*
	Bettina×Salad Blue	115	115	-0,71	<0,0001*
	Bettina×Hermes	90	90	-0,87	<0,0001*
	Bettina×Savanna	112	110	-0,84	<0,0001*
	Bettina×Nectar	133	117	-0,37	<0,0001*
	General	409	405	**-0,80**	**<0,0001***
$GP122_{564}$	Bettina×Galata	92	70	0,10	0,429*
	Bettina×Salad Blue	115	107	-0,02	0,860
	Bettina×Hermes	90	78	0,09	0,419*
	Bettina×Savanna	112	89	-0,10	0,366*
	Bettina×Nectar	73	41	-0,04	0,788
	General	482	285	**0,02**	**0,626**
$GP122_{718}$	Bettina×Galata	92	70	---	---
	Bettina×Salad Blue	115	107	-0,06	0,518
	Bettina×Hermes	78	90	0,11	0,358*
	Bettina×Savanna	112	88	-0,16	0,147*
	Bettina×Nectar	73	16	-0,16	0,554
	General	470	371	**-0,27**	**<0,0001**
RysC3	Bettina×Galata	82	82	-0,83	<0,0001*
	Bettina×Salad Blue	115	115	---	---
	Bettina×Hermes	89	89	---	---
	Bettina×Savanna	112	75	---	---
	Bettina×Nectar	73	73	---	---
	General	471	434	**-0,83**	**<0,0001**

4.4 Transmission Potential of Two PVY Strains by Aphids

A total of 180 plants, of which 90 were *N. tabacum* and the rest were *S.tuberosum*, were inoculated with $PVY^{NTN\ (A)}$ and $PVY^{N:O}$ strains separately by *M. persicae, A. fabae* and *A. gossypii.* Twenty days after inoculation typical virus symptoms were observed on some of the tested plants. Afterward, DAS-ELISA using polycolonal antibody revealed that a total of 47 samples become infected with PVY.

4.4.1 Transmission potential of $PVY^{NTN\ (A)}$

$PVY^{NTN\ (A)}$ was inoculated to *N. tabaccum* plants which were numbered from 46 to 90. The plant numbers 46-60 were inoculated with *A. gossypii,* 61-75 with *A. fabae* and 76-90 with *M. persicae.* Furthermore, the plants numbered from 91 to 135 belonged to *S. tuberosum*. The plants numbered as 91-105 were innoculated by using *M. persicae,* 106-120 by using *A. gossypii* and 121-135 by using *A. fabae.* The DAS-ELISA OD values of the tested sample are given in Table 3.10.

A total of 39 plants were found to be infected with PVY in $PVY^{NTN\ (A)}$ transmitted plants according to DAS-ELISA test results. Among them, 16 plants belonged to *N. tabacum* and 23 to *S. tuberosum.* The transmission potential of $PVY^{NTN\ (A)}$ was recorded 80.00% and 33.33% by *M. persicae* and *A. fabae* in *N. tabacum,* respectively. Whereas, *A. gossypii* did not transmit the $PVY^{\ NTN\ (A)}$ strain using *N. tobacum* plants.. In *S. tuberosum* plants, the transmission potential of $PVY^{NTN(A)}$ was 80.00% and 66.67% by *A. gossypii* and *M. persicae,* respectively (Figure 4.10). *A. fabae* did not transmit the $PVY^{\ NTN\ (A)}$ in *S. tuberosum*.

Table 4.7. The DAS-ELISA OD values of *N. tabacum* and *S. tuberosum* of PVY$^{NTN(A)}$ inoculated plants by using *A. fabae*, *M. persicae* and *A. gossypii*

Inoculated plant number	ELISA Sample Number	OD value 0,200< positive	Inoculated plant number	ELISA Sample Number	OD value 0,246 < positive	Inoculated plant number	ELISA Sample Number	OD value 0,246< positive
Buffer control		0.102	82	37	0.759	121	76	0.100
Negative control		0.097	83	38	0.231	122	77	0.097
Positive control		0.235	84	39	0.256	123	78	0.103
46	1	0.096	85	40	0.855	124	79	0.098
47	2	0.095	86	41	0.199	125	80	0.095
48	3	0.095	87	42	0.096	126	81	0.171
49	4	0.097	88	43	0.691	127	82	0.098
50	5	0.096	89	44	0.199	128	83	0.096
51	6	0.104	90	45	0.798	129	84	0.034
52	7	0.101	91	46	2.223	130	85	0.043
53	8	0.097	92	47	2.016	131	86	0.095
54	9	1.100	93	48	1.930	132	87	0.099
55	10	0.097	94	49	1.450	133	88	0.101
56	11	0.096	95	50	2.332	134	89	0.105
57	12	0.097	96	51	0.108	135	90	0.099
58	13	0.095	97	52	2.117			
59	14	0.099	98	53	1.910			
60	15	0.099	99	54	1.184			
61	16	0.098	100	55	1.610			
62	17	0.098	101	56	1.026			
63	18	0.234	102	57	1.173			
64	19	0.100	103	58	0.105			
65	20	0.091	104	59	0.095			
66	21	0.100	105	60	0.762			
67	22	0.097	106	61	0.411			
68	23	1.195	107	62	1.232			
69	24	0.101	108	63	0.099			
70	25	0.097	109	64	1.894			
71	26	0.786	110	65	1.837			
72	27	0.243	111	66	1.384			
73	28	0.337	112	67	0.400			
74	29	0.106	113	68	1.426			
75	30	0.100	114	69	2.414			
76	31	0.312	115	70	0.099			
77	32	0.096	116	71	1.095			
78	33	0.792	117	72	0.098			
79	34	0.323	118	73	0.667			
80	35	0.098	119	74	1.373			
81	36	0.250	120	75	1.020			

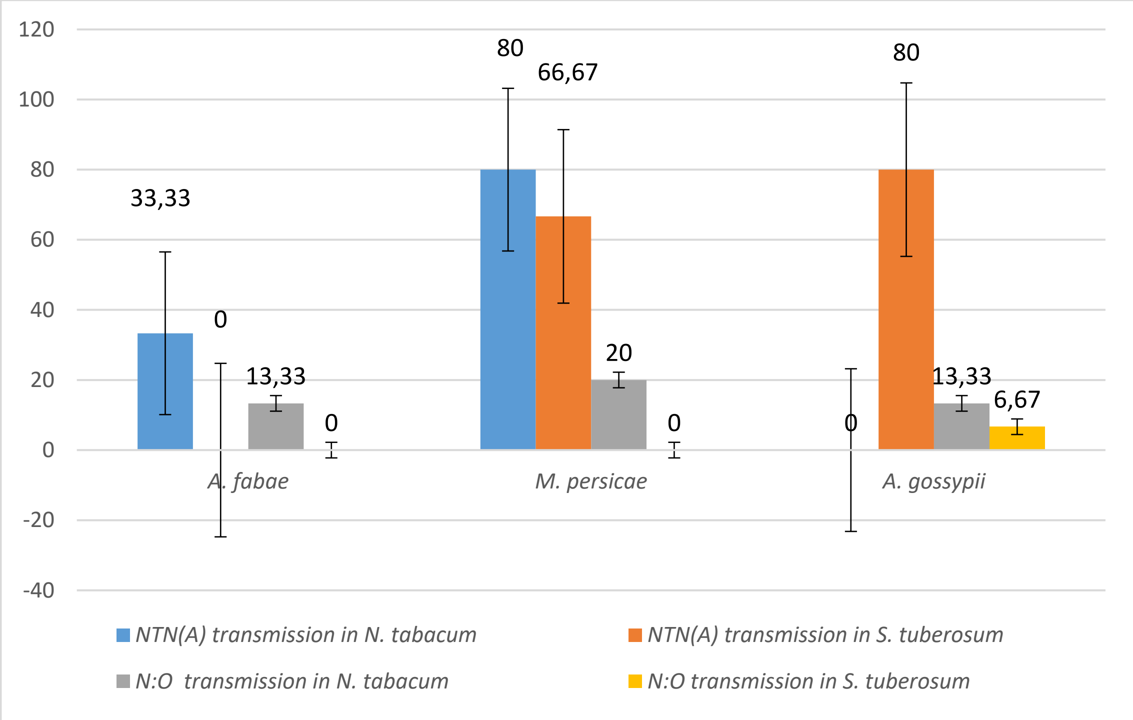

Figure 4.9. Transmission potential of PVY$^{NTN(A)}$ and PVY$^{N:O}$ in *N. tabacum* and *S. tuberosum* by *A. fabae*, *M. persicae* and *A. gossypii*

4.4.1.1 Confirmation of vector transmission of PVY$^{NTN\ (A)}$ by RT-IC-multiplex PCR

DAS-ELISA positive samples were tested by IC- RT-multiplex PCR to verify the presence of PVY$^{NTN\ (A)}$ strain in the plants (Figure 4.11). The samples numbered as 1-16 belonged to *N. tabacum*. Among them, the numbers 1-5 were confirmed the transmission by *A. fabae*, 6-16 by *M. persicae* in *N. tabacum* by providing strain specific fragments of 441+633+1307 bp, whereas, the numbers 17-27 indicating the transmission by *M. persicae* and 28-39 by *A. gossypii* in *S.tuberosum* providing the same strain specific fragments as above. Furthermore, PVY$^{NTN\ (A)}$ positive sample is shown as sample 40 in Figure 4.10.

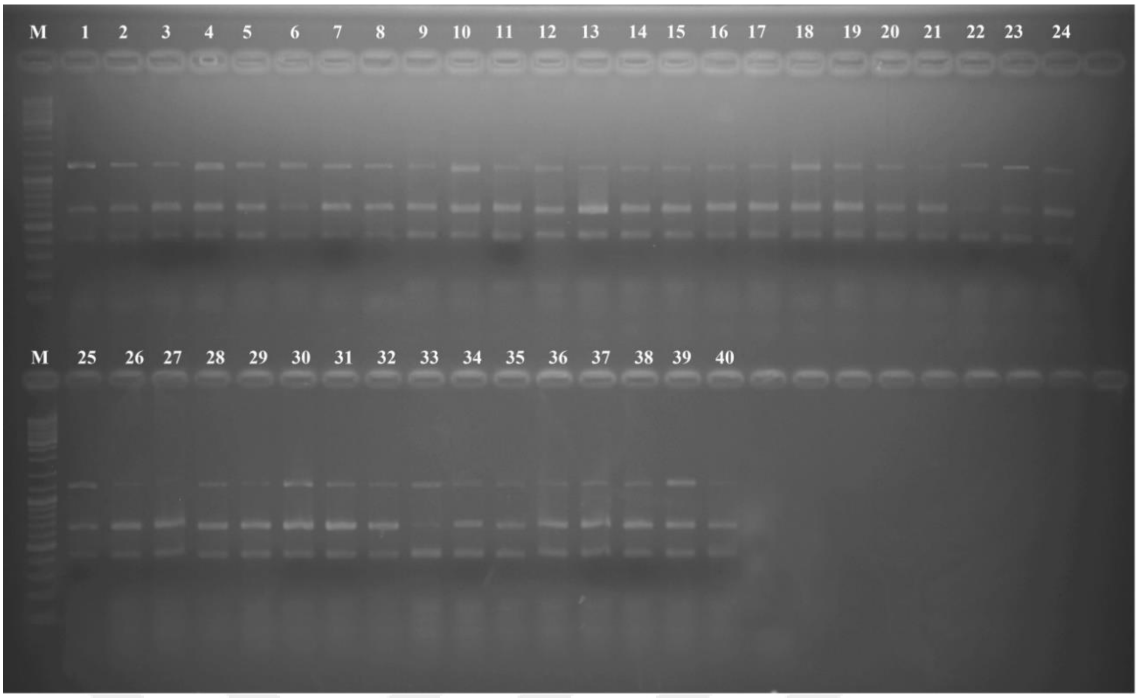

Figure 4.10. The results of IC-RT-multiplex PCR performed by using DAS- ELISA positive sample *N. tobaccum* and *S.tuberosum* plants after aphid transmission of PVY$^{NTN(A)}$. Line 1-16: *Nicotiana tabaccum* cv. *Xanthi*-nc, 17-39: *Solanum tuberosum* cv Desire. 1-5: *Aphis fabae*, 6-27: *Myzus persicae*, 28-39: *Aphis gossypii*

4.4.2 Transmission potential of PVY$^{N:O}$

PVY$^{N:O}$ plants inoculated numbered from 1 to 45 belonged to *N. tabacum*. The plants numbered as 1-15 were innoculated by *A. fabae,* 16-30 by *M. persicae* and 31-45 by *A. gossypii*. Furthermore, plant numbered from 136 to 180 belonged to *S. tuberosum*. The plant numbered from 136 to 150 were inoculated by *A. fabae,* 151 to 165 by *A. gossypii* and 166 to 180 by *M. persicae.* The DAS-ELISA OD values of the tested samples are given in Table 3.11.

According to DAS-ELISA results, 8 samples were found to be infected with PVY$^{N:O}$ after vector transmission. Of these, 7 plants beloned to *N. tabacum* and 1 to *S.tuberosum.* In *N. tabacum* plants, transmission potential of PVY$^{N:O}$ was recorded 20.00 % by *M. persicae* and 13.33 % by both *A. gossypii* and *A. fabae.* Whereas, transmission potential of PVY$^{N:O}$ was recorded as 6.67 % by *A. gossypii* in *S.tuberosum* (Figure 4.10). PVY$^{N:O}$ transmission by *M. persicae* and *A. fabae* was not detected in *S. tuberosum*.

4.4.2.1 Confirmation of vector transmission $PVY^{N:O}$ by IC- RT-multiplex PCR

IC- RT-multiplex PCR confirmed the presence of $PVY^{N:O}$ in DAS-ELISA positive plants. The sample numbers 1-7 belonging to *N. tabacum,* 8 to *S. tuberosum,* and 9 used as $PVY^{N:O}$ positive control revealed the amplification of $PVY^{N:O}$ strain specific 441+633+835 bp fragments (Figure 4.12).

Among the tested samples of 1-2 showed $PVY^{N:O}$ transmission by *Aphis fabae, 3-5* by *M. persicae,* 6-7 by *A. gossypii* in *N. tobacum*,whereas, the sample 8 by *M. persicae* in *S.tuberosum*. (Figure 4.11).

Table 4.8. The DAS-ELISA OD values of $PVY^{N:O}$ inoculated *N. tabacum and S. tuberosum* plants by using *A. fabae*, *M. persicae* and *A. gossypii*

Inoculated plant number	ELISA Sample Number	OD value 0,291> positive	Inoculated plant number	ELISA Sample Number	OD value 0,291> positive	Inoculated plant number	ELISA Sample Number	OD value 0,246> positive
Buffer control		0.299	37	37	0.287	166	76	0.271
Negative control		0.288	38	38	0.300	167	77	0.278
Positive control		0.585	39	39	0.289	168	78	0.275
1	1	0.330	40	40	0.317	169	79	0.277
2	2	0.308	41	41	0.424	170	80	0.420
3	3	0.304	42	42	0.495	171	81	0.287
4	4	0.304	43	43	0.315	172	82	0.276
5	5	0.300	44	44	0.291	173	83	0.278
6	6	0.306	45	45	0.292	174	84	0.283
7	7	0.312	91	136	0.288	175	85	0.285
8	8	0.456	92	137	0.282	176	86	0.281
9	9	0.333	93	138	0.285	177	87	0.283
10	10	0.320	94	139	0.252	178	88	0.285
11	11	0.302	95	140	0.208	179	89	0.281
12	12	0.459	96	141	0.292	180	90	0.296
13	13	0.298	97	142	0.294			
14	14	0.299	98	143	0.279			
15	15	0.298	99	144	0.286			
16	16	0.308	100	145	0.286			
17	17	0.478	101	146	0.286			
18	18	0.460	102	147	0.276			
19	19	0.470	103	148	0.294			
20	20	0.344	104	149	0.292			
21	21	0.291	105	150	0.288			
22	22	0.297	106	151	0.288			
23	23	0.277	107	152	0.281			
24	24	0.328	108	153	0.284			
25	25	0.148	109	154	0.287			
26	26	0.151	110	155	0.287			
27	27	0.134	111	156	0.280			
28	28	0.188	112	157	0.329			
29	29	0.285	113	158	0.282			
30	30	0.284	114	159	0.272			
31	31	0.284	115	160	0.294			
32	32	0.300	116	161	0.332			
33	33	0.247	117	162	0.277			
34	34	0.302	118	163	0.291			
35	35	0.298	119	164	0.278			
36	36	0.330	120	165	0.271			

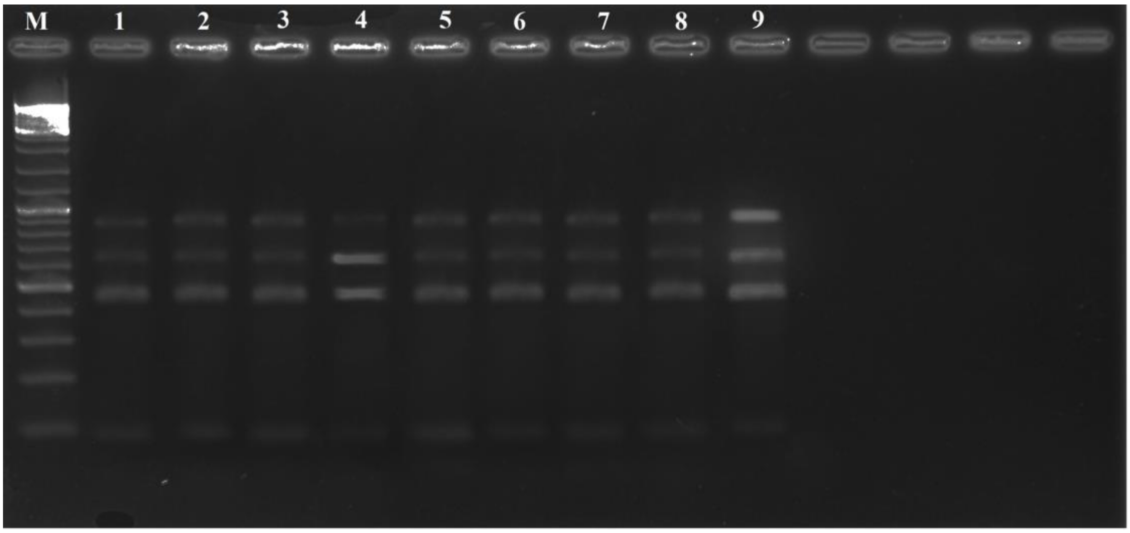

Figure 4.11. The results of IC-RT-multiplex PCR performed using DAS-ELISA positive *N. tabacum* and *S.tuberosum* after aphid transmission of $PVY^{N:O}$.
Lines 1-7: *N. tabacum,* 8: *S. tuberosum,* 9: $PVY^{N:O}$ positive control. 1-2: *Aphis fabae,* 3-5: *M. persicae*, 6-7: *A. gossypii* and 8: *M. persicae.*

CHAPTER V

DISCUSSION

PVY is among the destructive plant viruses causing severe damage to economically important crops including potato (Thompson, 1997). Potato crop is severely infected by PVY in Turkey (Bostan and Haliloglu, 2004; Yardimci et al., 2014; Yardımci et al., 2015). Unlike other plant pathogens, no direct methods are currently available to control viral agents including PVY. The role of detection and identification methods has a critical importance in virus disease management (Naidu et al., 2003). The quick and easiest detection of viruses from vegetative propagules, seeds, insect vectors and plant material is possible by enzyme-linked immunosorbent assay (ELISA) (Clark and Adams 1977). There are various reports about the successful application of ELISA for the detection of PVY, its strains and mix infection with other viruses (Banttari and Goodwin, 1985; Gugerli, 1978; Gugerli and Gehriger, 1980; General, 1989). By using ELISA, the presence of important potato viruses including PVY has been reported from various regions of Turkey (Arli-Sokmen et al., 2005; Bostan and Haliloglu, 2004; Ozaslan et al., 2006; Yardımcı et al., 2015). The detection and identification of PVY and its strains by using ELISA from the potato crop in the province of Niğde indicates that this region is also severely affected by this virus (Bolat, 2017).

The results of this study are confirmatory with studies of Bostan and Haliloglu, (2004), who have also reported the presence of PVY^N and PVY^O in the tested potato samples. However, PVY^C is not yet detected in the tested potato samples of the Turkey. Despite PVY an important problem to the potato industry of Turkey, sufficient information about the prevalent strains in the potato crop is still missing. Bolat, (2017) reported first time recombinant and the mix strains infection of various PVY strains from potato crop in Turkey. The serological strain identification results in this study and the detection of mixed strain infection of PVY suggest that it has potential to damage the potato crop in Turkey. Moreover, the identification of mixed strain infections during this study may be attributed to continuous usage of uncertified seeds from distributors and cultivation of preserved tuber from previous crop by farmers (Çalişkan et al., 2010). It is observed that the unchecked and continuous cultivation of uncertified virus infected seeds are among the major reasons for multiplying inoculum of PVY and mixed infection in potato fields of Niğde. There are various reports about the presence of PVY strains from various potato producing countries around the world (Chick Ali et al., 2013; Chang et al., 2015; Chikh-Ali et al., 2016; Fakhrabad et al., 2012; McDonald and Kristjansson, et al., 1993; Ramírez-Rodríguez et al., 2009; Schubert et al., 2007). Bahrami-Kamangar et al. (2009) has reported the presence of different strains including PVY^{O}, PVY^{N}, PVY^{NTN}, PVY^{NWi} in the potato crop of Iran. Anfoka et al. (2015) has reported the recombinant strains including $PVYY^{NTN\text{-}NW}$ infecting potato crop in Jordan. The results obtained in this study using IC-RT-multiplex PCR suggest that $PVY^{NTN\ (A)}$ is the most prevalent recombinant strain.

Europe is an important of potato seed exporter to Turkey, The Middle East and other countries of the world (Anonymous, 2017; Anfoka et al., 2015). The presence of recombinant strains may have resulted in the introduction of infected potato seeds to these regions while importing seeds. The results of this study would help in driving the management practices regulated by quarantine measure of Turkey.

Breeding the virus resistant potato varieties is among effective method for combating PVY (Solomon-Blackburn and Barker, 2001). The conventional breeding has an important role in developing potato varieties resistant to PVY (Felcher and Douches 2012). The advancement in breeding methods uses diverse genetic resources in a potato breeding programs followed by the use of MAS in accelerating the selection (Wenzel, 2006). A number of wild potato species with diverse *Ry* genes are known, but only few of them have been used in effective breeding programs (Brigneti et al., 1997; Celebi-Toprak et al., 2002; Flis et al., 2005; Hämäläinen et al., 1998; Hosaka et al., 2001; Kasai et al., 2000; Song et al., 2005; Takács et al., 1999). *Solanum stoloniferum* derivatives are frequently used for the introgression of PVY extreme resistance (Soloman- Blackburn and Barker, 2001). The use of DNA markers is one of the important, cost reducing methodology with increasing efficiency and precision (Barone 2004). Markers like CAPS (cleaved amplified polymorphic sequence) including GP122 and SSR (single sequence repeat) such as STM0003 are being used against Ry_{sto} gene located on chromosome XI (Song *et al.* 2005; Tiwari and Singh, 2013) for the purpose. The validation of the tested markers is necessary for calculating cost effective ratio while dealing with MAS (Lopez et al., 2009).

According to Heldak et al. (2007) the presence of amplicon at 110 bp during PCR product amplification with SSR marker STM0003 confirms the presence of *Ry*$_{sto}$ resistance gene. The results of this study shows that during analysing parents allele amplification was observed at 110 bp in potato cv. Bettina only. The result was confirmatory with Cernák et al. (2008) and Lacomme et al. (2017) who reported Bettina cultivar possessing *Ry*$_{sto}$ resistance gene and is reported to be resistant against PVY. In the marker validation studies of this research, the progenies of crosses having resistant alleles from Bettina cultivar were used as a resistance source, they showed the identical results when crosses were analyzed using STM0003.

The results of this study showed amplification of CAPs markers GP122$_{564}$ and GP122$_{718}$ at all expected levels. According to Witek et al. (2006), PCR product amplified using GP122$_{564}$, after cutting with *Eco*RV enzyme showed a 564 bp length associated with *Ry*$_{sto.}$ Whereas, two fragments around 400-500 bp were observed in the tested parents of this study. According to Flis et al. (2005) amplified product using GP122$_{718,}$when cut using *Eco*RV enzyme provided 718 bp amplicon associated with *Ry*$_{sto}$ resistance gene (Flis et al., 2005). The results of this study, gave amplified product using GP122$_{718}$ and when it was cut using *Eco*RV enzyme provided three fragments around the sizes of 310, 470 and 718 bp in all analyzed parents. Therefore, GP122$_{564}$ and GP122$_{718}$ markers showed incompatibility in relation to phenotypic studies. The results further showed that GP122564 and GP122$_{718}$ markers fail to differentiate the resistant and susceptible parents and the progenies of the tested crosses.

Kasai et al. (2000) indicated RysC3 marker's association with the *Ry*$_{adg}$ resistance gene, if the amplified amplicons were 321 bp in length. The results of this finding showed that when the parent cultivars were analyzed with this marker, the expected amplicon size was only obtained in cv. Galata. These results confirmed the presence of *Ry*$_{adg}$ resistance gene in Galata cultivar. Therefore, RysC3 marker was not suggested for the other clones for detecting PVY resistance. Furthermore, this hypothesis was confirmed by analyzing all clones using RysC3 marker.

Bettina cultivar is reported and evaluated as a resistant source for PVY (Heldák et al., 2007; Hinrichs et al., 1998; Hinrichs-Berger et al., 2000; Schubert et al., 2005). Among the tested parent cultivars, Bettina was evaluated resistant cultivar against PVY after repeated phenotypic and genotypic analyses and can be safely suggested as a resistance source for induction PVY resistance. The results of this study suggested that, due to the codominance characteristic of microsatellite markers, polymorphism between tested genotypes was easily determinable. The presence of resistant allele in the tested progenies of crosses was confirmed by the presence and absence of the amplification of the relevant allele/s. The results obtained from the genotypic and phenotypic analysis of the tested progenies of crosses, STM0003 marker linked to Ry_{sto} allele was evaluated as the most effective marker for screening of these populations.

Among the tested parents Nectar is evaluated as producing high number of tubers with quality skin. This variety is reported resistant to various disease and nematode infections (A'Hara et al., 2011). No literature about Nectar and Savana related resistance studies associated to PVY has been reported previously. The results of this study evaluated both cv. Savana and Nectar as the most sensitive varieties against PVY infection. During the course of this study, no tuber was obtained after virus inoculation after the first year harvest in hybrid of cv. Bettina × cv. Savanna. The results of the second year DAS-ELISA results suggested the highest tuber sensitivity against PVY in Bettina × Nectar. It can be suggested that cv. Savanna and cv. Nectar were evaluated as one of the two most sensitive parent. Additionally, minimum PVY tuber sensitivity was recorded in Bettina × Galata. This could be due to the presence of two resistant genes Ry_{sto} inherited from cv. Bettina (Hinriches et al., 1998) and Ry_{adg} from cv. Galata. The introduction of resistant cultivars is considered as practical approach for controlling virus infection in potato (Hussain et al., 2016.). The results of this study strongly suggest to replace the susceptible varieties with virus resistant ones after integrating them in breeding program to combat PVY infection. Simultaneously, the virus transmission can be controlled subject to local availability or import of resistant cultivars from other regions.

The results of this study suggest, *M. persicae* as an effective vector of both tested PVY strains In agreement with Brunt, A.A (2001) and Sertkaya and Sertkaya, (2005), who reported *M. persicae* as the most important vector transmitting PVY efficiently. They further suggest significant role of colonizing and non-colonizing aphid vectors can play an important role in transmission of PVY (Karasev and Gray, 2013; Mondal et al., 2016).

Although, the strain $PVY^{NTN (A)}$ is the recombinant of PVY^{NTN}, but still the results of this study are in agreement with Boukhris-Bouhachem et al. (2011) who reported *M. persicae* and *A. gossypii* as efficient vector and *A. fabae* as less efficient vector for transmitting PVY^{NTN} among the tested aphid species. Furthermore, the rate of virus transmission varies and lack of consensus may be attributed to aphid species, life stage of vectors, cultivar of potato and virus isolates (Basky and Almási, 2005; Boiteau et al., 1998; Cervantes and Alvarez, 2011; Davis et al., 2005; Kaliciak and Syller, 2009; Mello et al., 2011; Shrestha et al., 2014; Verbeek et al., 2010).

Variable results were obtained with reference to recombinant isolates transmitted more efficiently compared to non-recombinant strains (Mondal et al., 2016; Syller and Grupa, 2014). Additionally various transmission experiments using aphids have failed to support a general conclusion that differential transmission by aphids is a significant factor driving the recent emergence of recombinant strains (Mondal and Gray, 2017). The excessive use of insecticide results in reduction in colonizing aphid populations and majority of PVY spread is attributable to non-colonizing vector species (Davis et al., 2005; DiFonzo et al., 1997). The emergence of mixed infections may be attributed to the fact that during host finding, aphids prefer to visit and feed on multiple plants. During the process of traveling across a potato field, it is likely to encounter plants infected with different PVY strains showing either single or mixed infections (Mondal et al., 2016).

The use of chemical control is most frequent and commonly practiced method for controlling insect pests. Simultaneously, the detrimental usage of insecticides may induce problems such as destruction of natural enemies, resurgence of insecticide resistance, outbreaks of secondary pests, increasing cost of cultivation, and bio magnification of pesticide residues in food (Afzal et al., 2015; Armes et al., 1997; Smith and Jackson, 1975), The control of virus insect vectors (aphids) together with insecticides and mineral oils can be effective for controlling PVY infection (Hussain et al., 2016). There are various reports about reduction in transmission of PVY by spraying mineral oil for controlling insect vector on solanaceous and other crops (Wang and Pirone, 1996; Wrobel, 2012). The application of mineral oil along with chemical control can be effective and environmental friendly to maintain the aphid population at low level. The application of mineral oil enables to reduce viral transmission in plants by delaying the stylet penetration on plant host (Powell et al., 1998). According to Olubayo et al. (2010), the use of synthetic insecticides such as Dimethoate and Bifenthrin and application of mineral oil were significantly effective in reducing aphid infestations.

CHAPTER VI

CONCLUSION

Unkikewise other plant pathogens, no direct methods are currently available to control virus infections including PVY. The role of detection and identification methods has a critical importance in virus disease management. PVY causes significant damage to potato in Turkey. There is no direct strategy to protect plants against this virus. Development of strategies to combat PVY is desirable. This study reports some strategies to check and control the problems related to PVY in potato crop.

In order to prepare inoculum source plants the PVY positive plants samples were used as inoculum source possessing PVY^{O}, PVY^{N} and PVY^{O+C} strains. PVY^{C} was not detected in the tested samples. Furthermore, selected PVY isolates when subjected to molecular strain typing using IC-RT-multiplex PCR also confirmed the presence of $PVY^{NTN(A)}$ and $PVY^{N:O}$.

The tested parents cv. Hermes, cv. Salad Blue, cv. Galata, cv. Savanna and cv. Nectar were evaluated as virus-sensitive after phenotypic studies. The cv. Savanna and cv. Nectar were judged as most sensitive parents against PVY infection. Among the five crosses (Bettina × Hermes, Bettina × Nectar, Bettina × Savanna, Bettina × Galata and Bettina × Salad Blue) Bettina × Galata hybrids responded with maximum number of virus resistant progenies. The STM0003 marker was found as most effective selector of resistant progenies among the studied crosses due to its compatibility with phenotypic studies during the selection of PVY on the tested crosses.

M. persicae was evaluated as efficient vector for $PVY^{NTN\ (A)}$ and $PVY^{N:O}$ in both *S. tuberosum* and *N. tabacum. A. gossypii* transmitted $PVY^{N:O}$ in *S. tuberosum* and *N. tabacum* was also evaluated as effective vector for $PVY^{NTN\ (A)}$ in *S. tuberosum. A. fabae* was evaluated as least effective vector for recognition of $PVY^{NTN\ (A)}$ and $PVY^{N:\ O}$.

It is suggested that use of better pest management practices along with effective, reliable and time saving resistant screening tools (like tested markers in this study) can be beneficial in long lasting PVY management programs and breeding of potato.

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Appendix-A DAS-ELISA OD values of tested 63 samples for the detection of PVY^C, PVY^N and PVY^{O+C} **(Early Spring)**

Sample Number	ELISA Number	PVY^N	PVY^C	PVY^{O+C}	Sample Number	ELISA Number	PVY^N	PVY^C	PVY^{O+C}
Positive Control					05	11	0.182	0.147	0.160
						11	0.171	0.139	1.031
Negative Control		0.164	0.143	0.166	06	12	0.187	0.142	0.176
		0.169	0.141	0.161		12	0.182	0.132	0.197
Buffer Control		0.165	0.133	0.154	07	13	0.186	0.134	0.192
		0.170	0.134	0.156		13	0.210	0.145	0.206
1	1	0.221	0.183	0.198	08	14	0.193	0.167	0.199
	1	0.174	0.168	0.205		14	0.190	0.160	0.184
4	2	0.198	0.152	0.197	11	15	0.226	0.145	0.177
	2	0.205	0.152	0.194		15	0.229	0.151	0.176
6	3	0.180	0.162	0.171	13	16	0.201	0.133	0.181
	3	0.176	0.145	0.182		16	0.182	0.137	0.185
7	4	0.234	0.64	0.232	14	17	0.271	0.132	0.174
	4	0.243	0.183	0.237			0.222	0.141	0.184
11	5	0.238	0.164	0.196	19	18	0.190	0.165	0.181
	5	0.219	0.163	0.175			0.180	0.161	0.162
12	6	0.211	0.186	0.190	22	19	0.181	0.147	0.161
	6	0.224	0.157	0.172			0.178	0.154	0.156
16	7	0.188	0.154	0.175	23	20	0.192	0.143	0.184
	7	0.186	0.155	0.189			0.197	0.135	0.202
22	8	0.170	0.140	0.175	25	21	0.186	0.135	0.194
	8	0.174	0.142	0.218			0.181	0.140	0.296
03	9	0.174	0.136	0.179	26	22	0.225	0.182	0.188
	9	0.190	0.143	0.183			0.245	0.165	0.745
02	10	0.194	0.168	0.180	27	23	0.175	0.154	0.169
	10	0.215	0.151	0.167			0.222	0.139	0.209
28	24	0.180	0.146	0.172	59	40	0.210	0.213	0.193
	24	0.183	0.153	0.280		40	0.261	0.212	0.198
38	25	0.221	0.149	0.182	60	41	0.191	0.228	0.198
	25	0.191	0.160	0.187		41	0.177	0.228	0.195
39	26	0.188	0.186	0.176	62	42	0.174	0.329	0.213
	26	0.186	0.181	0.162		42	0.184	0.391	0.174
40	27	0.189	0.169	0.165	70	43	0.185	0.308	0.165
	27	0.178	0.157	0.229		43	0.183	0.257	0.159
41	28	0.182	0.142	0.190	71	44	0.198	0.240	0.174
	28	0.187	0.143	0.183		44	0.186	1.808	0.175
43	29	0.179	0.149	0.173	75	45	0.188	0.243	0.174
	29	0.177	0.144	0.180		45	0.187	0.266	0.180

Appendix-A (Sequel) DAS-ELISA OD values of tested 63 samples for the detection of PVY^{C}, PVY^{N} and PVY^{O+C} **(Early Spring)**

Sample Number	ELISA Number	PVY^{N}	PVY^{C}	PVY^{O+C}	Sample Number	ELISA Number	PVY^{N}	PVY^{C}	PVY^{O+C}
44	30	0.185	0.211	0.182	76	46	0.179	0.142	0.209
	30	0.176	0.200	0.166		46	0.176	0.139	0.188
45	31	0.179	0.205	0.171	94	55			
	31	0.176	0.174	0.171		55			
46	32	0.174	0.169	0.173	100	56	0.176	0.137	0.189
	32	0.185	0.157	0.184			0.178	0.139	0.193
48	33	0.203	0.172	0.186	103	57	0.184	0.144	0.201
	33	0.230	0.170	0.182			0.182	0.133	0.193
49	34	0.179	0.239	0.186	105	58	0.178	0.146	0.182
	34	0.175	0.218	0.168			0.175	0.128	0.185
50	35	0.192	0.271	0.177	107	59	0.188	0.133	0.184
	35	0.174	0.203	0.176			0.183	0.140	0.189
51	36	0.195	0.180	0.176	109	60	0.192	0.141	0.185
	36	0.195	0.179	0.178			0.181	0.142	0.212
52	37	0.202	0.195	0.187	114	61	0.183	0.139	0.194
	37	0.173	0.201	0.181			0.186	0.134	0.177
56	38	0.187	0.287	0.192	115	62	0.172	0.130	0.181
	38	0.174	0.355	0.171			0.172	0.133	0.194
57	39	0.171	0.320	0.166	116	63	0.203	0.141	0.192
	39	0.202	0.211	0.170			0.201	0.140	0.204
83	47	0.186	0.150	0.193					
	47	0.172	0.137	0.186					
84	48	0.179	0.142	0.194					
	48	0.179	0.143	0.203					
87	49	0.203	0.141	0.220					
	49	0.203	0.141	0.232					
88	50	0.180	0.138	0.188					
	50	0.173	0.133	0.184					
90	51	0.200	0.140	0.191					
	51	0.183	0.133	0.188					
91	52	0.184	0.135	0.210					
	52	0.204	0.131	0.209					
92	53	0.185	0.139	0.215					
	53	0.181	0.135	0.193					
93	54								
	54								

Appendix-A (Sequel) DAS-ELISA OD values of tested 63 samples for the detection of PVY^{C}, PVY^{N} and PVY^{O+C} **(Late spring)**

Sample Number	ELISA number	OD values of O+C	Mean	OD values of PVY^{C}	Mean	OD values of PVY^{N}	Mean
1	1	0,219	0,2175	0,127	0,1195	1	1,2235
	1	0,216		0,125		1	
3	2	0,218	0,1685	0,114	0,122	0,66	0,705
	2	0,119		0,121		0,75	
4	3	0,129	0,132	0,123	0,7235	0,721	0,727
	3	0,135		0,127		0,733	
5	4	0,14	0,14	1,32	0,7	0,388	0,3945
	4	0,14		1,28		0,401	
6	5	0,144	0,1475	0,12	0,125	0,974	0,994
	5	0,151		0,12		1,014	
8	6	0,125	0,131	0,13	0,135	0,517	0,5005
	6	0,137		0,134		0,484	
9	7	0,121	0,1275	0,136	0,12	0,944	0,9035
	7	0,134		0,121		0,863	
10	8	0,132	0,1355	0,119	0,1235	0,962	1,015
	8	0,139		0,125		1,068	
16	9	0,126	0,129	0,122	0,1345	0,8	0,805
	9	0,132		0,133		0,81	
17	10	0,127	0,124	0,136	0,1225	0,237	0,299
	10	0,121		0,125		0,361	
19	11	0,145	0,1395	0,12	0,137	0,357	0,368
	11	0,134		0,149		0,379	
20	12	0,123	0,126	0,125	0,12	0,648	0,665
	12	0,129		0,121		0,682	
21	13	0,122	0,1285	0,119	0,1295	0,509	0,517
	13	0,135		0,125		0,525	
22	14	0,128	0,131	0,134	0,1305	0,448	0,4575
	14	0,134		0,143		0,467	
23	15	0,127	0,1275	0,118	0,1235	0,456	0,4545
	15	0,128		0,125		0,453	
24	16	0,126	0,126	0,122	0,117	0,954	0,9075
	16	0,126		0,118		0,861	
25	17	0,132	0,125	0,116	0,138	0,396	0,3965
	17	0,118		0,121		0,397	
26	18	0,109	0,113	0,155	0,1375	0,58	0,578
	18	0,117		0,138		0,576	
28	19	0,128	0,1265	0,137	0,1265	0,603	0,712
	19	0,125		0,124		0,821	
30	20	0,122	0,123	0,129	0,1215	0,958	0,8865
	20	0,124		0,121		0,815	
32	21	0,128	0,126	0,122	0,736	0,803	0,76
	21	0,124		0,122		0,717	

Appendix-A (Sequel) DAS-ELISA OD values of tested 63 samples for the detection of PVY^{C}, PVY^{N} and PVY^{O+C} **(Late spring)**

Sample Number	ELISA number	OD values of O+C	Mean	OD values of PVYC	Mean	OD values of PVYN	Mean
33	22	0,126	0,127	1,35	0,1295	0,456	0,459
	22	0,128		0,133		0,462	
34	23	0,149	0,179	0,126	0,124	2	1222,5
	23	0,209		0,13		2.443	
36	24	0,132	0,137	0,118	0,1205	0,49	0,482
	24	0,142		0,12		0,474	
37	25	0,137	0,134	0,121	0,1265	1,201	1,2185
	25	0,131		0,123		1,236	
39	26	0,14	0,133	0,13	0,1325	0,544	0,6606
	26	0,126		0,133		0,7772	
40	27	0,122	0,119	0,132	0,131	1,411	1,3645
	27	0,116		0,135		1,318	
41	28	0,122	0,124	0,127	0,1195	1,653	1,613
	28	0,126		0,117		1,573	
42	29	0,127	0,124	0,122	0,1255	2,929	2,7175
	29	0,121		0,124		2,506	
43	30	0,183	0,204	0,127	0,1345	0,519	0,5245
	30	0,225		0,13		0,53	
45	31	0,121	0,122	0,139	0,1195	0,698	0,752
	31	0,123		0,12		0,806	
46	32	0,121	0,123	0,119	0,1245	2,367	2,3405
	32	0,125		0,129		2,314	
47	33	0,132	0,128	0,12	0,121	1,392	1,449
	33	0,124		0,119		1,506	
48	34	0,177	0,1695	0,123	0,117	0,31	0,3085
	34	0,162		0,115		0,307	
49	35	0,153	0,139	0,119	0,117	2,745	2,62
	35	0,125		0,119		2,495	
50	36	0,117	0,119	0,115	0,1265	1,279	1,124
	36	0,121		0,127		0,969	
51	37	0,146	0,1735	0,126	0,12	1,125	1,374
	37	0,201		0,122		1,623	
53	38	0,13	0,1355	0,118	0,117	1,362	1,3125
	38	0,141		0,115		1,263	
54	39	0,125	0,123	0,119	0,1215	0,831	0,776
	39	0,121		0,122		0,721	
55	40	0,121	0,1205	0,121	0,1215	0,873	0,8775
	40	0,12		0,123		0,882	
59	41	0,119	0,1195	0,12	0,118	0,802	0,8035
	441	0,12		0,121		0,805	

Appendix-A (Sequel) DAS-ELISA OD values of tested 63 samples for the detection of PVY^{C}, PVY^{N} and PVY^{O+C} **(Late spring)**

Sample Number	ELISA number	OD values of O+C	Mean	OD values of PVYC	Mean	OD values of PVYN	Mean
61	42	0,13	0,127	0,115	0,114	2,636	2,812
	42	0,124		0,114		2,988	
63	43	0,124	0,1185	0,114	0,121	0,511	0,55
	43	0,113		0,123		0,589	
64	44	0,118	0,1245	0,119	0,128	1,444	1,339
	44	0,131		0,128		1,234	

Appendix-B DAS-ELISA OD values for the detection of PVY in 479 progenies of tested crosses

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD value
Buffer control		0.115	15	15	0.094
		0.099			
Negative control		0.107		15	0.098
		0.108			
Positive control		0.119	16	16	1.387
		0.107			
1	1	0.990		16	1.406
	1	1.000	17	17	0.111
2	2	1.358		17	0.099
	2	1.541	18	18	1.527
3	3	1.505		18	1.555
	3	1.494	19	19	0.095
				19	0.099
4	4	1.101	20	20	0.099
				20	0.109
	4	1.103	21	21	1.138
5	5	0.142		21	0.994
	5	0.102	22	22	0.105
6	6	0.099		22	0.103
	6	0.159	23	23	0.100
7	7	0.107		23	0.098
	7	0.100	24	24	1.277
8	8	0.100		24	1.389
	8	0.113	25	25	0.098
9	9	1.581		25	0.097
	9	1.604	26	26	1.341
10	10	0.185		26	1.326
	10	0.146	27	27	1.582
11	11	1.386		27	1.584
	11	1.279	28	28	1.109
12	12	0.098		28	1.149
	12	0.110	29	29	0.100
13	13	1.688		29	0.096
	13	1.689	30	30	1.374
14	14	1.301		30	1.419
	14	1.330			

Appendix-B (Sequel) DAS-ELISA OD values for the detection of PVY in 479 progenies of tested crosses

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
31	31	0.099			
	31	0.110			
32	32	0.099			
	32	0.100			
33	33	0.097			
	33	0.096			
34	34	0.111			
	34	0.100			
35	35	1.078			
	35	1.153			
36	36	0.098			
	36	0.100			
37	37	0.098			
	37	0.099			
38	38	1.433			
	38	1.440			
39	39	0.100			
	39	0.104			
40	40	1.052			
	40	1.113			
41	41	1.380			
	41	0.952			
42	42	1.278			
	42	1.282			
43	43	1.584			
	43	1.507			
44	44	1.109			
	44	0.923			
45	45	1.142			
	45	1.087			

Appendix-B (Sequel) DAS-ELISA OD values for the detection of PVY in 479 progenies of tested crosses

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
Buffer control		0.096	60	60	1.416
		0.098			
Negative control		0.099		60	1.440
		0.096			
Positive control		0.113	61	61	0.102
		0.111			
46	46	0.106		61	0.103
	46	0.109	62	62	1.686
47	47	0.105		62	1.621
	47	0.109	63	63	1.296
48	48	1.349		63	1.299
	48	1.254	64	64	1.024
				64	1.177
49	49	0.109	65	65	1.460
				65	1.501
	49	0.108	66	66	0.102
50	50	1.234		66	0.103
	50	1.201	67	67	1.643
51	51	1.330		67	1.634
	51	1.355	68	68	1.239
52	52	1.010		68	1.378
	52	1.121	69	69	1.566
53	53	1.310		69	1.532
	53	1.266	70	70	0.104
54	54	1.446		70	0.109
	54	1.435	71	71	0.098
55	55	1.259		71	0.102
	55	1.265	72	72	1.267
56	56	1.394		72	1.249
	56	1.492	73	73	0.100
57	57	0.102		73	0.099
	57	0.106	74	74	0.098
58	58	1.532		74	0.098
	58	1.591	75	75	0.104
59	59	0.107		75	0.104
	59	0.111			

Appendix-B (Sequel) DAS-ELISA OD values for the detection of PVY in 479 progenies of tested crosses

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
76	76	0.102			
	76	0.104			
77	77	1.094			
	77	1.112			
78	78	0.101			
	78	0.101			
79	79	0.099			
	79	0.104			
80	80	0.101			
	80	0.105			
81	81	1.129			
	81	1.096			
82	82	0.100			
	82	0.101			
83	83	0.102			
	83	0.111			
84	84	0.105			
	84	0.107			
85	85	0.105			
	85	0.108			
86	86	0.102			
	86	0.108			
87	87	0.798			
	87	0.783			
88	88	0.116			
	88	0.104			
89	89	0.106			
	89	0.105			
90	90	1.054			
	90	1.021			

Appendix-B (Sequel) DAS-ELISA OD values for the detection of PVY in 479 progenies of tested crosses

Sample Number	**ELISA Number**	**OD values**	**Sample Number**	**ELISA Number**	**OD values**
Buffer control		0.098	105	105	0.111
		0.099			
Negative control		0.097		105	0.095
		0.099			
Positive control		0.131	106	106	0.094
		0.116			
91	91	0.103		106	0.101
	91	0.101	107	107	0.996
92	92	0.100		107	0.433
	92	0.100	108	108	0.586
93	93	0.101		108	1.205
	93	0.102	109	109	1.026
				109	1.088
94	94	1.493	110	110	0.098
				110	0.098
	94	0.693	111	111	0.098
95	95	1.298		111	0.099
	95	1.327	112	112	0.105
96	96	1.350		112	0.099
	96	1.384	113	113	0.097
97	97	0.099		113	0.096
	97	0.103	114	114	1.282
98	98	1.299		114	1.295
	98	1.365	115	115	0.098
99	99	0.100		115	0.098
	99	0.098	116	116	1.157
100	100	0.110		116	1.189
	100	0.108	117	117	1.206
101	111	1.160		117	1.298
	111	1.212	118	118	0.099
102	102	1.293		118	0.099
	102	1.256	119	119	0.084
103	103	0.100		119	0.577
	103	0.097	120	120	0.107
104	104	1.203		120	0.100
	104	1.303			

Appendix-B (Sequel) DAS-ELISA OD values for the detection of PVY in 479 progenies of tested crosses

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
121	121	0.997			
	121	1.066			
122	122	1.086			
	122	1.058			
123	123	0.396			
	123	0.287			
124	124	0.603			
	124	1.042			
125	125	0.098			
	125	0.096			
126	126	1.321			
	126	0.947			
127	127	0.449			
	127	0.307			
128	128	0.589			
	128	0.600			
129	129	0.096			
	129	0.101			
130	130	0.931			
	130	0.867			
131	131	0.852			
	131	0.795			
132	132	0.101			
	132	0.097			
133	133	0.101			
	133	0.099			
134	134	0.706			
	134	0.686			
135	135	0.433			
	135	0.493			

Appendix-B (Sequel) DAS-ELISA OD values for the detection of PVY in 479 progenies of tested crosses

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
Buffer control		0.106	150	150	Out
		0.099			
Negative control		0.100		150	Out
		0.099			
Positive control		0.179	151	151	0.101
		0.126			
136	136	Out		151	0.101
	136	Out	152	152	0.102
137	137	0.112		152	0.098
	137	0.105	153	153	Out
138	138	0.107		153	Out
	138	0.108	154	154	0.100
				154	0.099
139	139	Out	155	155	0.101
				155	0.103
	139	Out	156	156	0.108
140	140	0.106		156	0.099
	140	0.102	157	157	0.113
141	141	Out		157	0.101
	141	Out	158	158	0.102
142	142	Out		158	0.099
	142	Out	159	159	0.100
143	143	Out		159	0.099
	143	Out	160	160	Out
144	144	Out		160	Out
	144	Out	161	161	0.119
145	145	0.113		161	0.101
	145	0.102	162	162	Out
146	146	Out		162	Out
	146	Out	163	163	Out
147	147	0.102		163	Out
	147	0.104	164	164	Out
148	148	Out		164	Out
	148	Out	165	165	0.110
149	149	2.678		165	0.123
	149	2.903			

Appendix-B (Sequel) DAS-ELISA OD values for the detection of PVY in 479 progenies of tested crosses

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
166	166	0.100			
	166	0.101			
167	167	0.102			
	167	0.110			
168	168	Out			
	168	0.918			
169	169	0.120			
	169	0.120			
170	170	Out			
	170	Out			
171	171	0.101			
	171	0.101			
172	172	0.098			
	172	0.105			
173	173	0,504			
	173	1.624			
174	174	0.100			
	174	0.100			
175	175	2.348			
	175	1.667			
176	176	0.101			
	176	0.104			
177	177	Out			
	177	Out			
178	178	0.104			
	178	0.106			
179	179	2.147			
	179	2.257			
180	180	0.111			
	180	0.123			

Appendix-B (Sequel) DAS-ELISA OD values for the detection of PVY in 479 progenies of tested crosses

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
Buffer control		0.262	195	195	0.107
		0.144			
Negative control		0.101		195	0.102
		0.116			
Positive control		0.542	196	196	0.121
		0,309			
181	181	0.112		196	0.152
	181	0.131	197	197	Out
182	182	Out		197	Out
	182	Out	198	198	0.106
183	183	0.129		198	0.106
	183	0.121	199	199	Out
				199	Out
184	184	0.170	200	200	Out
				200	Out
	184	0.113	201	201	Out
185	185	Out		201	Out
	185	Out	202	202	Out
186	186	Out		202	Out
	186	Out	203	203	0.113
187	187	Out		203	0.112
	187	Out	204	204	Out
188	188	0.130		204	Out
	188	0.116	205	205	0.124
189	189	Out		205	0.104
	189	Out	206	206	0.107
190	190	Out		206	0.105
	190	Out	207	207	0.230
191	191	Out		207	0.101
	191	Out	208	208	Out
192	192	Out		208	Out
	192	Out	209	209	0.273
193	193	0.097		209	0.172
	193	0.109	210	210	0.108
194	194	0.110		210	0.103
	194	0.099			

Appendix-B (Sequel) DAS-ELISA OD values for the detection of PVY in 479 progenies of tested crosses

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
211	211	Out			
	211	Out			
212	212	Out			
	212	Out			
213	213	Out			
	213	Out			
214	214	Out			
	214	Out			
215	215	Out			
	215	Out			
216	216	Out			
	216	Out			
217	217	0.219			
	217	0.125			
218	218	Out			
	218	Out			
219	219	Out			
	219	Out			
220	220	0.125			
	220	0.107			
221	221	0.110			
	221	0.105			
222	222	0.106			
	222	0.112			
223	223	Out			
	223	Out			
224	224	0.111			
	224	0.111			
225	225	Out			
	225	out			

Appendix-B (Sequel) DAS-ELISA OD values for the detection of PVY in 479 progenies of tested crosses

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
Buffer control		0.123	240	240	0.116
		0.106		240	0.107
Negative control		0.103	241	241	0.110
		0.103		241	0.120
Positive control		0.492	242	242	out
		0.530		242	out
226	226	0.134	243	243	0.142
	226	0.125		243	0.126
227	227	0.114	244	244	out
	227	0.119		244	out
228	228	0.118	245	245	0.111
	228	0.120		245	0.112
229	229	0.111	246	246	out
	229	0.122		246	out
230	230	0.131	247	247	out
	230	0.120		247	out
231	231	0.128	248	248	0.110
	231	0.118		248	0.110
232	232	0.118	249	249	out
	232	0.115		249	out
233	233	0.127	250	250	out
	233	0.123		250	out
234	234	out	251	251	0.128
	234	out		251	0.113
235	235	0.127	252	252	0.101
	235	0.119		252	0.110
236	236	out	253	253	out
	236	out		253	out
237	237	out	254	254	0.113
	237	out		254	0.123
238	238	0.120	255	255	0.120
	238	0.130		255	0.115
239	239	0.117			
	239	0.120			

Appendix-B (Sequel) DAS-ELISA OD values for the detection of PVY in 479 progenies of tested crosses

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
256	256	0.104			
	256	0.108			
257	257	0.109			
	257	0.106			
258	258	0.112			
	258	0.116			
259	259	out			
	259	out			
260	260	0.109			
	260	0.106			
261	261	0.104			
	261	0.109			
262	262	out			
	262	out			
263	263	0.127			
	263	0.112			
264	264	out			
	264	out			
265	265	out			
	265	out			
266	266	out			
	266	out			
267	267	0.119			
	267	0.123			
268	268	0.115			
	268	0.116			
269	269	out			
	269	out			
270	270	out			
	270	out			

Appendix-B (Sequel) DAS-ELISA OD values for the detection of PVY in 479 progenies of tested crosses

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
Buffer control		0.137	285	285	out
		0.158		285	out
Negative control		0.160	286	286	0.151
		0.133		286	0.201
Positive control		0.993	287	287	0.127
		0.244		287	0.109
271	271	out	288	288	out
	271	out		288	out
272	272	out	289	289	0.142
	272	out		289	0.122
273	273	0.116	290	290	0.125
	273	0.121		290	0.213
274	274	0.119	291	291	out
	274	0.122		291	out
275	275	0.122	292	292	out
	275	0.137		292	out
276	276	out	293	293	0.118
	276	out		293	0.219
277	277	out	294	294	out
	277	out		294	out
278	278	out	295	295	0.138
	278	out		295	0.108
279	279	out	296	296	out
	279	out		296	out
280	280	0.112	297	297	0.138
	280	0.160		297	0.256
281	281	0.188	298	298	out
	281	0.255		298	out
282	282	0.163	299	299	0.131
	282	0.194		299	0.110
283	283	out	300	300	0.112
	283	out		300	0.114
284	284	0.122			
	284	0.125			

Appendix-B (Sequel) DAS-ELISA OD values for the detection of PVY in 479 progenies of tested crosses

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
301	301	out			
	301	out			
302	302	out			
	302	out			
303	303	0.135			
	303	0.110			
304	304	out			
	304	out			
305	305	out			
	305	out			
306	306	out			
	306	out			
307	307	out			
	307	out			
308	308	0.111			
	308	0.101			
309	309	0.164			
	309	0.159			
310	310	out			
	310	out			
311	311	0.114			
	311	0.108			
312	312	0.113			
	312	0.114			
313	313	0.109			
	313	0.110			
314	314	out			
	314	out			
315	315	0.115			
	315	0.127			
316	316	0.123			
	316	0.124			

Appendix-B (Sequel) DAS-ELISA OD values for the detection of PVY in 479 progenies of tested crosses

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
Buffer control		0.119	331	331	2.570
		0.119			
Negative control		0.121		331	2.707
		0.120			
Positive control		2.726	332	332	0.678
		2.663			
317	317	2.651		332	0.659
	317	2.832	333	333	0.127
318	318	0.132		333	0.126
	318	0.126	334	334	Out
319	319	0.134		334	Out
	319	0.131	335	335	Out
				335	Out
320	320	0.112	336	336	1.661
				336	1.744
	320	0.131	337	337	2.241
321	321	0.962		337	2.315
	321	1.258	338	338	0.130
322	322	0.132		338	0.133
	322	0.126	339	339	0.120
323	323	0.130		339	0.124
	323	0.133	340	340	0.128
324	324	2.728		340	0.127
	324	2.806	341	341	2.816
325	325	0.126		341	2.826
	325	0.121	342	342	0.121
326	326	1.641		342	0.122
	326	1.688	343	343	0.759
327	327	0.127		343	0.787
	327	0.128	344	344	0.122
328	328	1.553		344	0.124
	328	1.463	345	345	0.119
329	329	2.828		345	0.129
	329	Out	346	346	0.124
330	330	1.472		346	0.126
	330	1.504			

Appendix-B (Sequel) DAS-ELISA OD values for the detection of PVY in 479 progenies of tested crosses

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
347	347	0,099	363	363	0,118
	347	0,106		363	0,106
348	348	0,098	364	364	0,118
	348	0,102		364	0,106
349	349	0,100	365	365	0.123
	349	0,101		365	0.122
350	350	2.101	366	366	0.125
	350	2.193		366	0.126
351	351	2.041	367	367	0,103
	351	2.103		367	0,103
352	352	1,00	368	368	0,121
	352	0,097		368	0,114
353	353	0,101	369	369	0,099
	353	0,098		369	0,098
354	354	0,099	370	370	2.390
	354	0,097		370	2.486
355	355	0.122	371	371	0.130
	355	0.121		371	0.128
356	356	Out	372	372	0,098
	356	Out		372	0,096
357	357	0,102	373	373	0,104
	357	0,096		373	0,098
358	358	0,101	374	374	0,100
	358	0,101		374	0,099
359	359	0,105	375	375	0.117
	359	0,100		375	0.119
360	360	0.123	376	376	1.284
				376	1.376
			377	377	0,104
				377	0,098
	360	0.120	378	378	0,234
				378	0,231
			379	379	0,109
				379	0,101
361	361	1.433	380	380	0.952
	361	1.402		380	0.924
362	362	0,100	381	381	1.493
	362	0,100		381	1.395

Appendix-B (Sequel) DAS-ELISA OD values for the detection of PVY in 479 progenies of tested crosses

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
Buffer control			396	396	0,128
				396	0,119
Negative control			397	397	0,105
				397	0,132
Positive control			398	398	0,214
382	382			398	0,108
	382	399	399	399	0,133
383	383			399	0,107
	383	400	400	400	0,115
384	384			400	0,117
	384	401	401	401	0,882
				401	0,998
385	38	402	402		
	385	403	403		
386	386				
	386	0,121	404		
387	387	0,105			
	387	0,104	405	405	0,184
388	388	0,105		405	0,116
	388	0,103	406		
389	389	0,104			
	389	0,107	407		
390	390	1,145			
	390	1.71	408		
391	391	0,116			
	391	0,120	409		
392	392	0,817			
	392	0,640	410	410	2,985
393	393	0,113		410	2,997
	393	0,104	411	411	0,137
394	394	0,529		411	0,116
	394	0,383	412	412	2,029
395	395	0,123		412	1,970
	395	0,123			

Appendix-B (Sequel) DAS-ELISA OD values for the detection of PVY in 479 progenies of tested crosses

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
Buffer control			427		
Negative control			428		
Positive control			429		
413	413				
	413		430	430	0,132
414	414			430	0,134
	414		431	431	0,165
415	415	2,029		431	0,162
	415	1,970	432	432	0.094
				432	0.097
416	416	0,151	433	433	0.111
				433	0.110
	416	0,116	434	434	0.111
417	417			434	0.110
	417		435	435	0,136
418	418			435	0,138
	418		436	436	0,119
419	419			436	0,129
	419		437	437	0,123
420	420	2,376		437	0.103
	420	2,422	438	438	0.274
421	421	Out		438	0.110
	421	out	439	439	0.109
422	422			439	0.103
	422		440	440	0,120
423	423			440	0,122
	423		441	441	
424	424			441	0,217
	424		442	442	0.104
425	425	0,118		442	0.100
	425	0,118	443	443	0.108
426	426	Out		443	0.108
	426	out			

Appendix-B (Sequel) DAS-ELISA OD values for the detection of PVY in 479 progenies of tested crosses

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
444	444	0,116	463	463	2.432
	444	0,118		463	2.523
445	445	0,750	464	464	2.386
	445	0,713		464	2.698
446	446	1,764	465	465	2.502
	446	1,897		465	2.129
447	447	1,705	466	466	2.100
	447	1,612		466	2.130
448	448	0.111	467	467	2.464
	448	0.111		467	2.616
449	449	2.075	468	468	0.115
	449	1.888		468	0.104
450	450	0.110	469	469	2.701
	450	0.117		469	2.523
451	451	2.407	470	470	0.112
	451	2.562		470	0.112
452	452	0.115	471	471	2.332
	452	0.109		471	2.378
453	453	0.137	472	472	0.101
	453	0.120		472	0,103
454	454	0.125	473	473	0,106
	454	0.123		473	0,103
455	455	1.461	474	474	1.842
	455	1.482		474	1.948
456	456	2.193	475	475	0.114
	456	2.317		475	0.101
457	457	0.104	476	476	0.105
	457	0.105		476	0.113
458	458	1.676	477	477	2.286
	458	1.707		477	2.101
			478	478	1.474
				478	1.700
459	459	2.980	479	479	0.106
	459	2.794		479	0.110
460	460	0.122	480	480	0.872
	460	0.105		480	0.877
461	461	1.897	481	481	0.112
	461	1.614		481	0.104
462	462	0.120	482	482	1.531
	462	0.116		482	1.567

Appendix-B (Sequel) DAS-ELISA OD values for the detection of PVY in 479 progenies of tested crosses

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
483	0.110		502		0.107
	0.106				0.113
484	1.644		503		0.095
	1.596				0.105
485	1.633		504		0.102
	1.796				0.099
486	0.143		505		1.393
	0.110				1.419
487	1.640		506		0.095
	1.702				0.105
488	0.103		507		0.104
	0.108				0.098
489	1.628		508		0.100
	1.569				0.098
490	0.110		509		Out
	0.098				Out
491	1.588		510		0.099
	1.685				0.102
492	0.122		511		1.244
	0.113				1.298
492	1.784		512		1.512
	1.713				1.574
493	1.781		513		0.099
	2.065				0.098
494	0.113		514		2.698
	0.111				2.739
495	0.112		515		0.099
	0.110				0.096
496	1.105		516		Out
	0.954				Out
			517		1.866
497	1.105				1.994
	0.954		518		0.099
498	0.135				0.095
499	0.108		519		0.095
	1.300				0.101
500	1.289		520		1.480
	1.649				1.541
501	1.547		521		2.728
	1.915				2.682

Appendix-B (Sequel) DAS-ELISA OD values for the detection of PVY in 479 progenies of tested crosses

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
522	522	2.038	541	541	0.0097
	522	1.964		541	0.101
523	523	0.098	542	542	2.289
	523	0.098		542	2.184
524	524	1.778			
	524	1.776			
525	525	0.109			
	525	0.096			
526	526	2.038			
	526	1.964			
527	527	1.334			
	527	1.478			
528	528	0.102			
	528	0.104			
529	529	2.790			
	529	2.604			
530	530	0.101			
	539	0.111			
531	531	Out			
	531	Out			
532	531	0.097			
	531	0.097			
533	533	0.098			
	533	0.098			
534	534	0.099			
	534	0.107			
535	535	0.104			
	535	0.095			
536	536	0.096			
	536	0.094			
537	537	0.100			
	537	0.097			
538	538	2.642			
	538	2.689			
539	539	0.104			
	539	0.100			
540	540	2.609			
	540	2.535			

Appendix-C DAS-ELISA OD values test conducting for testing parent's cultivars sensitivity against PVY infection

ELISA number	Tested cultivar	Plant Number	ELISA number	Tested cultivar	Plant Number	ELISA number	Tested cultivar	Plant Number
1	Bettina	B1	31	Slad Blue	S1	61	Savana	Sav7
2	Bettina	B2	32	Slad Blue	S2	62	Savana	Sav8
3	Bettina	B3	33	Slad Blue	S3	63	Savana	Sav9
4	Bettina	B4	34	Slad Blue	S4	64	Savana	Sav10
5	Bettina	B5	35	Slad Blue	S5	65	Savana	Sav11
6	Bettina	B6	36	Slad Blue	S6	66	Savana	Sav12
7	Bettina	B7	37	Slad Blue	S7	67	Savana	Sav13
8	Bettina	B8	38	Slad Blue	S8	68	Savana	Sav14
9	Bettina	B9	39	Slad Blue	S9	69	Nector	N1
10	Bettina	B10	40	Slad Blue	S10	70	Nector	N2
11	Bettina	B11	41	Slad Blue	S11	71	Nector	N3
12	Bettina	B12	42	Galata	G1	72	Nector	N4
13	Bettina	B13	43	Galata	G2	73	Nector	N5
14	Bettina	B14	44	Galata	G3	74	Nector	N6
15	Hermes	H1	45	Galata	G4	75	Nector	N7
16	Hermes	H2	46	Galata	G5	76	Nector	N8
17	Hermes	H3	47	Galata	G6	77	Nector	N9
18	Hermes	H4	48	Galata	G7	78	Nector	N10
19	Hermes	H5	49	Galata	G8	79	Nector	N11
20	Hermes	H6	50	Galata	G9	80	Nector	N12
21	Hermes	H7	51	Galata	G10	81	Nector	N13
22	Hermes	H8	52	Galata	G11	82	Nector	N14
23	Hermes	H9	53	Galata	G12			
24	Hermes	H10	54	Galata	G13			
25	Hermes	H11	55	Savana	Sav1			
26	Hermes	H12	56	Savana	Sav2			
27	Hermes	H13	57	Savana	Sav3			
28	Hermes	H14	58	Savana	Sav4			
29	Slad Blue	S1	59	Savana	Sav5			
30	Slad Blue	S2	60	Savana	Sav6			

Appendix-C (Sequel) DAS-ELISA OD values test conducting for testing parent's cultivars sensitivity against PVY infection

Sample Number	**ELISA Number**	**OD values**	**Sample Number**	**ELISA Number**	**OD values**
Buffer control		0.113	15	0,11	0,1105
		0.113		0,111	
Negative control		0.105	16	1,388	1,4305
		0.105		1,473	
Positive control		Out	17	1,158	1,1655
		Out			
1	0,165	21		1,173	
	0,105	22	18	0,136	0,648
2	0,106	23		1,16	
	0,105	24	19	0,109	0,613
3	0,138	25		1,117	
	0,119	26	20	1,597	1,5555
	0,12	27		1,514	
4	0,11		21	0,108	0,108
	0,111	0,112		0,108	
	0,113		22	0,109	0,104
5	0,118	0,1175		0,099	
	0,117		23	0,114	0,116
6	0,122	0,116		0,118	
	0,11		24	0,115	0,1125
7	0,117	0,1155		0,11	
	0,114		25	0,111	0,1095
8	0,21	0,158		0,108	
	0,106		26	0,114	0,1105
9	0,128	0,1645		0,107	
	0,201		27	0,126	0,118
10	0,111	0,112		0,11	
	0,113		28	1,132	1,139
11	0,109	0,109		1,146	
	0,109		29	0,111	0,112
12	0,121	0,117		0,113	
	0,113		30	0,109	0,109
13	0,113	0,1175		0,109	
	0,118		31	0,121	0,117
14	0,117	0,116		0,113	

Appendix-C (Sequel) DAS-ELISA OD values test conducting for testing parent's cultivars sensitivity against PVY infection

Sample Number	**ELISA Number**	**OD values**	**Sample Number**	**ELISA Number**	**OD values**
32	0,115	0,1125			
	0,11				
33	0,111	0,1095			
	0,108				
34	0,114	0,1105			
	0,107				
35	0,126	0,118			
	0,11				
36	1,132	1,139			
	1,146				
37	0,923	0,9075			
	0,892				
38	0,541	0,5645			
	0,588				
39	0,769	0,7925			
	0,816				
40	0,75	0,4081			
	0,0662				
41	0,697	0,7085			
	0,72				
42	0,12	0,115			
	0,11				
43	0,111	0,112			
	0,113				
44	0,118	0,1175			
	0,117				
45	0,122	0,116			
	0,11				

Appendix-C (Sequel) DAS-ELISA OD values test conducting for testing parent’s cultivars sensitivity against PVY infection

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
Buffer control		0.637	59	0,62	0,6485
		0.212		0,677	
Negative control		0.200	60	1,324	1,3765
		0.200		1,429	
Positive control		Out	61	1,6	1,594
		out			
46	0,21	0,158		1,588	2,1115
	0,106		62	2	2,5375
47	0,128	0,1645		2,223	
	0,201		63	2,351	2,636
48	0,111	0,112		2,724	
	0,113		65	2,693	2,5115
				2,579	
49	1,21	1,223	66	2,571	2,5115
				2,452	
	1,236		67	2,389	2,4805
50	1,597	1,6825		2,572	
	1,768		68	2,33	2,459
51	1,771	1,792		2,588	
	1,813		69	2,97	2,9495
52	2,379	2,419		2,929	
	2,459		70	0,108	0,116
53	1,431	1,3685		0,124	
	1, 306		71	0,112	0,121
54	1,289	1,3045		0,13	
	1,32		72	0,123	0,118
55	1,289	1,3045		0,113	
	1,32		73	0,122	0,533
56	0,14	0,1525		0,944	
	0,165		74	2	2
57	0,865	0,8145		2	
	0,764		75	1,801	1,723
58	2,053	2,1195		1,645	
	2,186		76	1,445	1,46
				1,475	

Appendix-C (Sequel) DAS-ELISA OD values test conducting for testing parent's cultivars sensitivity against PVY infection

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
77	1,982	1,9835			
	1,985				
78	1,449	1,513			
	1,577				
79	1,608	1,5415			
	1,475				
80	1,505	1,5895			
	1,674				
81	1,787	1,782			
	1,777				
82	1,315	1,3195			
	1,324				

Appendix-D DAS-ELISA OD values, tested conducting the sensitivty of tested genotypes plants during the second year

ELSI A No	Sample No	ELISA No	Sample Number	ELISA No	Sample Number
1	4a	47	46a	93	92d
2	4b	48	46b	94	93a
3	5a	49	46c	95	93b
4	5b	50	46d	96	93c
5	6a	51	47a	97	97a
6	6b	52	47c(no plant)	98	97b
7	7a	53	49a	99	97c
8	7b	54	49b	100	99a
9	7c	55	57a	101	100a
10	8a	56	57b	102	103b
11	8b	57	57c	103	103a
12	10a	58	61a	104	102a
13	10b	59	61b	105	105b
14	12a	60	66a	106	106a
15	12b	61	66b	107	106b
16	12c	62	66c	108	106c
17	12d	63	70a	109	110a
18	15a	64	70b	110	111a
19	15b	65	70c	111	111b
20	17a	66	71a	112	111c
21	17b	67	73a	113	112a
22	19a	68	73b	114	112b
23	19b	69	74a	115	113a
24	22a	70	75a	116	113b
25	22b	71	75b	117	115a
26	22c	72	75c	118	115b
27	22d	73	75d	119	115c
28	25a	74	76a	120	118a
29	25b	75	76b	121	120a
30	29a	76	76c	122	120b
31	29b	77	78a	123	120c
32	29c	78	78b	124	125a
33	31a	79	79a	125	125b
34	31b	80	80d	126	129a
35	31c	81	82a	127	132a
36	32a	82	83a	128	133a
37	32b	83	84a	129	133b
38	33a	84	85a	130	133c
39	33b	85	88a	131	137a
40	33c	86	89a	132	137b
41	34	87	89b	133	138a
42	36a	88	91a	134	140a
43	36b	89	91b	135	140b
44	37a	90	92a	136	140c
45	39a	91	92b	137	145a
46	39b	92	92c	138	147a

Appendix-D (Sequel) DAS-ELISA OD values, tested conducting the sensitivty of tested genotypes plants during the second year

ELSIA No	Sample No	ELISA No	Sample Number	ELISA No	Sample Number
139	147b	185	184a	231	238a
140	147c	186	184b	232	238b
141	151a	187	188a	233	238c
142	151b	188	188b	234	239a
143	152a	189	193a	235	239b
144	152b	190	193b	236	240b
145	152c	191	194a	237	240c
146	152d	192	195a	238	241a
147	154a	193	195b	239	241b
148	155a	194	196a	240	241c
149	155b	195	198a	241	243a
150	155c	196	203a	242	243b
151	156a	197	205a	243	243c
152	156b	198	205b	244	245a
153	157a	199	205c	245	245b
154	158a	200	206a	246	248a
155	158b	201	207a	247	248b
156	158c	202	209a	248	251a
157	159a	203	209b	249	251b
158	161a	204	210a	250	251c
159	161b	205	217a	251	252a
160	165a	206	217b	252	252b
161	165b	207	220a	253	254a
162	165c	208	220b	254	254b
163	166a	209	220c	255	255a
164	166b	210	220d	256	255b
165	166c	211	221a	257	256a
166	167a	212	221b	258	257a
167	168a	213	222a	259	258a
168	168b	214	222b	260	260a
169	169a	215	222c	261	261a
170	169b	216	222d	262	261b
171	171a	217	224a	263	267a
172	172a	218	224b	264	267b
173	174a	219	226a	265	268a
174	176a	220	226b	266	273a
175	176b	221	227a	267	273b
176	178a	222	227b	268	274a
177	178b	223	227c	269	274b
178	180a	224	227d	270	274c
179	181a	225	229a	271	275a
180	181b	226	229b	272	275b
181	181c	227	232a	273	280a
182	181d	228	230a	274	280b
183	183a	229	232b	275	280b
184	183b	230	233a	276	280c

Appendix-D (Sequel) DAS-ELISA OD values, tested conducting the sensitivty of tested genotypes plants during the second year

ELSIA No	Sample No	ELISA No	Sample Number	ELISA No	Sample Number
277	281a	323	325b	369	452a
278	281b	324	327a	370	453a
279	282a	325	327b	371	453b
280	282b	326	339a	372	454a
281	284a	327	340a	373	457a
282	286a	328	342a	374	457b
283	287a	329	342b	375	460a
284	283a	330	344a	376	460b
285	289a	331	344b	377	462a
286	290a	332	346a	378	462b
287	291b	333	350a	379	470a
288	293a	334	350b	380	472a
289	295a	335	365_1(a)	381	472b
290	297a	336	366a	382	473a
291	300a	337	371a	383	473b
292	300b	338	371b	384	475a
293	303a	339	375a	385	475b
294	303b	340	385a	386	475c
295	308a	341	386a	387	476a
296	308b	342	386b	388	476b
297	308c	343	386c	389	479a
298	309a	344	391a	390	481a
299	309b	345	395a	391	481b
300	311a	346	396a	392	481c
301	311b	347	400a	393	483a
302	312a	348	405a	394	483b
303	312b	349	406a	395	486a
304	312c	350	406b	396	486b
305	313a	351	411a	397	486c
306	313b	352	416a	398	490a
307	313c	353	425a	399	490b
308	315a	354	430a	400	490c
309	316a	355	430b	401	490d
310	316b	356	430c	402	492a
311	318a	357	431a	403	492b
312	319a	358	435a	404	495a
313	319b	359	435b	405	495b
314	320a	360	436a	406	496a
315	320b	361	436b	407	496b
316	322a	362	436c	408	496c
317	322b	363	441a	409	498a
318	322c	364	445a	410	502a
319	322d	365	448a	411	502b
320	323a	366	448b	412	502c
321	323b	367	450a	413	502d
322	325a	368	450b	414	502e

Appendix-D (Sequel) DAS-ELISA OD values, tested conducting the sensitivty of tested genotypes plants during the second year

ELSIA No	Sample No	ELISA No	Sample Number	ELISA No	Sample Number
415	503a	457	528b		
416	503b	458	528c		
417	503c	459	530a		
418	503d	460	530b		
419	504a	461	530c		
420	504b	462	530d		
421	506a	463	532a		
422	506b	464	532b		
423	506c	465	532c		
424	506d	466	532d		
425	507a	467	533a		
426	507b	468	533b		
427	507c	469	534a		
428	508a	470	534b		
429	508b	471	534c		
430	508c	472	534d		
431	510a	473	535a		
432	510b	474	535b		
433	510c	475	536a		
434	510d	476	536b		
435	513a	477	536c		
436	513b	478	536d		
437	515a	479	539a		
438	515b	480	541a		
439	518a	481	541b		
440	518b	482	541c		
441	518c				
442	518d				
443	519a				
444	519b				
445	519c				
446	519d				
447	522a				
448	522b				
449	522c				
450	523a				
451	523b				
452	523c				
453	525a				
454	525b				
455	525c				
456	528a				

Appendix-D (Sequel) DAS-ELISA OD values, tested conducting the sensitivty of tested genotypes plants during the second year

Sample Number	**ELISA Number**	**OD values**
Buffer control		0.111
		0.105
Negative control		0.108
		0.162
Positive control		0.109
		0.105
1	1	0.108
	1	0.109
2	2	0.108
	2	0.111
3	3	0.113
	3	0.126
4	4	0.117
	4	0.113
5	5	0.111
	5	0.103
6	6	0.111
	6	0.109
7	7	0.094
	7	0.107
8	8	0.106
	8	0.107
9	9	0.106
	9	0.107
10	10	0.111
	10	0.109
11	11	0.107
	11	0.107
12	12	0.109
	12	0.108
13	13	0.108
	13	0.106
14	14	0.115
	14	0.107

Sample Number	**ELISA Number**	**OD values**
15	15	0.116
	15	0.104
16	16	0.108
	16	0.110
17	17	0.108
	17	0.104
18	18	0.108
	18	0.107
19	19	0.111
	19	0.110
20	20	0.107
	20	0.108
21	21	0.113
	21	0.107
22	22	0.109
	22	0.104
23	23	0.106
	23	0.103
24	24	0.112
	24	0.110
25	25	0.109
	25	0.112
26	26	0.108
	26	O.111
27	27	0.108
	27	0.104
28	28	0.109
	28	0.123
29	29	0.106
	29	0.104
30	30	0.112
	30	0.106

Appendix-D (Sequel) DAS-ELISA OD values, tested conducting the sensitivty of tested genotypes plants during the second year

Sample Number	**ELISA Number**	**OD values**	**Average**	**Sample Number**	**ELISA Number**	**OD values**	**Average**
31	31	0.109					
	31	0.109					
32	32	0.111					
	32	0.111					
33	33	0.106					
	33	0.115					
34	34	0.121					
	34	0.106					
35	35	0.109					
	35	0.105					
36	36	0.107					
	36	0.109					
37	37	0.105					
	37	0.111					
38	38	0.182					
	38	0.108					
39	39	O.112					
	39	0.125					
40	40	0.108					
	40	0.112					
41	41	0.108					
	41	0.110					
42	42	0.115					
	42	0.124					
43	43	0.130					
	43	0.106					
44	44	0.140					
	44	0.122					
45	45	0.107					
	45	0.119					

Appendix-D (Sequel) DAS-ELISA OD values, tested conducting the sensitivty of tested genotypes plants during the second year

Sample Number	ELISA Number	OD values	Average	Sample Number	ELISA Number	OD values	Average
Buffer control		0.107		60	60	0.106	
		0.0.107					
Negative control		0.104			60	0.104	
		0.102					
Positive control		0.123		61	61	0.106	
		0.124					
46	46	0.100			61	0.105	
	46	0.100		62	62	0.105	
47	47	0.106			62	0.108	
	47	0.100		63	63	0.108	
48	48	0.110			63	0.105	
	48	0.110		64	64	0.105	
					64	0.106	
49	49	0.103		65	65	0.107	
					65	0.110	
	49	0.138		66	66	0.108	
50	50	0.113			66	0.107	
	50	0.102		67	67	0.106	
51	51	0.109			67	0.103	
	51	0.103		68	68	0.103	
52	52	0.105			68	0.105	
	52	0.104		69	69	0.106	
53	53	0.107			69	0.103	
	53	0.102		70	70	0.104	
54	54	0.107			70	0.105	
	54	0.114		71	71	0.104	
55	55	0.108			71	0.103	
	55	0.104		72	72	0.103	
56	56	0.104			72	0.103	
	56	0.105		73	73	0.105	
57	57	0.104			73	0.108	
	57	0.104		74	74	0.106	
58	58	0.103			74	0.108	
	58	0.108		75	75	0.103	
59	59	0.108			75	0.103	
	59	0.104					

Appendix-D (Sequel) DAS-ELISA OD values, tested conducting the sensitivty of tested genotypes plants during the second year

Sample Number	**ELISA Number**	**OD values**	**Sample Number**	**ELISA Number**	**OD value 0,246> pozitif**
76	76	0.101			
	76	0.101			
77	77	0.106			
	77	0.106			
78	78	0.103			
	78	0.112			
79	79	0.105			
	79	0.102			
80	80	0.102			
	80	0.101			
81	81	0.104			
	81	0.104			
82	82	0.104			
	82	0.106			
83	83	0.103			
	83	0.103			
84	84	0.101			
	84	0.103			
85	85	0.102			
	85	0.103			
86	86	0.104			
	86	0.107			
87	87	0.106			
	87	0.108			
88	88	0.107			
	88	0.107			
89	89	0.108			
	89	0.116			
90	90	0.104			
	90	0.107			

Appendix-D (Sequel) DAS-ELISA OD values, tested conducting the sensitivty of tested genotypes plants during the second year

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
Buffer control		0.105	105	105	0.103
Buffer control		0.104	105	105	0.102
Negative control		0.107	106	106	0.102
Negative control		0.105	106	106	0.105
Positive control		0.117	107	107	0.104
Positive control		0.118	107	107	0.104
91	91	0.117	108	108	0.107
91	91	0.105	108	108	0.101
92	92	0.111	109	109	0.102
92	92	0.105	109	109	0.099
93	93	0.112	110	110	0.101
93	93	0.107	110	110	0.101
94	94	0.105	111	111	0.104
94	94	0.113	111	111	0.106
95	95	0.105	112	112	0.105
95	95	0.105	112	112	0.102
96	96	0.104	113	113	0.102
96	96	0.104	113	113	0.101
97	97	0.104	114	114	0.100
97	97	0.102	114	114	0.101
98	98	0.104	115	115	0.105
98	98	0.107	115	115	0.104
99	99	0.105	116	116	0.104
99	99	0.106	116	116	0.102
100	100	0.108	117	117	0.102
100	100	0.103	117	117	0.100
101	111	Out	118	118	0.103
101	111	Out	118	118	0.102
102	102	0.104	119	119	0.104
102	102	0.104	119	119	0.110
103	103	0.104	120	120	0.110
103	103	0.109	120	120	0.103
104	104	0.107			
104	104	0.102			

Appendix-D (Sequel) DAS-ELISA OD values, tested conducting the sensitivty of tested genotypes plants during the second year

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
121	121	0.102			
	121	0.100			
122	122	0.102			
	122	0.101			
123	123	0.106			
	123	0.112			
124	124	0.110			
	124	0.103			
125	125	0.101			
	125	0.092			
126	126	0.105			
	126	0.103			
127	127	0.103			
	127	0.114			
128	128	0.112			
	128	0.106			
129	129	0.101			
	129	0.098			
130	130	0.102			
	130	0.103			
131	131	0.103			
	131	0.109			
132	132	0.108			
	132	0.106			
133	133	0.102			
	133	0.104			
134	134	0.103			
	134	0.105			
135	135	0.108			
	135	0.116			

Appendix-D (Sequel) DAS-ELISA OD values, tested conducting the sensitivty of tested genotypes plants during the second year

Sample Number	**ELISA Number**	**OD values**
Buffer control		0.114
		0.112
Negative control		0.116
		0.113
Positive control		0.123
		0.133
136	136	0.117
	136	0.122
137	137	0.127
	137	0.123
138	138	0.132
	138	0.119
139	139	0.124
	139	0.135
140	140	0.137
	140	0.111
141	141	0.137
	141	0.129
142	142	0.123
	142	0.108
143	143	0.118
	143	0.114
144	144	0.116
	144	0.120
145	145	0.145
	145	0.126
146	146	0.113
	146	0.230
147	147	0.108
	147	0.106
148	148	0.114
	148	0.118
149	149	0.137
	149	0.122

Sample Number	**ELISA Number**	**OD values**
150	150	0.117
	150	0.112
151	151	0.109
	151	0.110
152	152	0.113
	152	0.122
153	153	0.136
	153	0.130
154	154	0.147
	154	0.108
155	155	0.116
	155	0.111
156	156	0.115
	156	0.136
157	157	0.138
	157	0.117
158	158	0.113
	158	0.109
159	159	0.115
	159	0.129
160	160	0.119
	160	0.162
161	161	0.137
	161	0.117
162	162	0.118
	162	0.107
163	163	0.110
	163	0.113
164	164	0.115
	164	0.127
165	165	0.136
	165	0.120

Appendix-D (Sequel) DAS-ELISA OD values, tested conducting the sensitivty of tested genotypes plants during the second year

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
166	166	0.112			
	166	0.110			
167	167	Out			
	167	Out			
168	168	Out			
	168	Out			
169	169	0.124			
	169	0.121			
170	170	0.131			
	170	0.111			
171	171	0.115			
	171	0.115			
172	172	0.113			
	172	0.119			
173	173	0.125			
	173	0.119			
174	174	0.116			
	174	0.108			
175	175	0.119			
	175	0.124			
176	176	0.126			
	176	0.142			
177	177	0.169			
	177	0.152			
178	178	0.139			
	178	0.140			
179	179	0.137			
	179	0.139			
180	180	0.151			
	180	0.165			

Appendix-D (Sequel) DAS-ELISA OD values, tested conducting the sensitivty of tested genotypes plants during the second year

Sample Number	ELISA Number	OD values
Buffer control		0.113
		0.112
Negative control		0.118
		0.120
Positive control		0.111
		0.112
181	181	0.126
	181	0.119
182	182	0.111
	182	0.116
183	183	0.129
	183	0.126
184	184	0.120
	184	0.128
185	185	0.113
	185	0.110
186	186	0.118
	186	0.110
187	187	0.114
	187	0.108
188	188	0.112
	188	0.110
189	189	0.112
	189	0.113
190	190	0.116
	190	0.117
191	191	0.113
	191	0.109
192	192	0.110
	192	0.110
193	193	0.112
	193	0.112
194	194	0.119
	194	0.111

Sample Number	ELISA Number	OD values
195	195	0.109
	195	0.109
196	196	0.112
	196	0.110
197	197	0.110
	197	0.112
198	198	0.119
	198	0.111
199	199	0.112
	199	0.108
200	200	0.111
	200	0.115
201	201	0.111
	201	0.113
202	202	0.113
	202	0.110
203	203	0.112
	203	0.110
204	204	0.113
	204	0.108
205	205	0.107
	205	0.114
206	206	0.121
	206	0.115
207	207	0.113
	207	0.110
208	208	0.110
	208	0.113
209	209	0.117
	209	0.120
210	210	0.117
	210	0.112

Appendix-D (Sequel) DAS-ELISA OD values, tested conducting the sensitivty of tested genotypes plants during the second year

Sample Number	**ELISA Number**	**OD values**	**Sample Number**	**ELISA Number**	**OD values**
211	211	0.109			
	211	0.109			
212	212	0.112			
	212	0.110			
213	213	0.114			
	213	0.110			
214	214	0.112			
	214	0.110			
215	215	0.111			
	215	0.107			
216	216	0.108			
	216	0.106			
217	217	0.111			
	217	0.109			
218	218	0.111			
	218	0.111			
219	219	0.109			
	219	0.107			
220	220	0.109			
	220	0.109			
221	221	0.110			
	221	0.115			
222	222	0.121			
	222	0.117			
223	223	0.113			
	223	0.107			
224	224	0.108			
	224	0.109			
225	225	0.112			
	225	0.116			

Appendix-D (Sequel) DAS-ELISA OD values, tested conducting the sensitivty of tested genotypes plants during the second year

Sample Number	ELISA Number	OD values
Buffer control		0.107
		0.106
Negative control		0.121
		0.127
Positive control		0.107
		0.109
226	226	0.122
	226	0.115
227	227	0.128
	227	0.126
228	228	0.131
	228	0.123
229	229	0.125
	229	0.122
230	230	0.140
	230	0.135
231	231	0.122
	231	0.120
232	232	0.127
	232	0.124
233	233	0.117
	233	0.117
234	234	0.120
	234	0.122
235	235	0.123
	235	0.115
236	236	0.121
	236	0.119
237	237	0.121
	237	0.121
238	238	0.120
	238	0.124
239	239	0.127
	239	0.125

Sample Number	ELISA Number	OD values
240	240	0.124
	240	0.124
241	241	0.123
	241	0.123
242	242	0.122
	242	0.124
243	243	0.125
	243	0.126
244	244	0.122
	244	0.121
245	245	0.114
	245	0.111
246	246	0.119
	246	0.121
247	247	0.115
	247	0.117
248	248	0.131
	248	0.121
249	249	0.122
	249	0.120
250	250	0.120
	250	0.121
251	251	0.127
	251	0.123
252	252	0.130
	252	0.126
253	253	0.124
	253	0.125
254	254	0.120
	254	0.119
255	255	0.129
	255	0.126

Appendix-D (Sequel) DAS-ELISA OD values, tested conducting the sensitivty of tested genotypes plants during the second year

Sample Number	**ELISA Number**	**OD values**	**Sample Number**	**ELISA Number**	**OD values**
256	256	0.121			
	256	0.120			
257	257	0.119			
	257	0.116			
258	258	0.117			
	258	0.115			
259	259	0.118			
	259	0.116			
260	260	0.117			
	260	0.118			
261	261	0.118			
	261	0.117			
262	262	0.119			
	262	0.118			
263	263	0.117			
	263	0.118			
264	264	0.127			
	264	0.124			
265	265	0.129			
	265	0.123			
266	266	0.120			
	266	0.113			
267	267	0.119			
	267	0.125			
268	268	0.119			
	268	0.117			
269	269	0.123			
	269	0.124			
270	270	0.121			
	270	0.121			

Appendix-D (Sequel) DAS-ELISA OD values, tested conducting the sensitivty of tested genotypes plants during the second year

Sample Number	**ELISA Number**	**OD values**	**Sample Number**	**ELISA Number**	**OD values**
Buffer control		0.109	285	285	0.117
		0.107		285	0.115
Negative control		0.118	286	286	0.110
		0.116		286	0.108
Positive control		0.116	287	287	0.109
		0.117		287	0.113
271	271	0.111	288	288	0.112
	271	0.116		288	0.121
272	272	0.116	289	289	0.114
	272	0.119		289	0.110
273	273	0.117	290	290	0.112
	273	0.115		290	0.109
274	274	0.115	291	291	0.110
	274	0.116		291	0.111
275	275	0.117	292	292	0.112
	275	0.111		292	0.119
276	276	0.113	293	293	0.110
	276	0.113		293	0.108
277	277	No sample	294	294	0.110
	277	No sample		294	0.110
278	278	0.112	295	295	0.111
	278	0.111		295	0.108
279	279	Out	296	296	0.112
	279	Out		296	0.116
280	280	Out	297	297	0.113
	280	Out		297	0.110
281	281	Out	298	298	0.110
	281	Out		298	0.107
282	282	0.113	299	299	0.114
	282	0.111		299	0.110
283	283	0.111	300	300	0.111
	283	0.114		300	0.119
284	284	0.114			
	284	0.135			

Appendix-D (Sequel) DAS-ELISA OD values, tested conducting the sensitivty of tested genotypes plants during the second year

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
301	301	0.112			
	301	0.111			
302	302	0.106			
	302	0.103			
303	303	0.108			
	303	0.107			
304	304	0.110			
	304	0.122			
305	305	0.114			
	305	0.116			
306	306	0.112			
	306	0.109			
307	307	0.110			
	307	0.111			
308	308	0.115			
	308	0.124			
309	309	0.120			
	309	0.114			
310	310	0.115			
	310	0.111			
311	311	0.114			
	311	0.113			
312	312	0.134			
	312	0.139			
313	313	0.151			
	313	0.149			
314	314	0.114			
	314	0.113			
315	315	0.115			
316	316	0.116			
	316	0.115			

Appendix-D (Sequel) DAS-ELISA OD values, tested conducting the sensitivty of tested genotypes plants during the second year

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
Buffer control		0.106	331	331	0.112
		0.104		331	0.112
Negative control		0.109	332	332	0.141
		0.105		332	0.141
Positive control		0.106	333	333	0.200
		0.108		333	0.200
317	317	0.166	334	334	0.200
	317	0.119		334	0.200
318	318	0.118	335	335	0.124
	318	0.117		335	0.125
319	319	0.114	336	336	0.129
	319	0.113		336	0.119
320	320	0.135	337	337	0.121
	320	0.128		337	0.132
321	321	0.128	338	338	0.127
	321	0.122		338	0.142
322	322	0.125	339	339	0.132
	322	0.119		339	0.126
323	323	0.123	340	340	0.117
	323	0.120		340	0.138
324	324	0.120	341	341	0.123
	324	0.119		341	0.121
325	325	0.125	342	342	0.129
	325	0.190		342	0.158
326	326	0.122	343	343	0.125
	326	0.117		343	0.114
327	327	0.122	344	344	0.142
	327	0.119		344	0.139
328	328	0.119	345	345	0.125
	328	0.360		345	0.130
329	329	0.119	346	346	0.118
	329	0.124		346	0.116
330	330	0.118			
	330	0.117			

Appendix-D (Sequel) DAS-ELISA OD values, tested conducting the sensitivty of tested genotypes plants during the second year

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
347	347	0.118			
	347	0.117			
348	348	0.151			
	348	0.117			
349	349	0.120			
	349	0.120			
350	350	0.120			
	350	0.121			
351	351	0.138			
	351	0.144			
352	352	0.119			
	352	0.129			
353	353	0.156			
	353	0.118			
354	354	0.199			
	354	0.114			
355	355	0.126			
	355	0.116			
356	356	0.140			
	356	0.141			
357	357	0.121			
	357	0.124			
358	358	0.125			
	358	0.121			
359	359	0.127			
	359	0.119			
360	360	0.131			
	360	0.125			
361	361	0.122			
	361	0.122			

Appendix-D (Sequel) DAS-ELISA OD values, tested conducting the sensitivty of tested genotypes plants during the second year

Sample Number	**ELISA Number**	**OD values**
Buffer control		0.103
		0.102
Negative control		0.123
		0.16
Positive control		0.108
		0.116
362	362	0.115
	362	0.111
363	363	0.109
	363	0.105
364	364	Out
	364	Out
365	365	0.110
	365	0.118
366	366	0.107
	366	0.103
367	367	0.110
	367	0.107
368	368	0.104
	368	0.102
369	369	0.104
	369	0.103
370	370	0.105
	370	0.110
371	371	0.108
	371	0.106
372	372	0.104
	372	0.132
373	373	0.106
	373	0.103
374	374	0.105
	374	0.110
375	375	0.106
	375	0.103

Sample Number	**ELISA Number**	**OD values**
376	376	0.106
	376	0.238
377	377	0.124
	377	0.104
378	378	0.104
	378	0.105
379	379	0.112
	379	0.104
380	380	0.104
	380	0.173
381	381	0.107
	381	0.104
382	382	0.106
	382	0.109
383	383	0.113
	383	0.106
384	384	0.116
	384	0.127
385	385	0.113
	385	0.105
386	386	0.106
	386	0.109
387	387	0.110
	387	0.108
388	388	0.127
	388	0.159
389	389	0.135
	389	0.123
390	390	0.103
	390	0.107

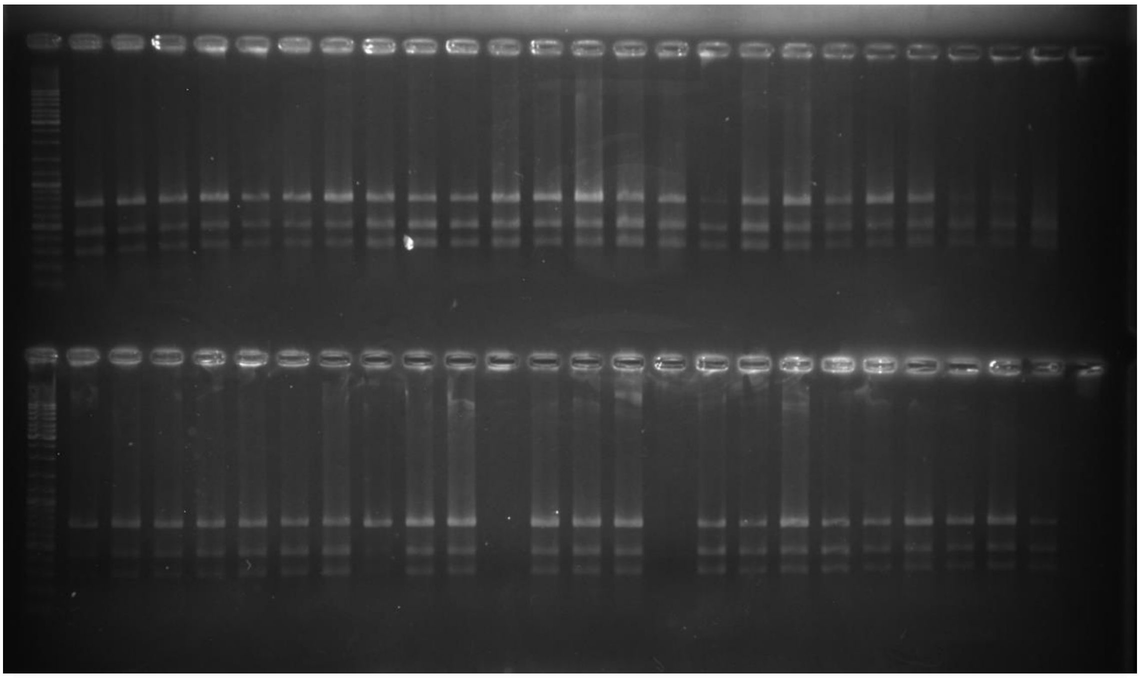
Appendix-D (Sequel) DAS-ELISA OD values, tested conducting the sensitivty of tested genotypes plants during the second year

Sample Number	ELISA Number	OD values	Sample Number	ELISA Number	OD values
391	391	0.110			
	391	0.104			
392	392	0.128			
	392	0.127			
393	393	0.139			
	393	0.127			
394	394	0.105			
	394	0.110			
395	395	0.112			
	395	0.107			
396	396	0.144			
	396	0.134			
397	397	0.122			
	397	0.120			
398	398	0.126			
	398	0.109			
399	399	0.113			
	399	0.108			
400	400	0.110			
	400	0.103			
401	401	0.129			
	401	0.110			
402	402	0.106			
	402	0.105			
403	403	0.111			
	403	0.108			
404	404	0.110			
	404	0.107			
405	405	0.108			
	405	0.108			
406	406	0.112			
	406	0.119			

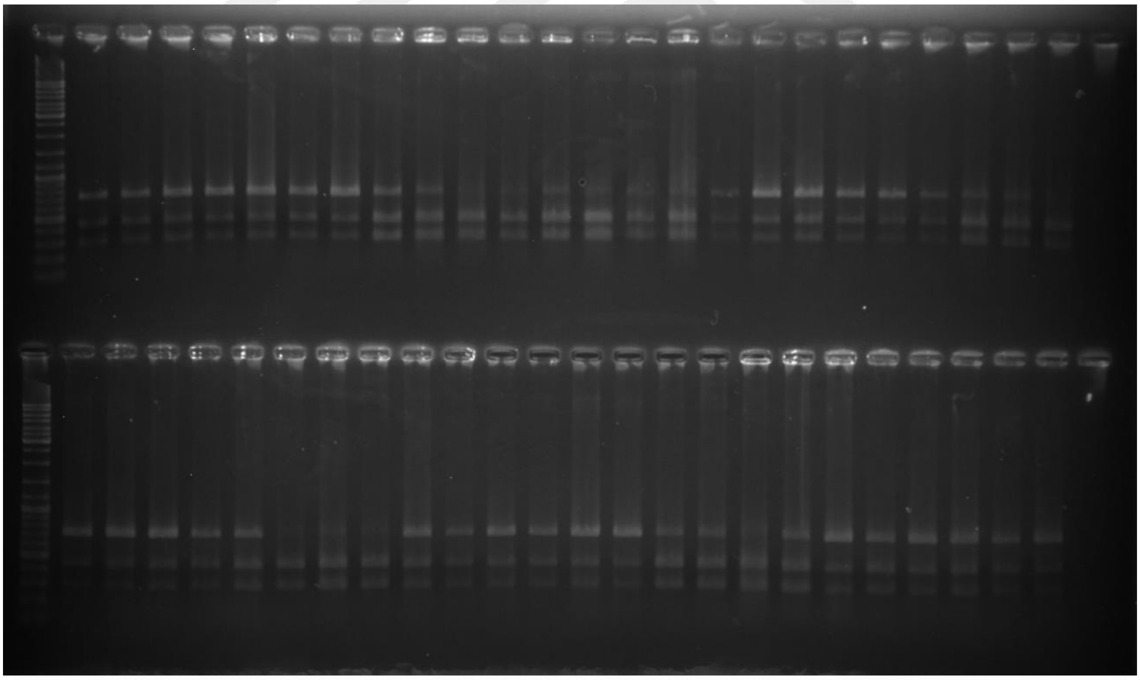
Appendix-D (Sequel) DAS-ELISA OD values, tested conducting the sensitivty of tested genotypes plants during the second year

Sample Number	**OD values**	**Sample Number**	**OD values**	**Sample Number**	**OD values**
Buffer control	0.104	439	0.110	474	0.952
	0.104				
Negative control	0.110	440	0.113	475	0.106
	0.109				
Positive control	0.108	441	0.104	476	0.101
	0.127				
407	0.104	442	0.109	477	0.107
408	0.104	443	0.108	478	0.103
409	0.108	444	0.106	479	0.115
410	0.106	445	0.107	480	0.107
411	0.105	446	0.108	481	0.104
412	0.105	447	0.119	482	0.109
413	0.104	448	0.107	407	0.104
414	0.125	449	0.106	408	0.102
415	0.104	450	0.107	409	0.113
416	0.103	451	0.101	410	0.107
417	0.110	452	0.100	411	0.109
418	0.101	453	0.101	412	0.112
419	0.103	454	0.102	20	0.109
420	0.116	455	0.113	20	0.106
421	0.105	456	0.120	21	0.105
422	0.106	457	0.104	21	0.106
423	0.111	458	0.102	22	0.106
424	0.172	459	0.103	22	0.119
425	0.110	460	0.102	23	0.112
426	0.105	461	0.104	23	0.126
427	0.105	462	0.108		
428	0.105	463	0.112		
429	out	464	0.107		
430	0.109	465	0.104		
431	0.121	466	0.103		
432	0.166	467	0.113		
433	0.110	468	0.107		
434	0.108	469	0.109		
435	0.106	470	0.105		
436	0.105	471	0.113		
437	0.107	472	0.114		
438	0.110	473	0.999		

Appendix-E Marker assisted analysis of cross progenies using $GP122_{718}$

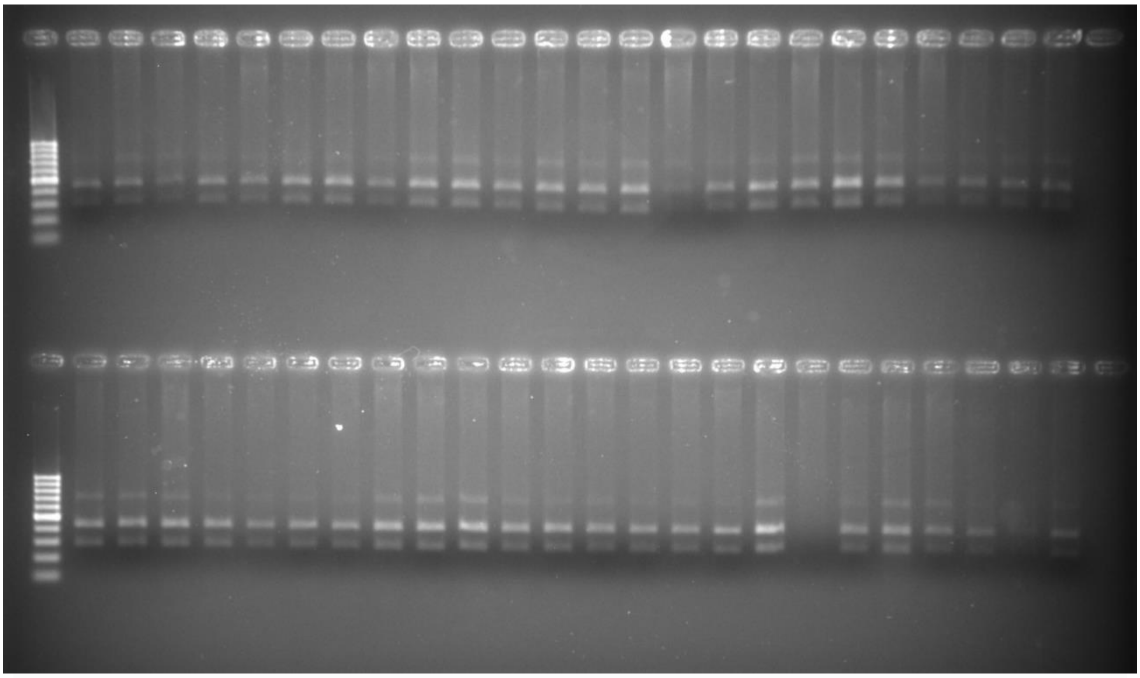


*Each image shows 48 samples belonging to the progenies of tested crosses analysed using $GP122_{718}$

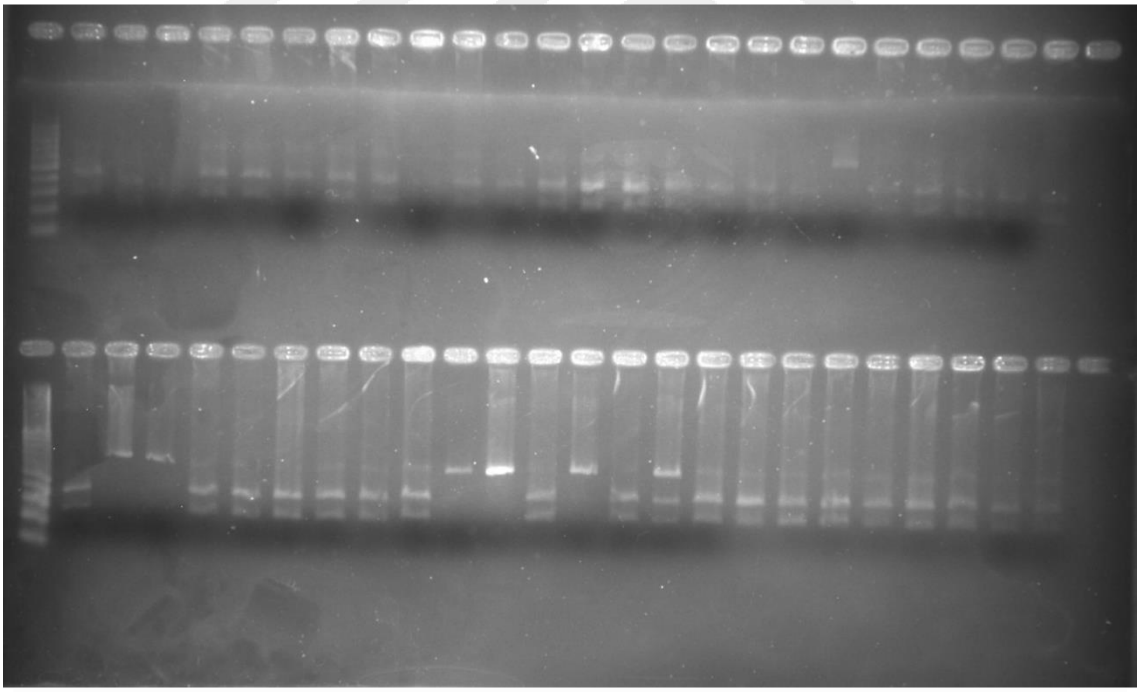


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using $GP122_{718}$

Appendix-E Marker assisted analysis of cross progenies using $GP122_{718}$

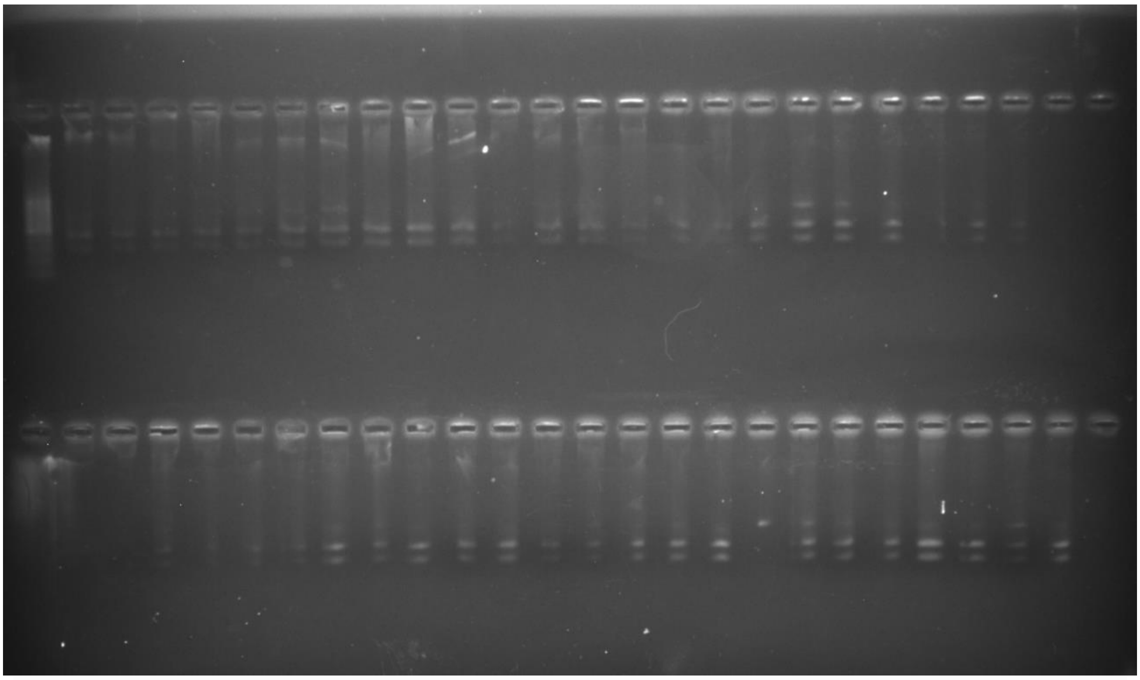


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using $GP122_{718}$

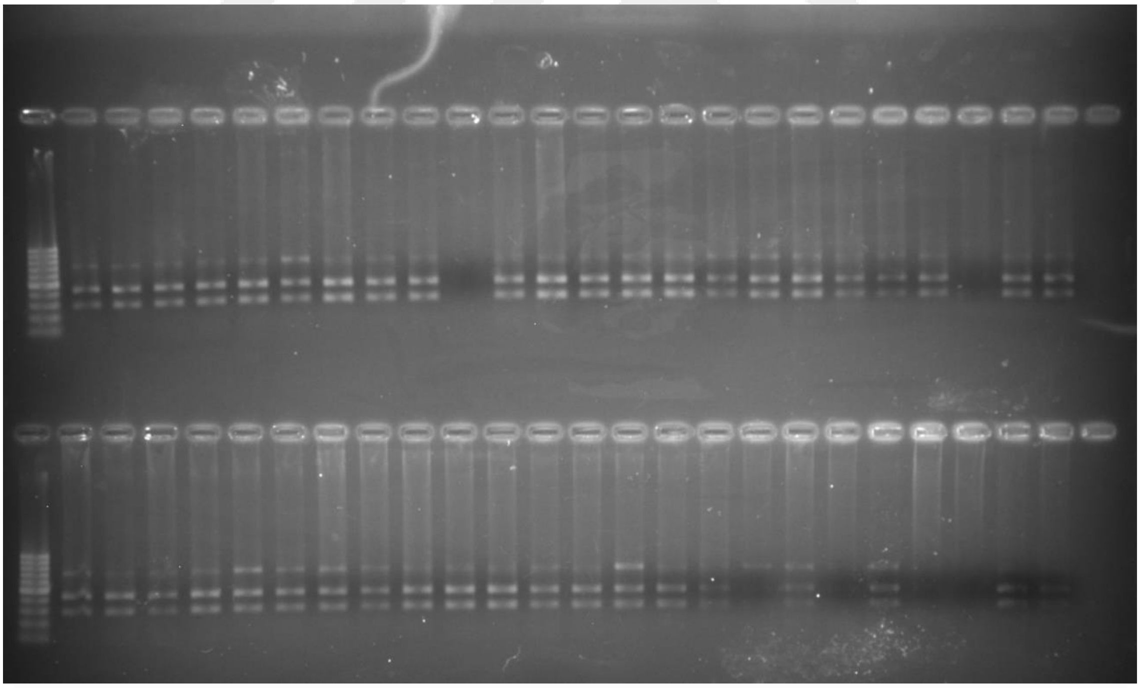


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using $GP122_{718}$

Appendix-E Marker assisted analysis of cross progenies using $GP122_{718}$

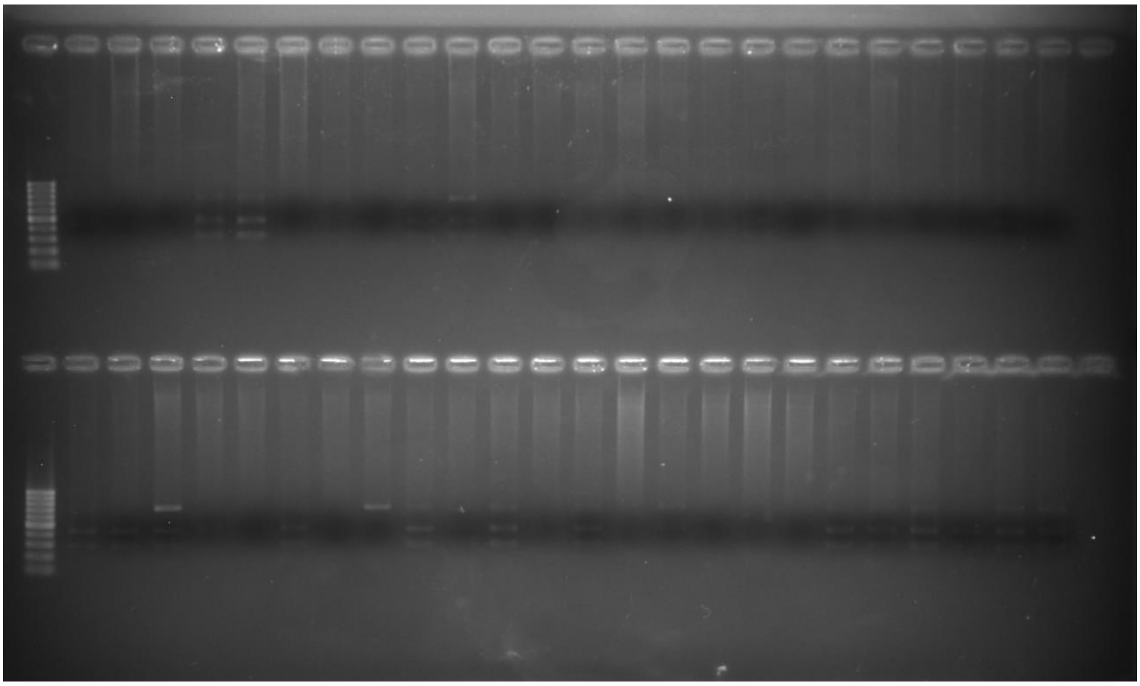


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using $GP122_{718}$

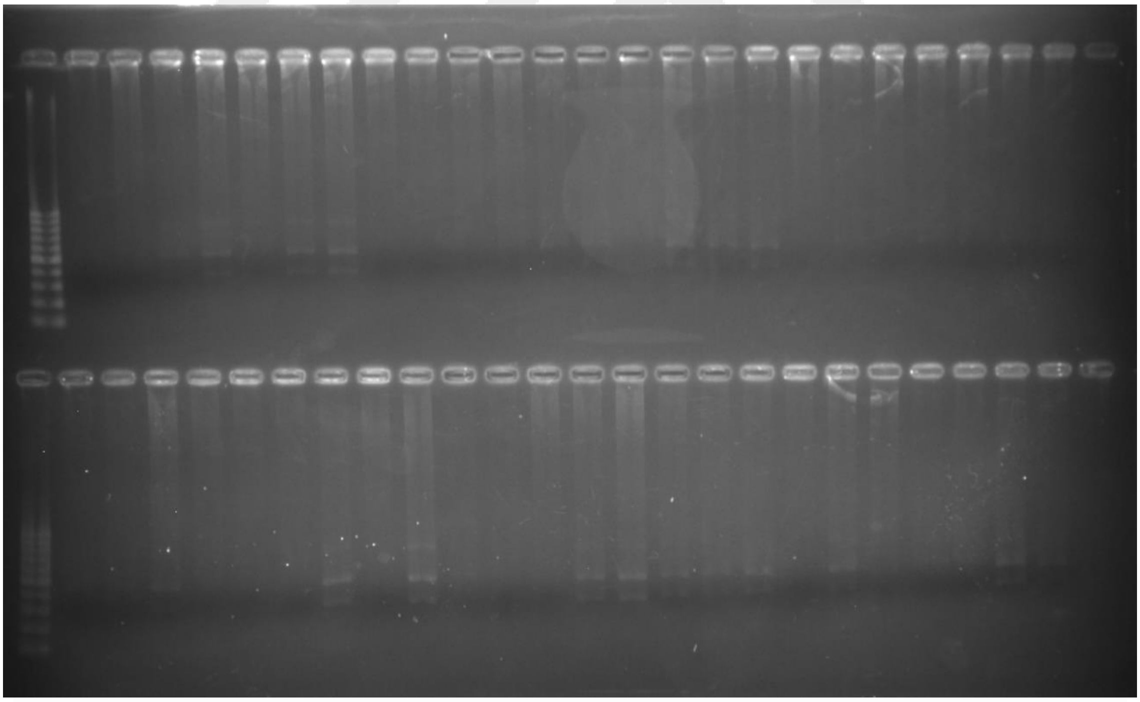


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using $GP122_{718}$

Appendix-E Marker assisted analysis of cross progenies using $GP122_{718}$

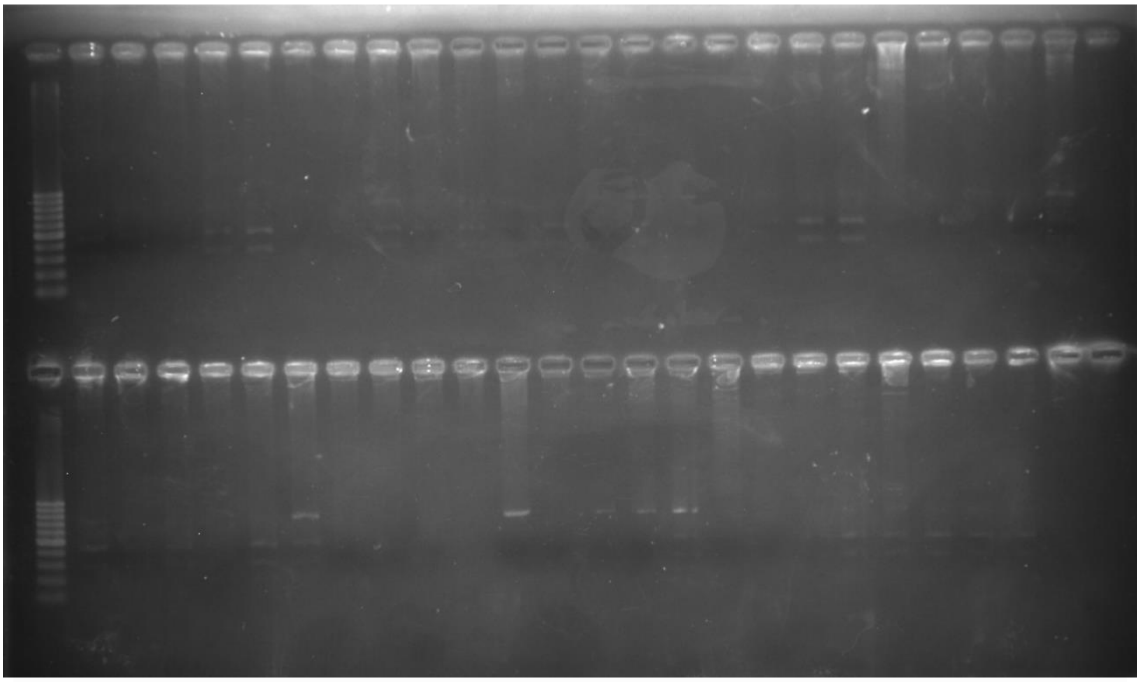


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using $GP122_{718}$

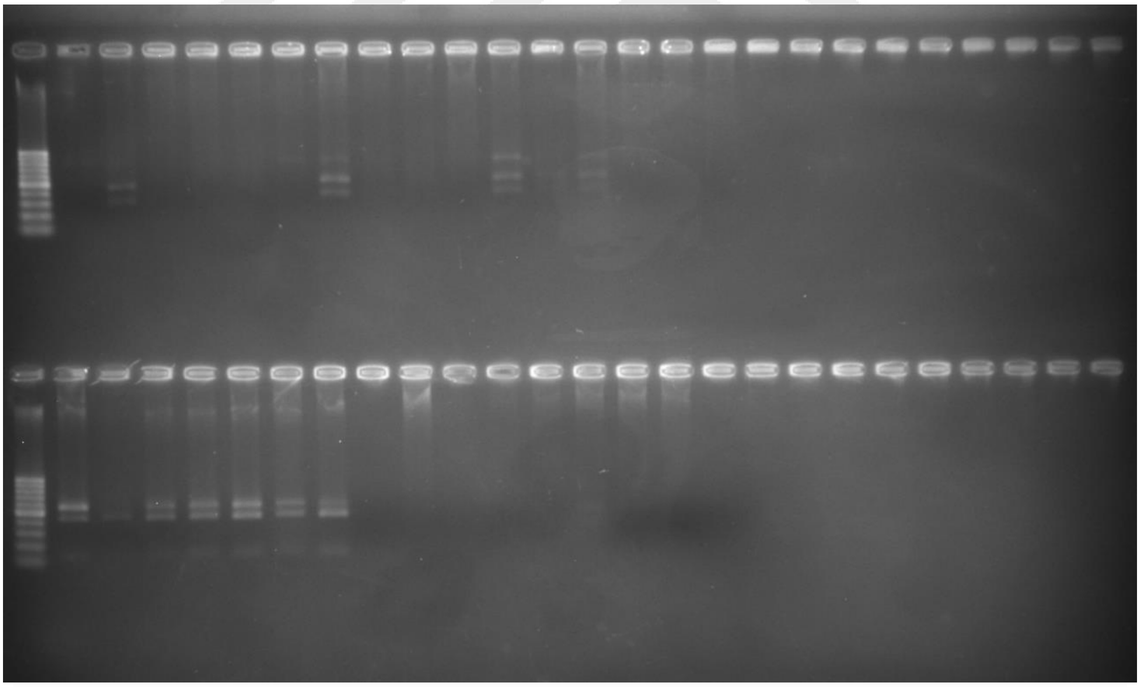


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using $GP122_{718}$

Appendix-E Marker assisted analysis of cross progenies using $GP122_{718}$

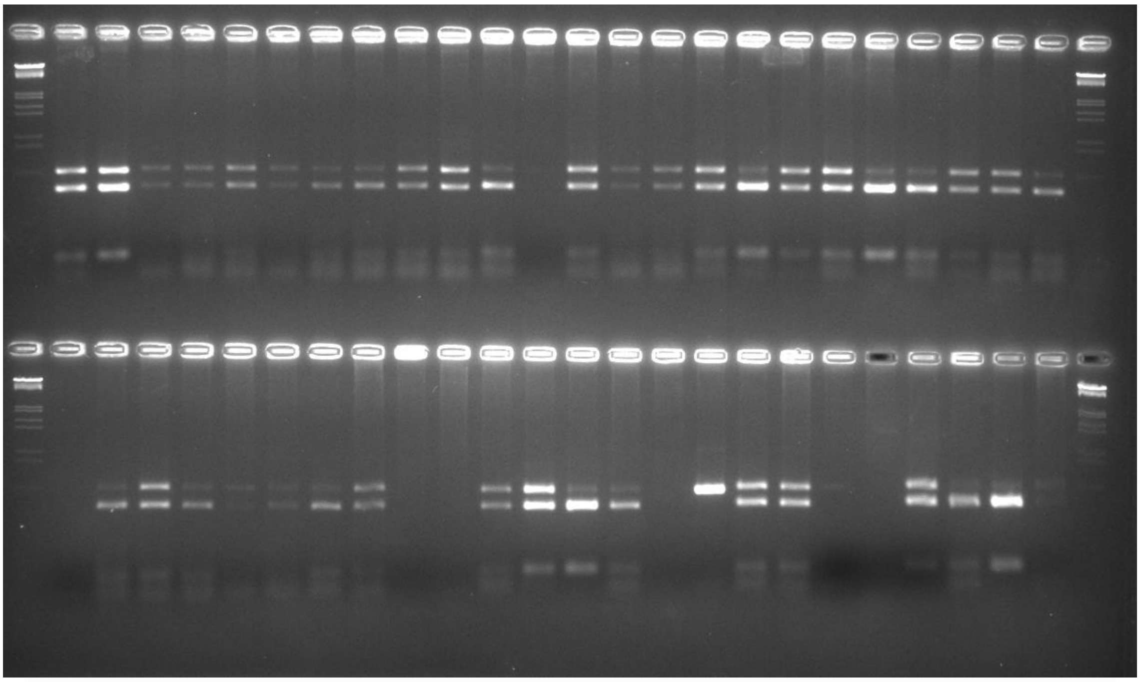


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using $GP122_{718}$

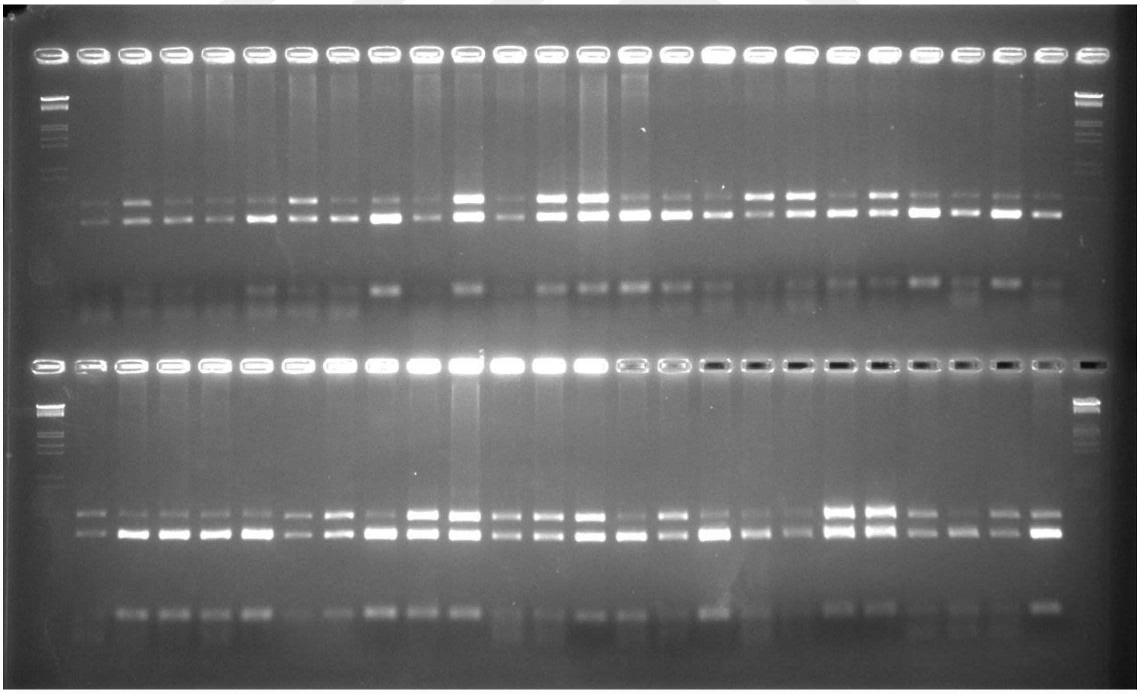


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using $GP122_{718}$

Appendix-F Marker assisted analysis of cross progenies using $GP122_{564}$

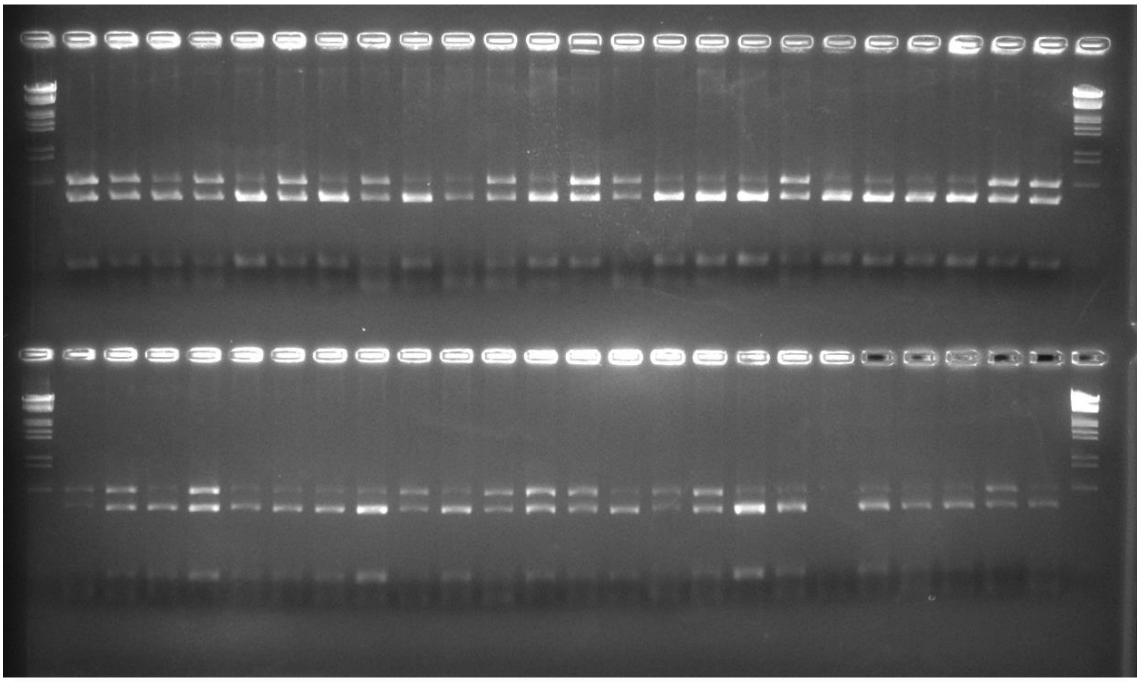


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using $GP122_{564}$

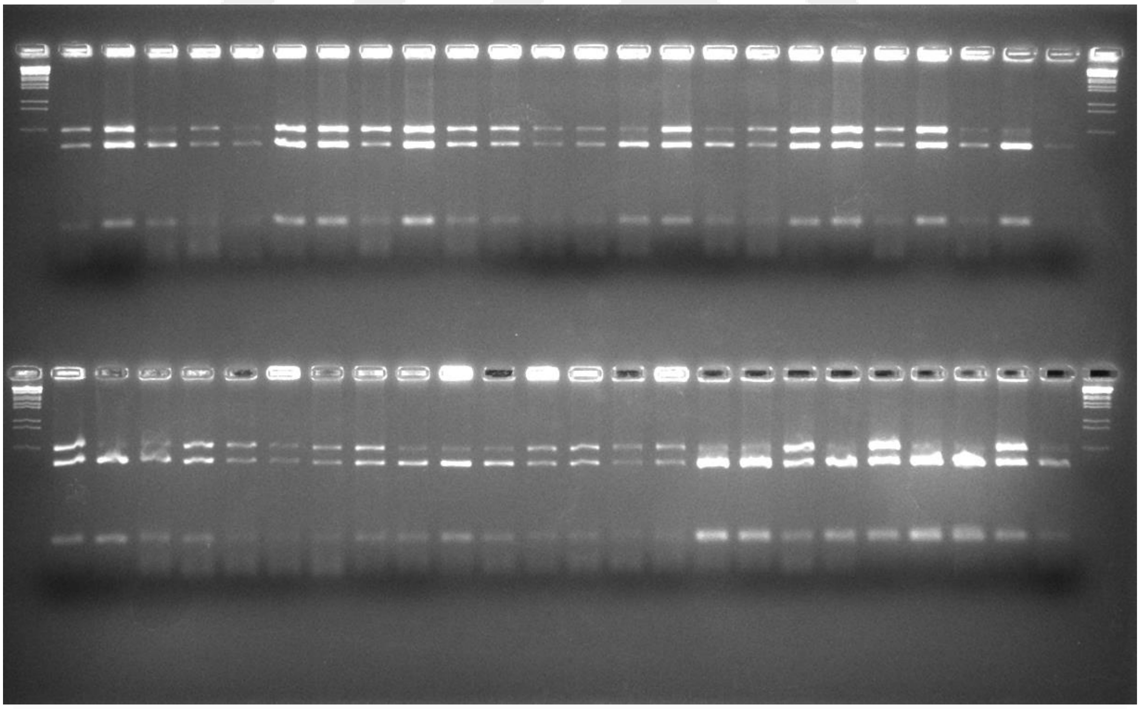


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using $GP122_{564}$

Appendix-F Marker assisted analysis of cross progenies using GP122$_{564}$

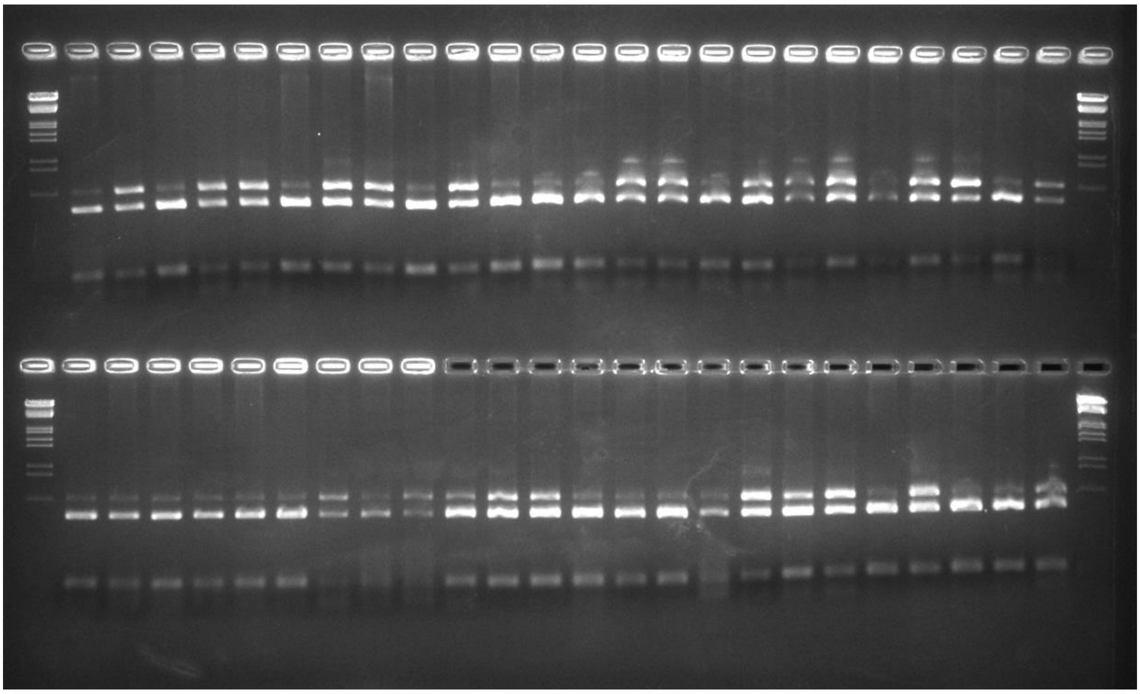


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using GP122$_{564}$

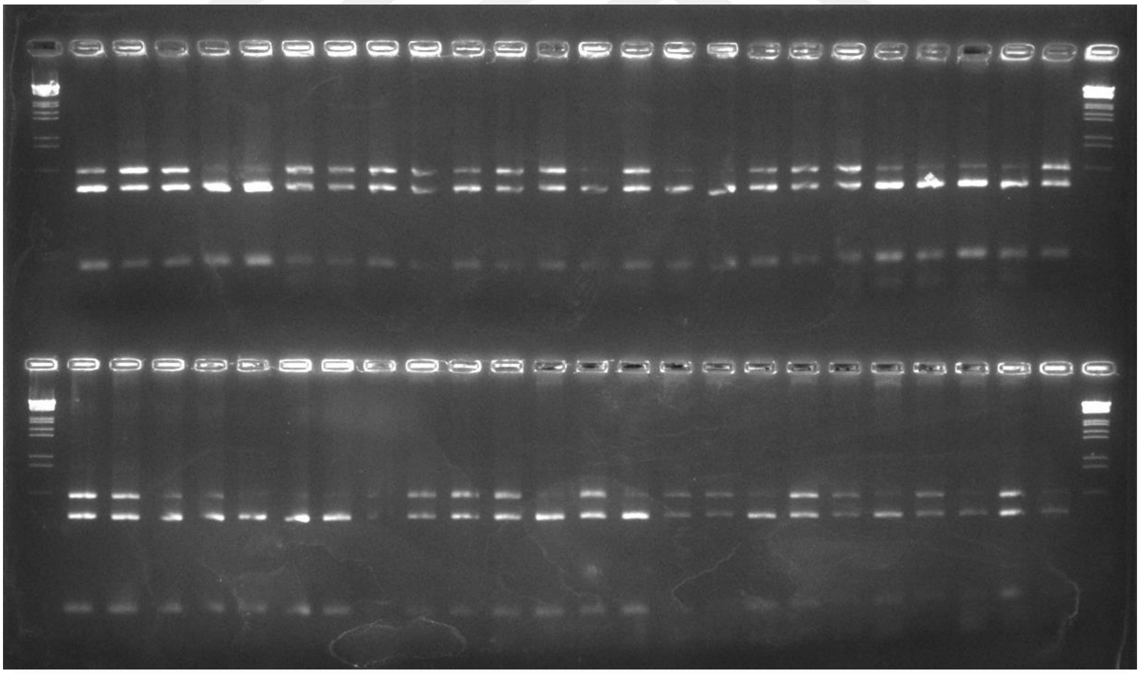


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using GP122$_{564}$

Appendix-F Marker assisted analysis of cross progenies using $GP122_{564}$

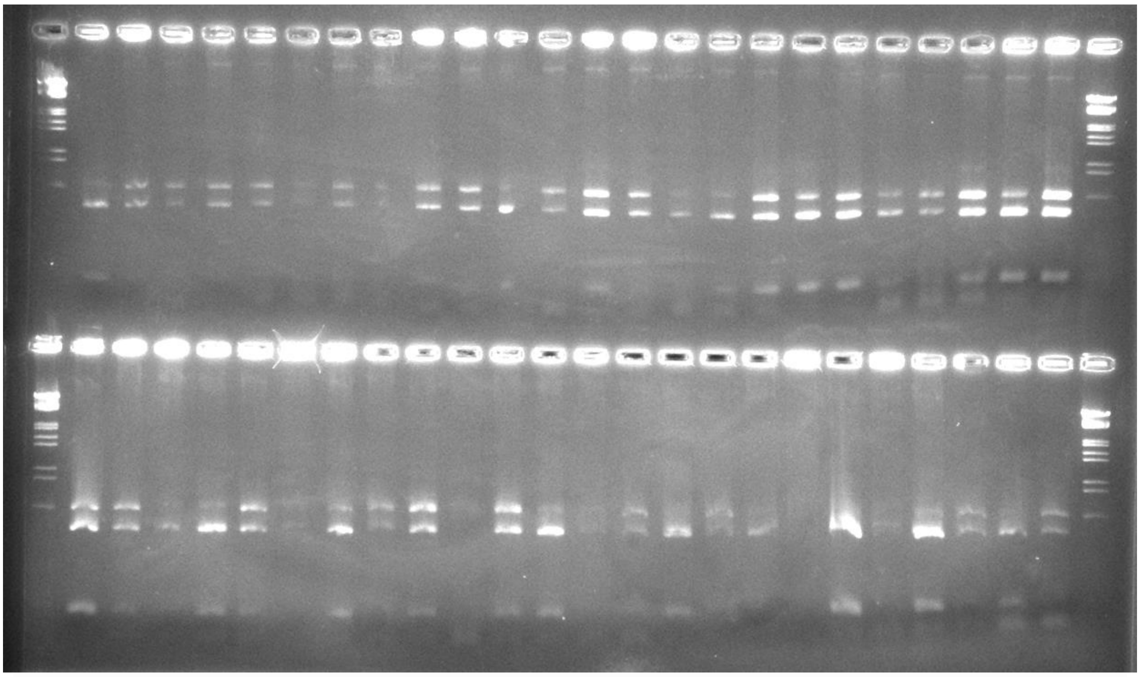


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using $GP122_{564}$

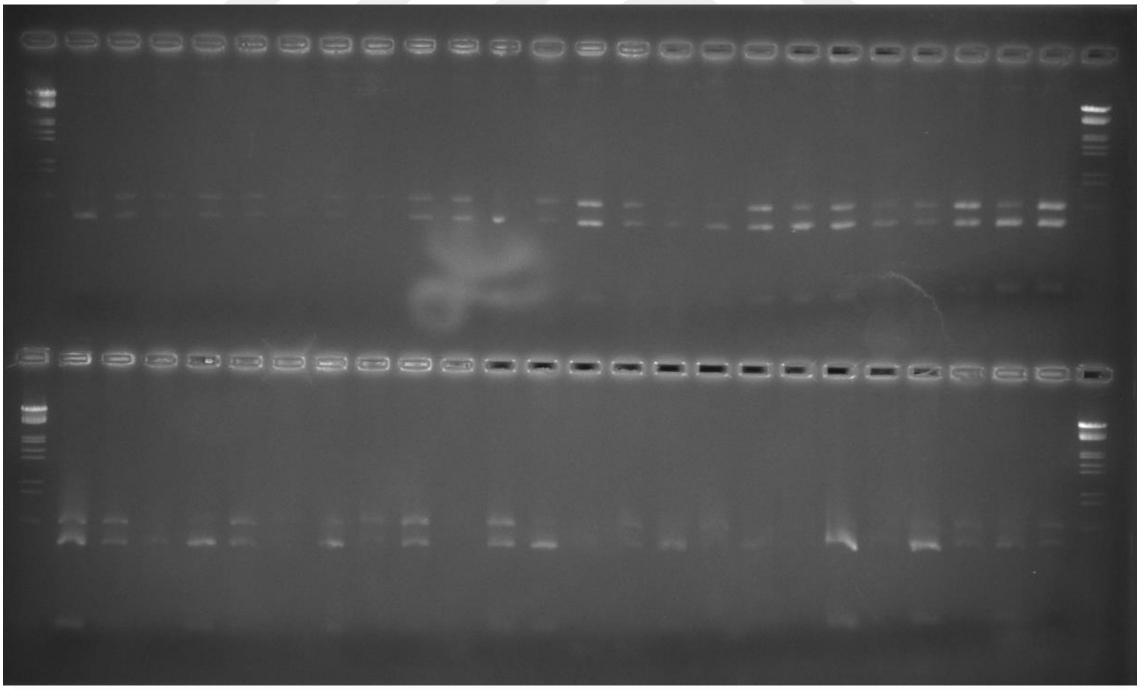


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using $GP122_{564}$

Appendix-F Marker assisted analysis of cross progenies using $GP122_{564}$

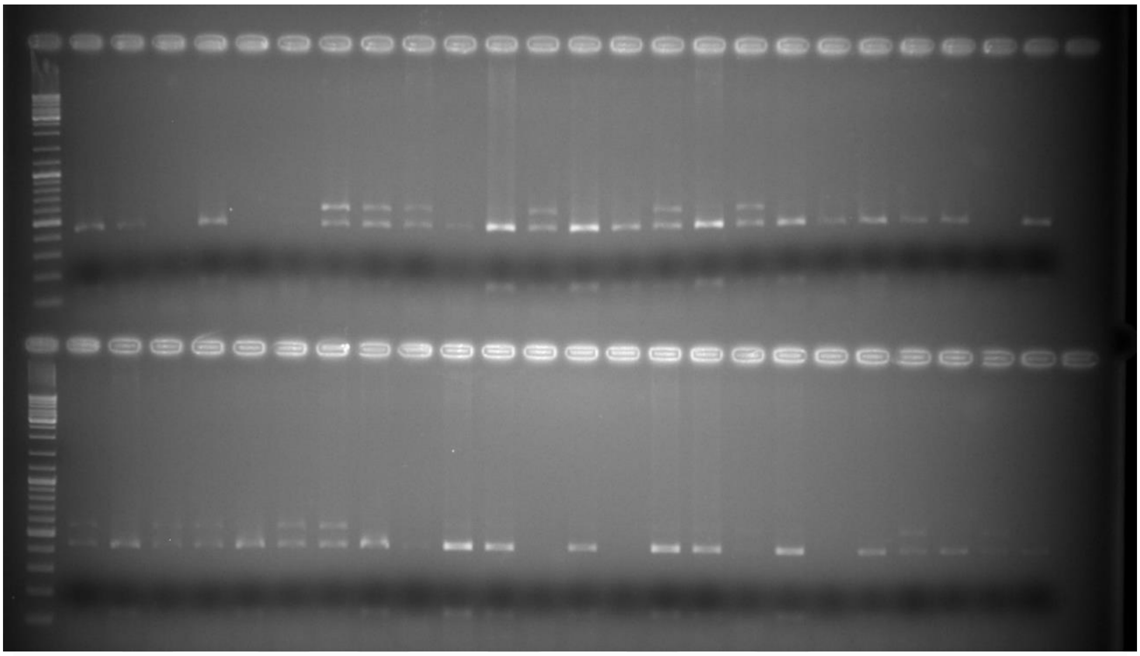


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using $GP122_{564}$

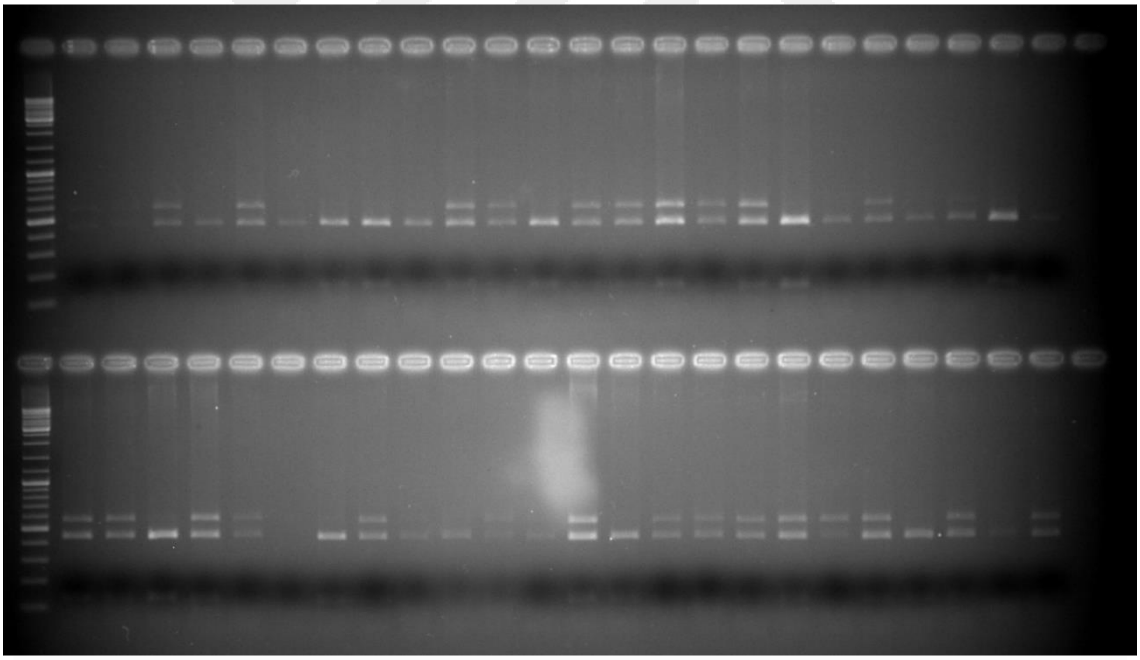


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using $GP122_{564}$

Appendix-F Marker assisted analysis of cross progenies using $GP122_{564}$

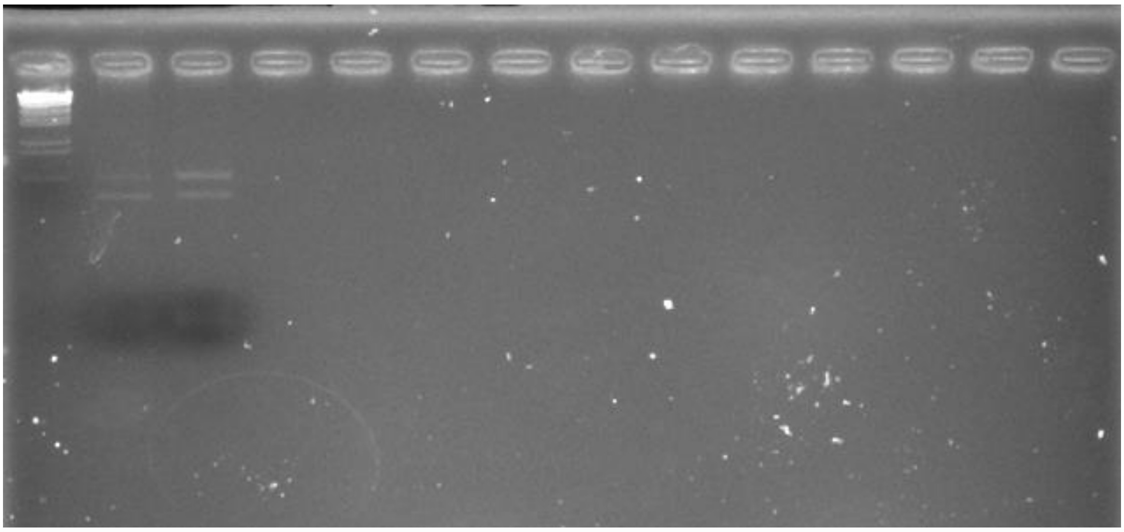


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using $GP122_{564}$



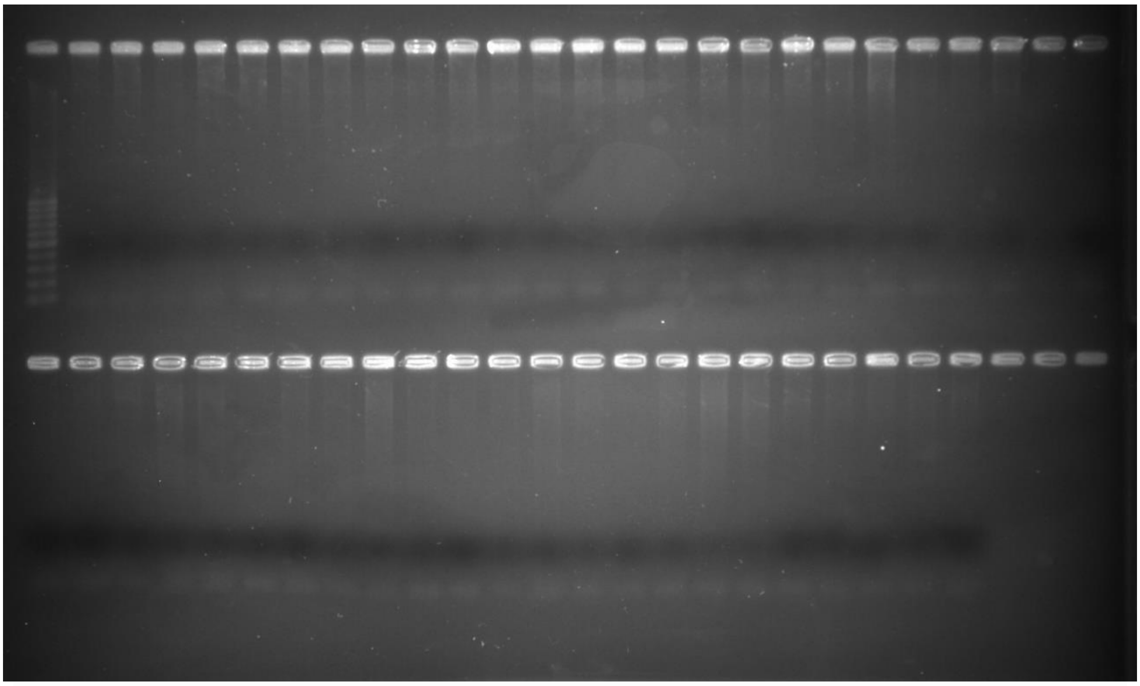
*Each image showing 48 samples belongs to the progenies of tested crosses analysed using $GP122_{564}$

Appendix-F Marker assisted analysis of cross progenies using $GP122_{564}$

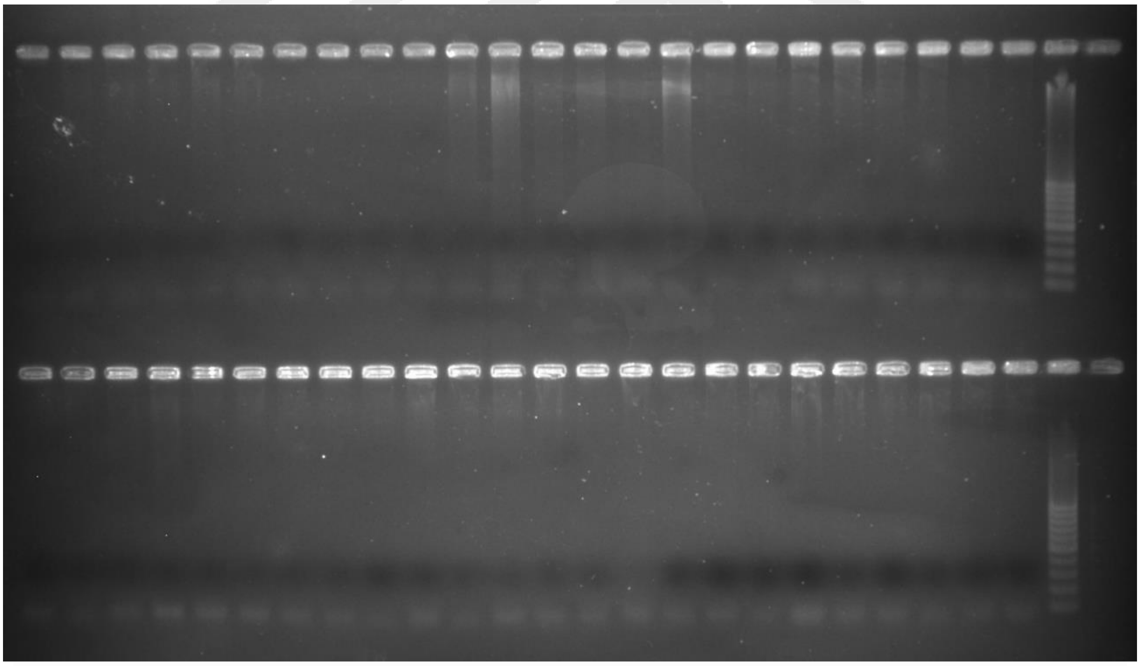


*Each image showing samples belongs to the progenies of tested crosses analysed using $GP122_{564}$

Appendix-G Marker assisted analysis of cross progenies using RysC

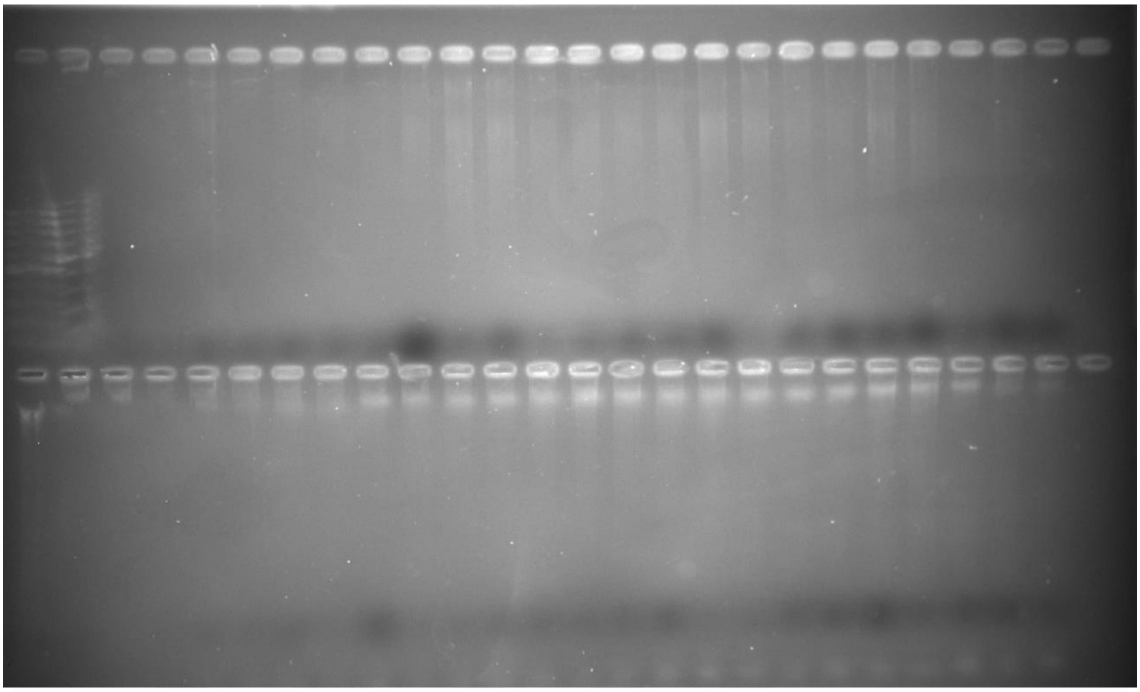


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using RysC

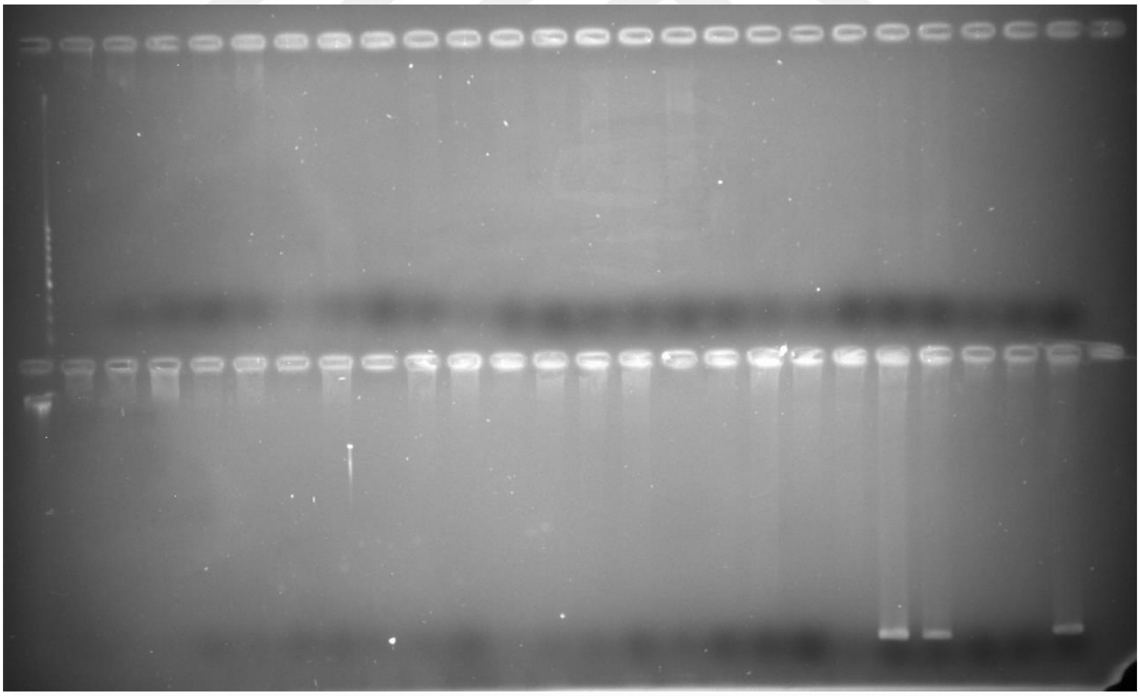


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using RysC

Appendix-G Marker assisted analysis of cross progenies using RysC

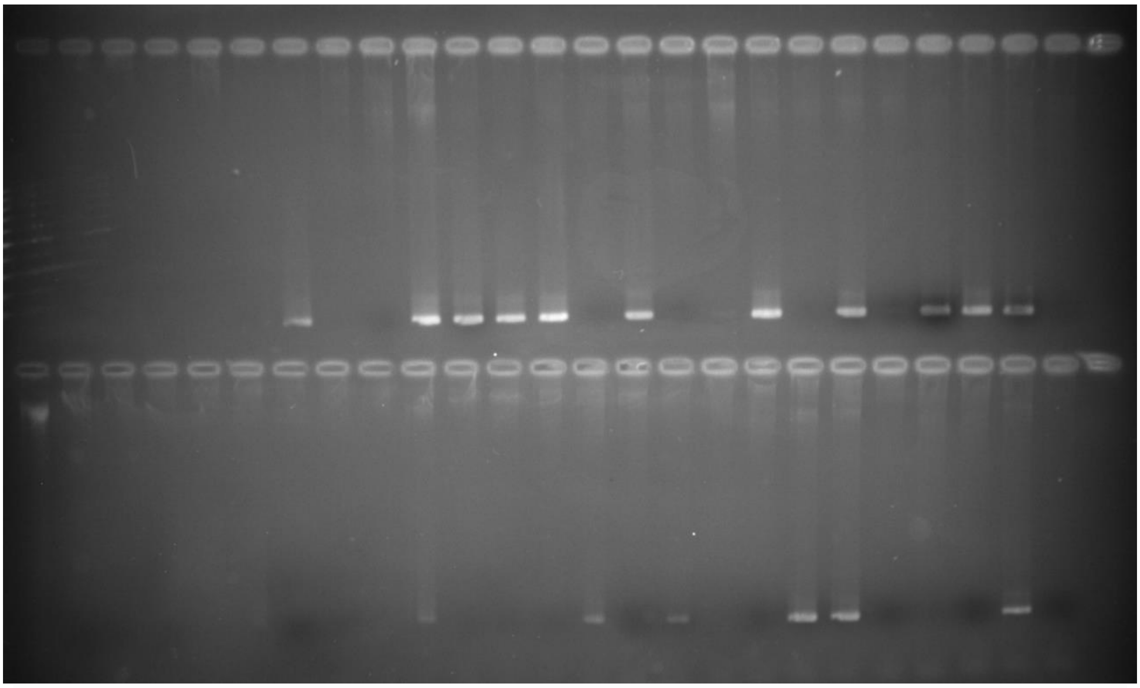


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using RysC

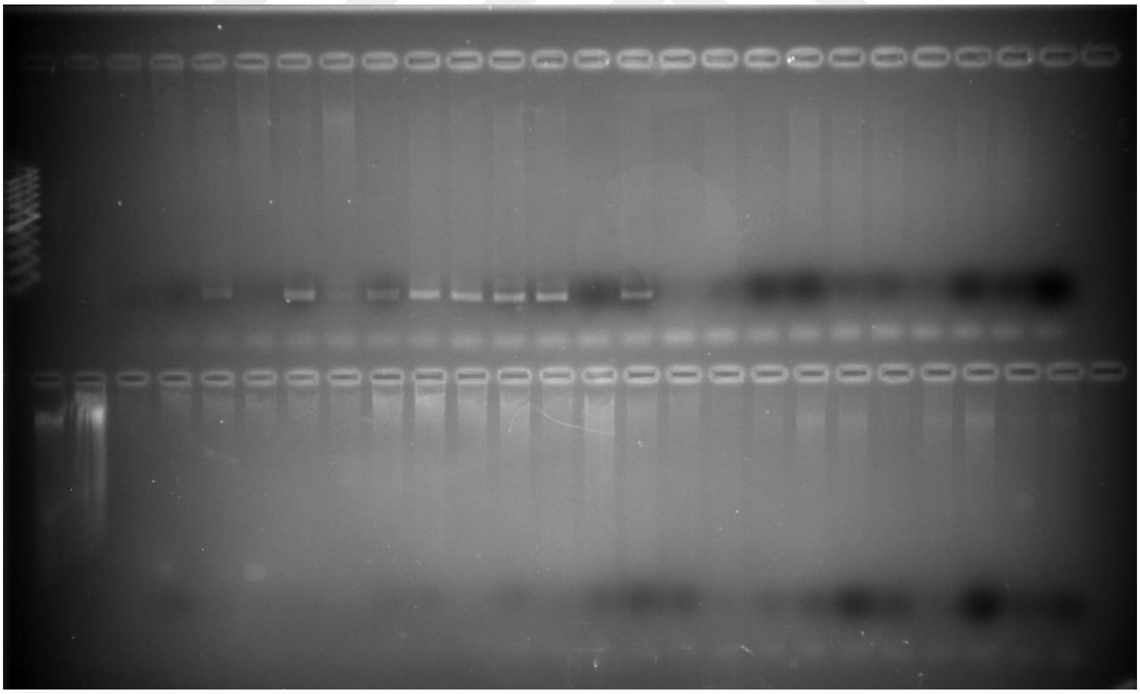


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using RysC

Appendix-G Marker assisted analysis of cross progenies using RysC

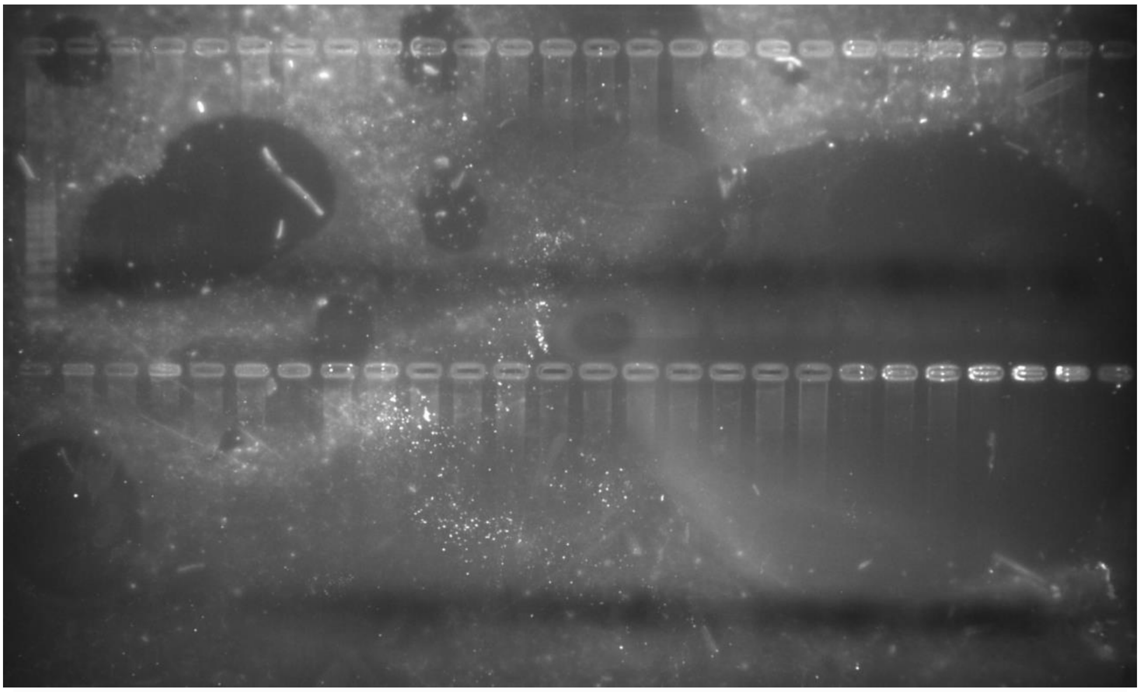


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using RysC

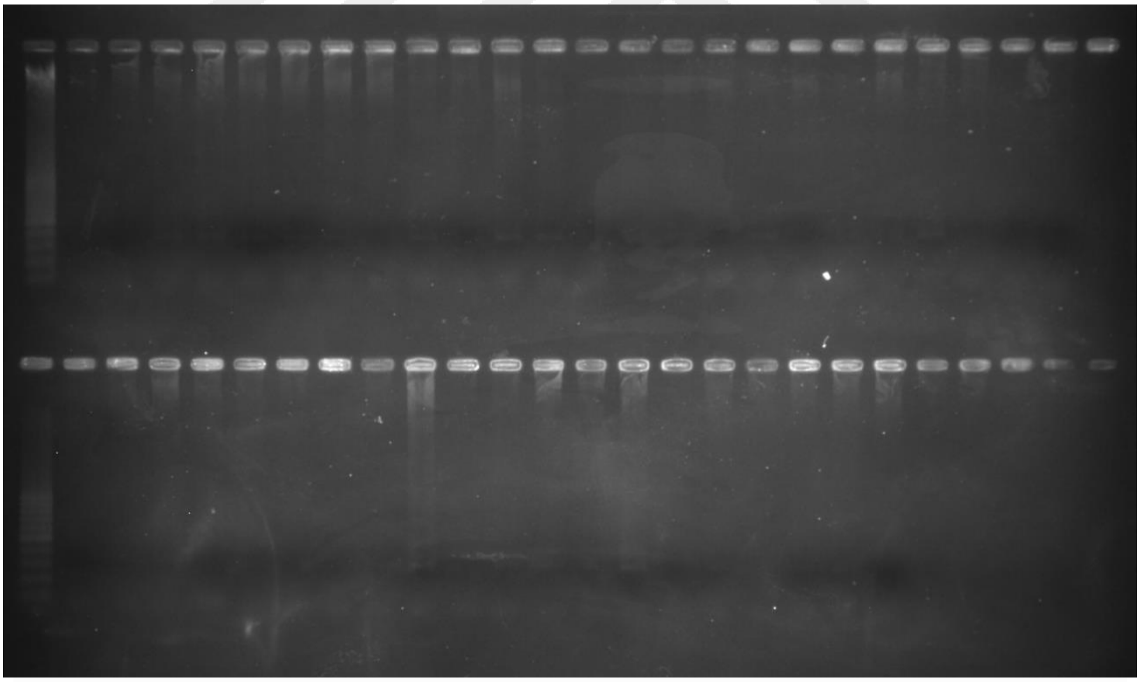


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using RysC

Appendix-G Marker assisted analysis of cross progenies using RysC

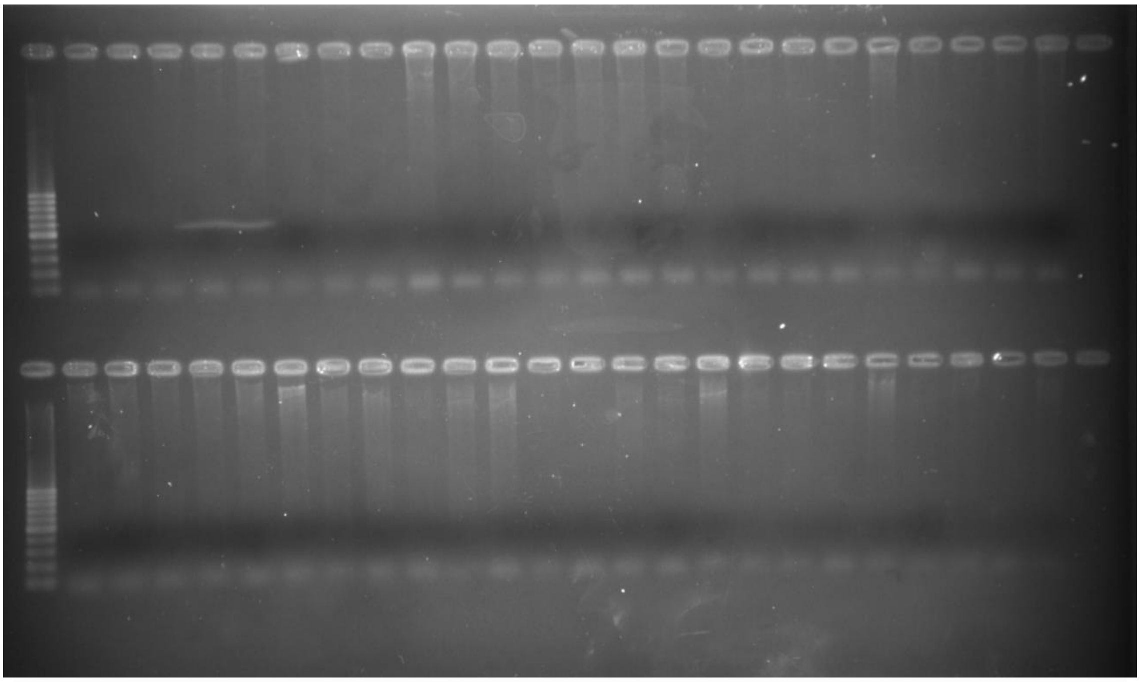


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using RysC

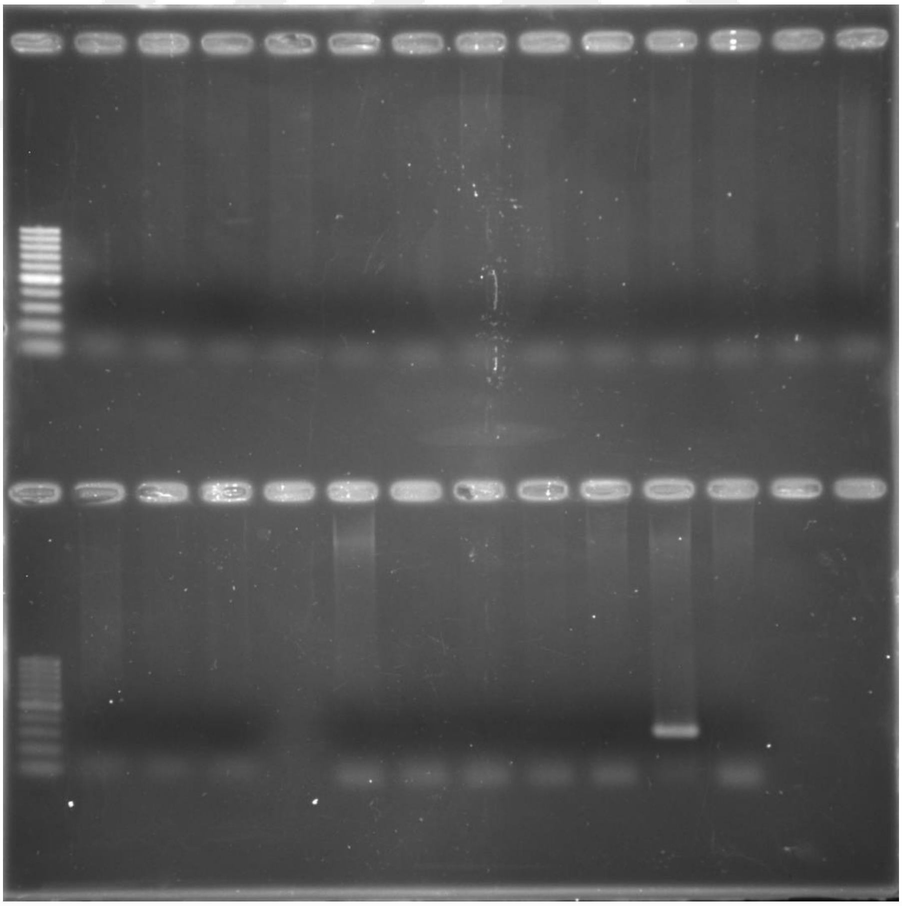


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using RysC

Appendix-G Marker assisted analysis of cross progenies using RysC

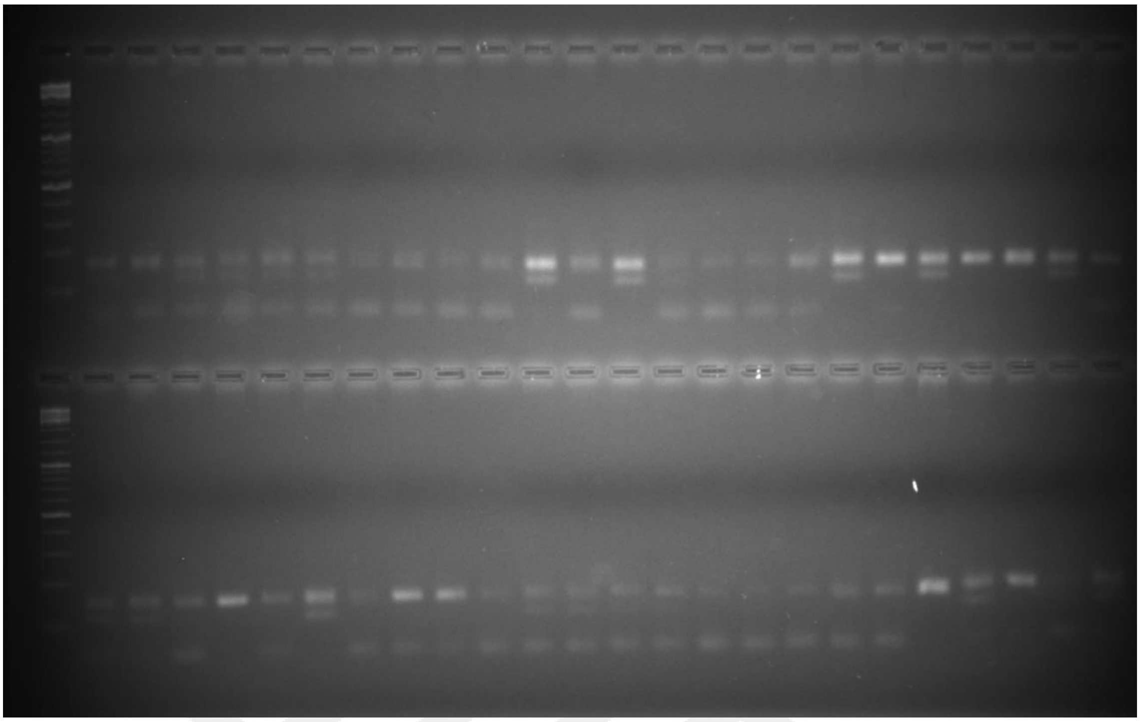


*Each image showing 48 samples belongs to the progenies of tested crosses analysed using RysC

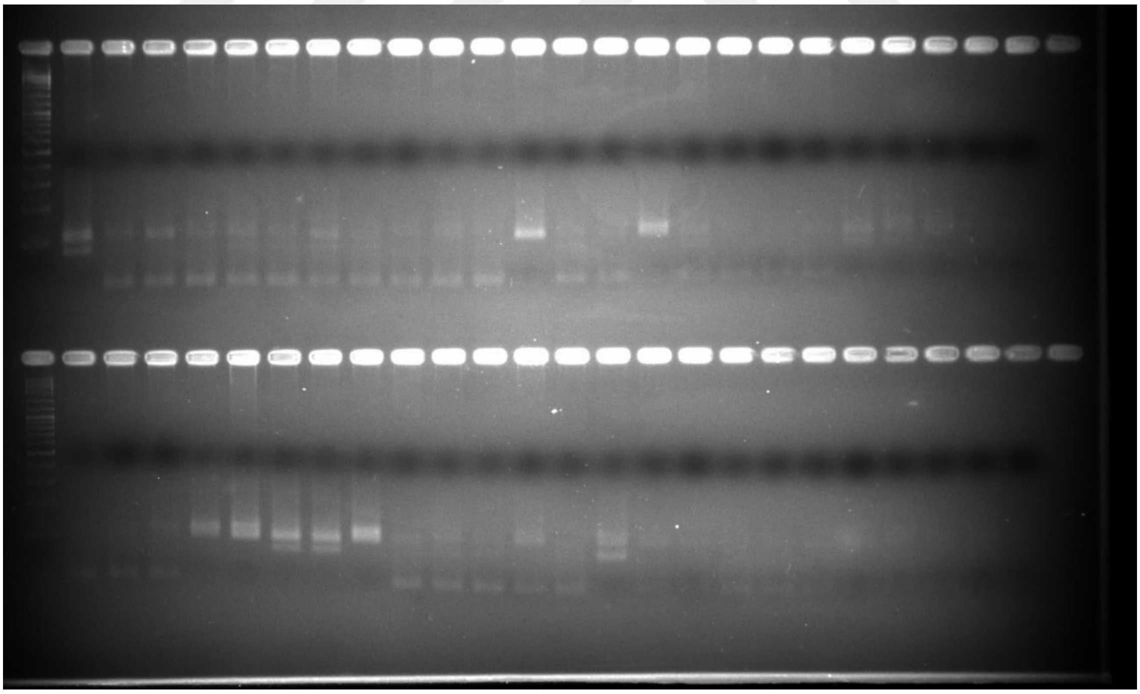


*Each image showing samples belongs to the progenies of tested crosses analysed using RysC

Appendix-H Marker assisted analysis of cross progenies using STM0003

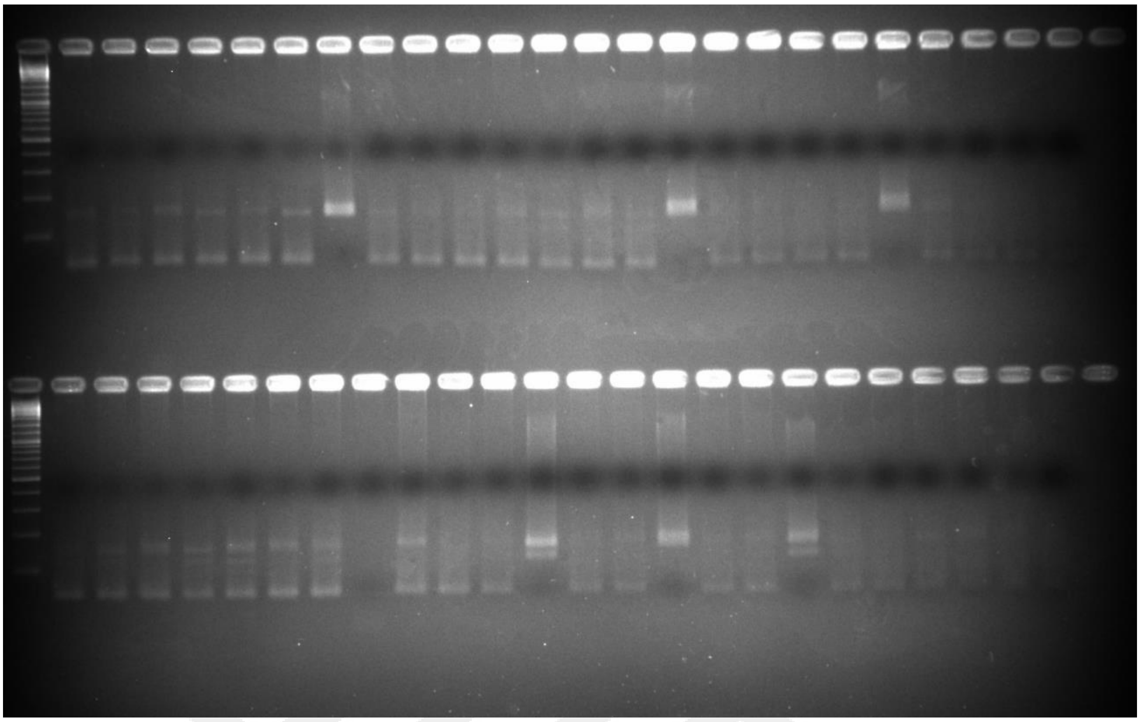


*Each image showing samples belongs to the progenies of tested crosses analysed using STM0003

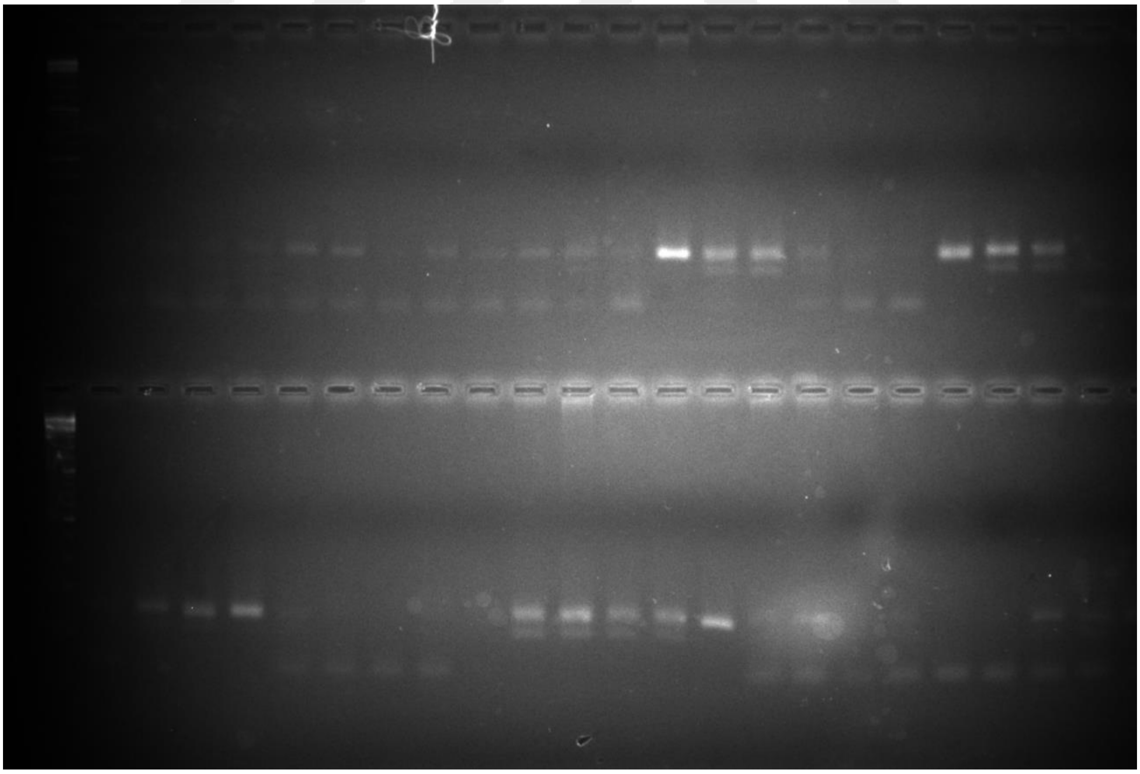


*Each image showing samples belongs to the progenies of tested crosses analysed using STM0003

Appendix-H Marker assisted analysis of cross progenies using STM0003

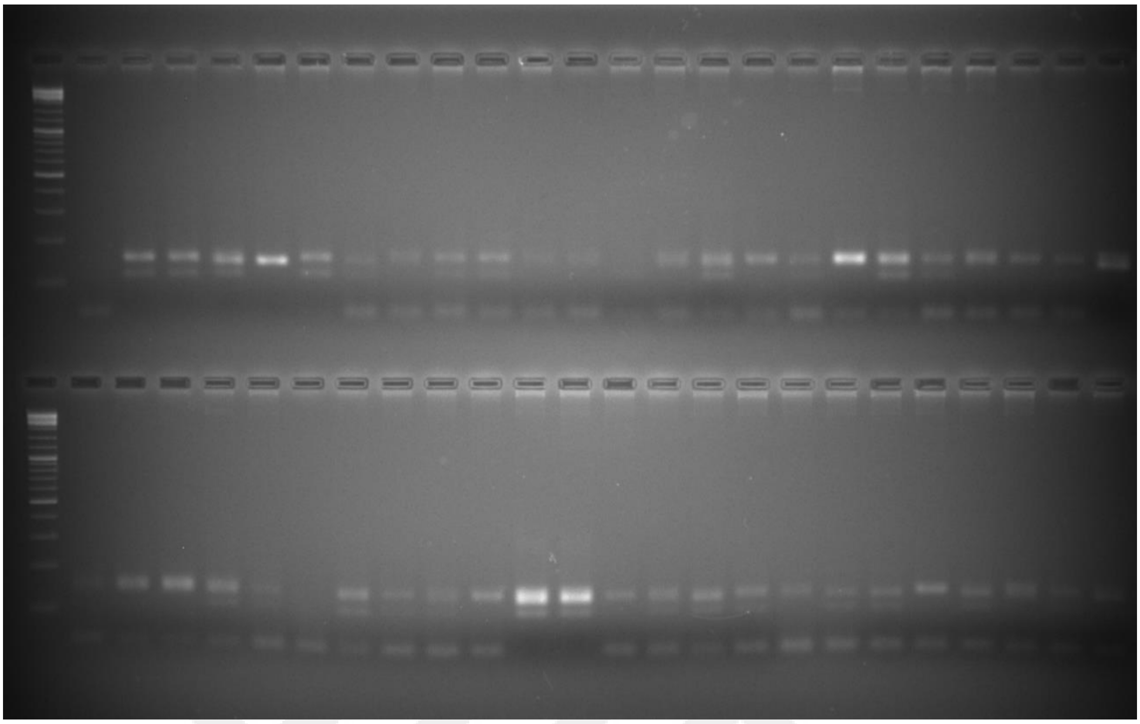


*Each image showing samples belongs to the progenies of tested crosses analysed using STM0003

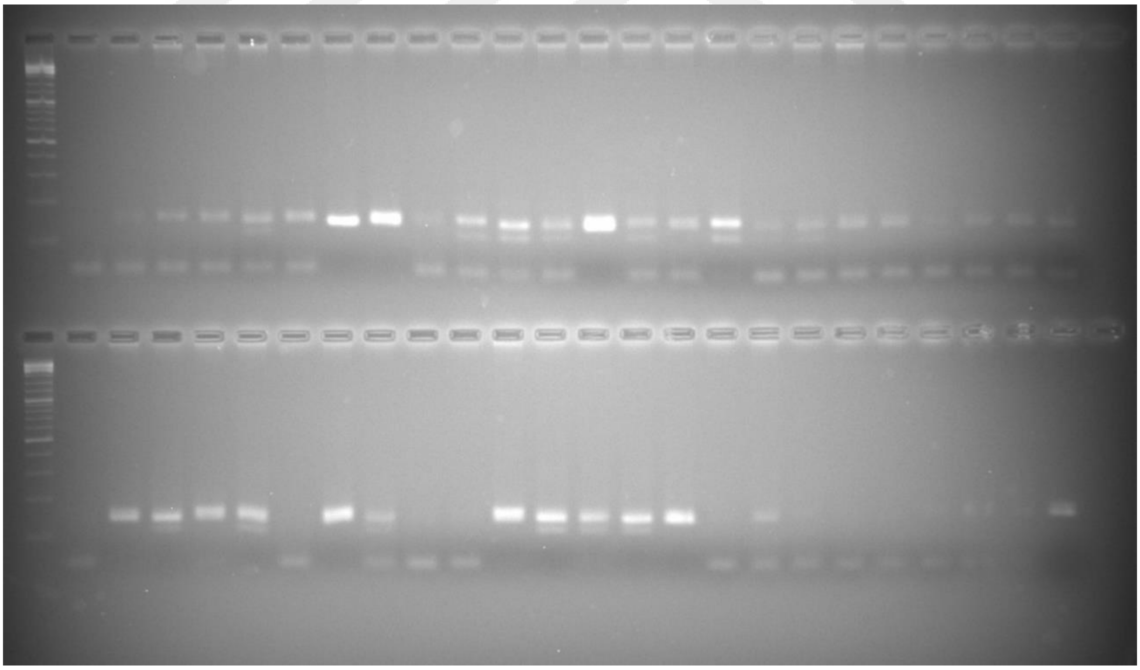


*Each image showing samples belongs to the progenies of tested crosses analysed using STM0003

Appendix-H Marker assisted analysis of cross progenies using STM0003

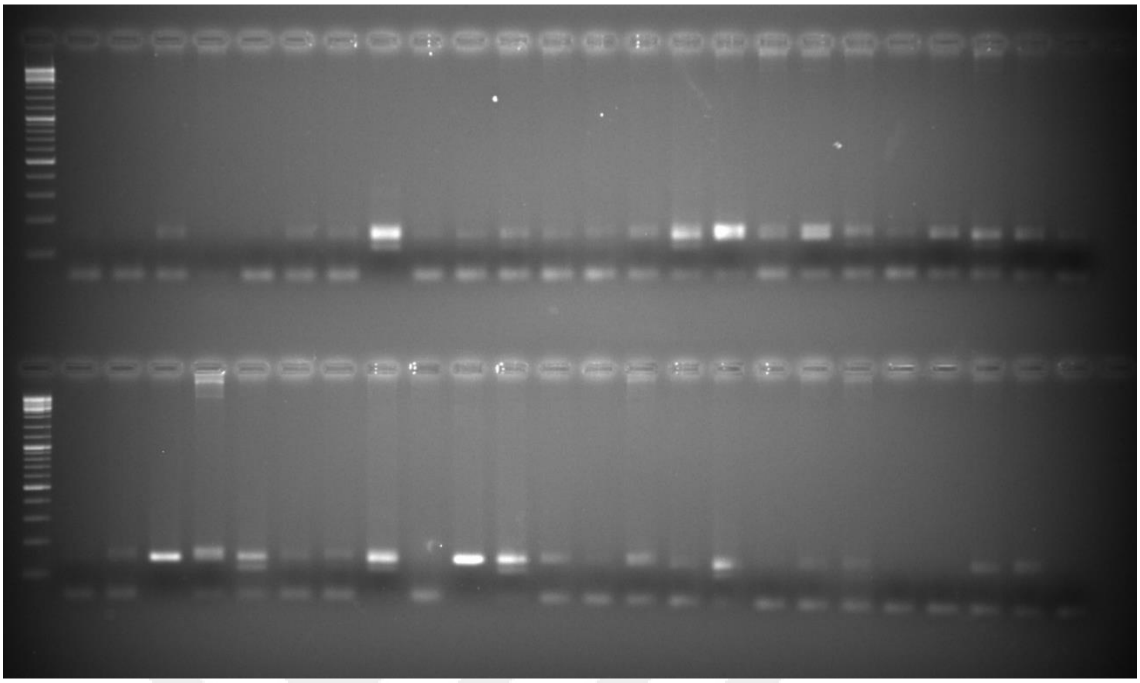


*Each image showing samples belongs to the progenies of tested crosses analysed using STM0003

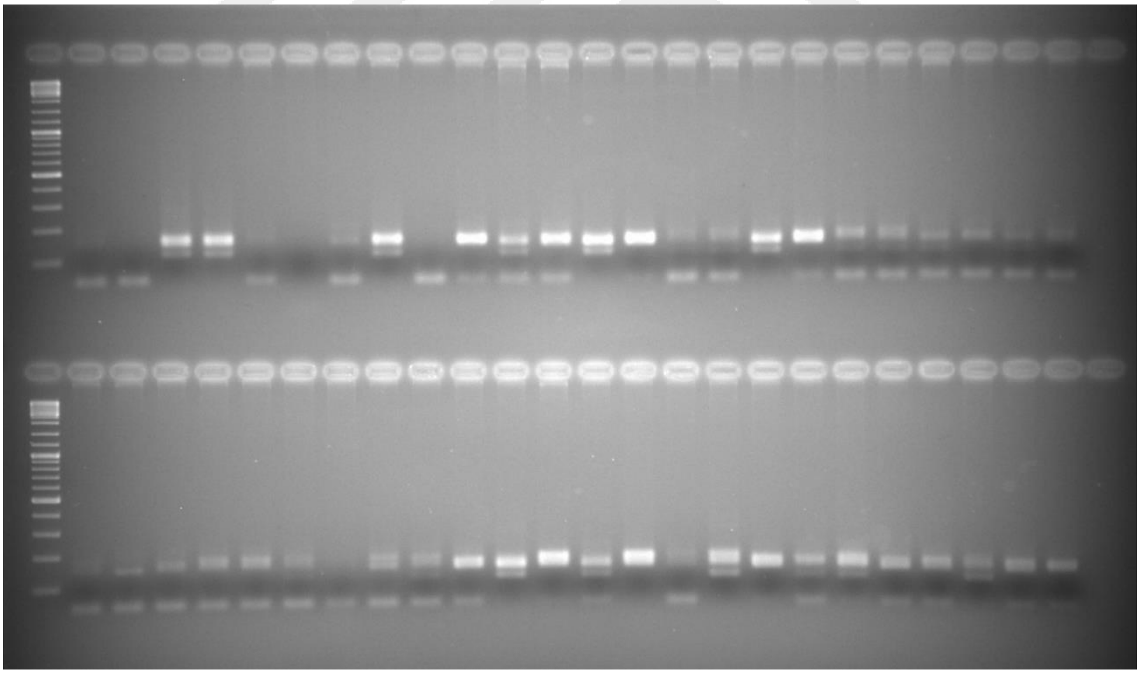


*Each image showing samples belongs to the progenies of tested crosses analysed using STM0003

Appendix-H Marker assisted analysis of cross progenies using STM0003

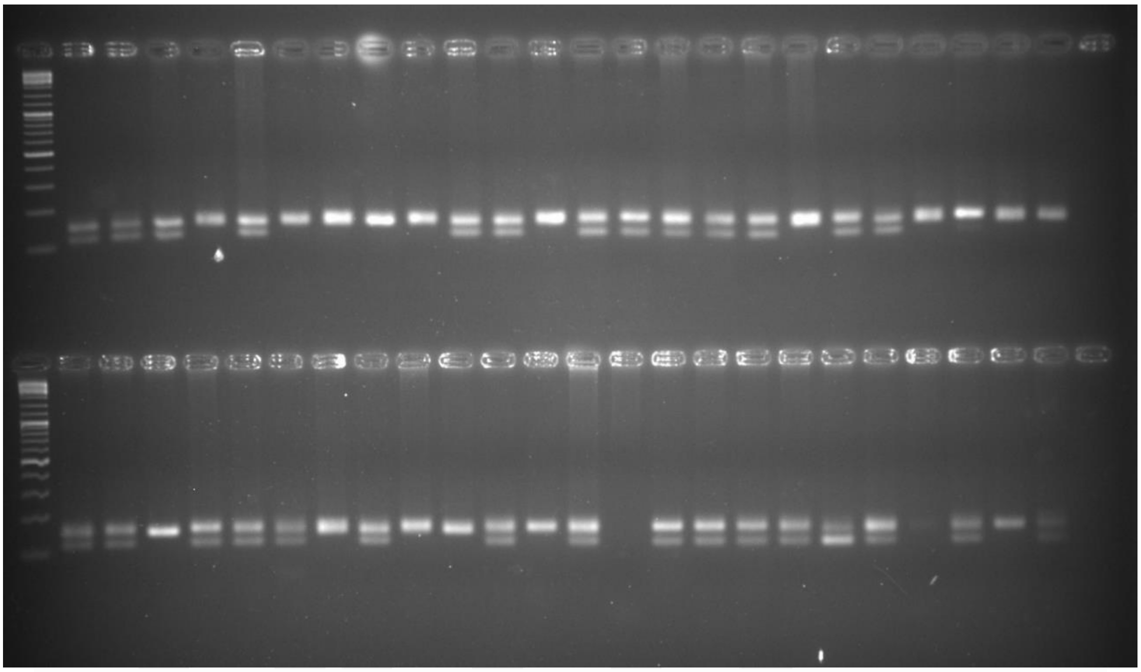


*Each image showing samples belongs to the progenies of tested crosses analysed using STM0003

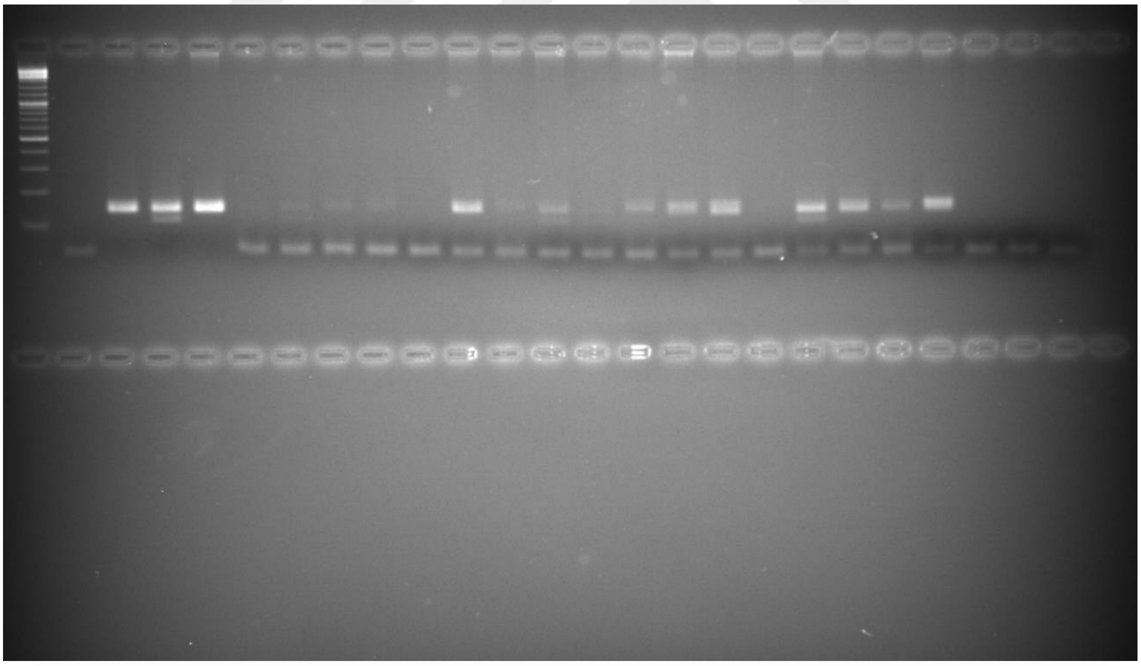


*Each image showing samples belongs to the progenies of tested crosses analysed using STM0003

Appendix-H Marker assisted analysis of cross progenies using STM0003

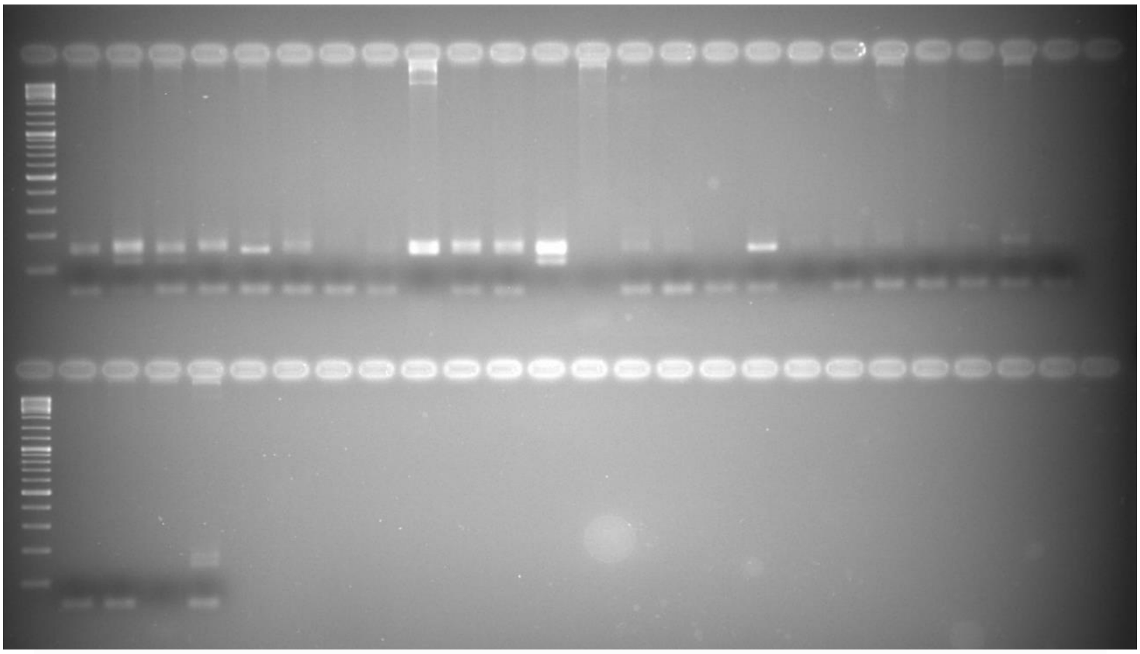


*Each image showing samples belongs to the progenies of tested crosses analysed using STM0003

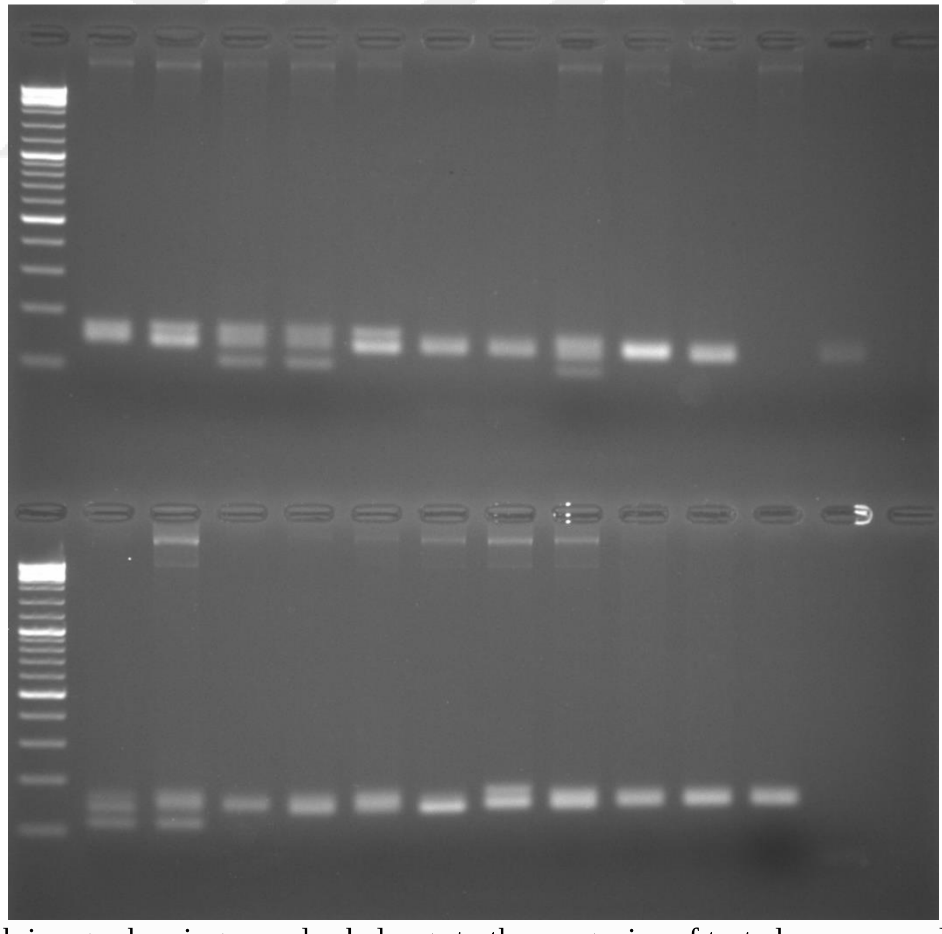


*Each image showing samples belongs to the progenies of tested crosses analysed using STM0003

Appendix-H Marker assisted analysis of cross progenies using STM0003

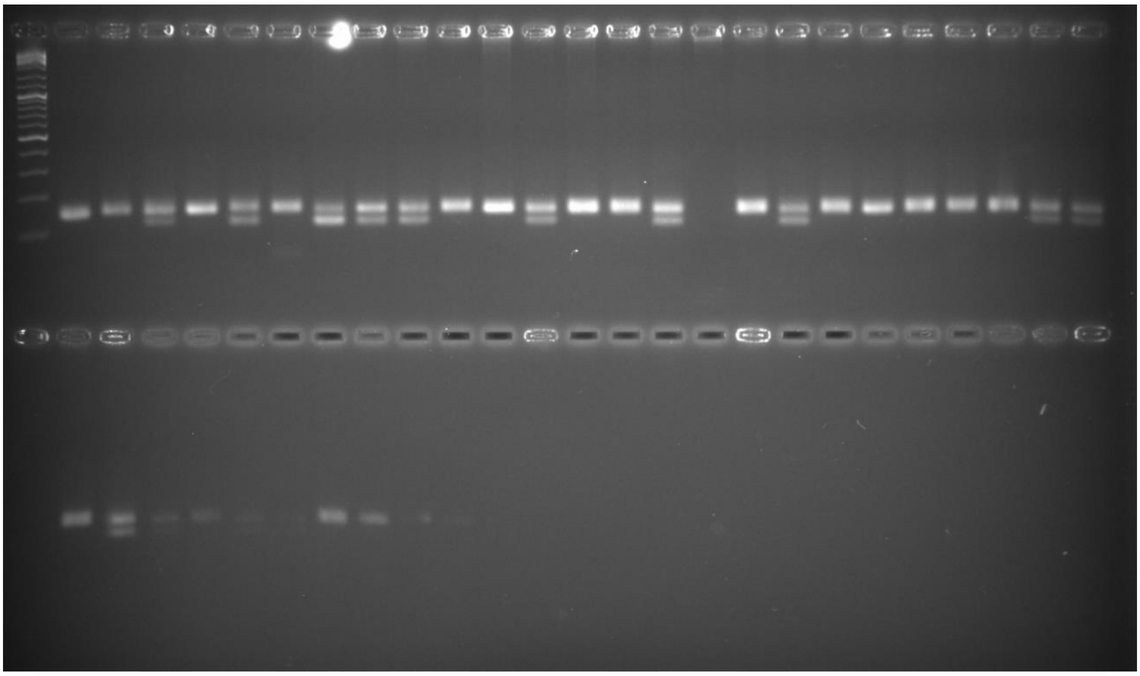


*Each image showing samples belongs to the progenies of tested crosses analysed using STM0003



*Each image showing samples belongs to the progenies of tested crosses analysed using STM0003

Appendix-H Marker assisted analysis of cross progenies using STM0003



*Each image showing samples belongs to the progenies of tested crosses analysed using STM0003

CURRICULUM VITAE

Mahmood Ayyaz was born on December 15, 1988 in Multan, Pakistan. He completed his higher secondary education from Government College of Multan, Multan in 2005. He earned his BSc Agriculture (Hons) Agriculture degree with major studies in Agriculture Entomology from University College of Agriculture, Bahauddin Zakariya University, Multan Pakistan during the session 2005-09. During September, 2009, he joined world renowned pesticide company Syngenta Pakistan as Agriculture Consultant Officer and worked for two year and seven months. Afterward worked as a Senior Agriculture Consultant Officer for Sungro Private Limited, Pakistan and performed his job duties at Multan for the period of one year. While working as Agriculture Consultant he earned his MSc Agriculture (Hons) Agriculture degree with major studies in Agriculture Entomology from University College of Agriculture, Bahauddin Zakariya University, Multan, Pakistan during the session 2010-12. During his 18 years of education tenure Mahmood Ayyaz was continuous scholarship holder and member of ‘‘Merit Based Scholarship for the Employee children’s of National Bank of Pakistan’’. Keeping continuing in his scholastic achievements; He won PhD fellowship (Graduate Scholarship Program for International Students-2215) awarded by The Scientific and Technological Research Council of Turkey (TÜBITAK) during the year 2013. While staying in Ankara he earned a 6 month Turkish language diploma from Turkish and Foreign Languages Research and Application Center (TÖMER) Ankara University, Ankara. He enrolled as PhD student in Department of Plant Production and Technologies under the supervision of Prof. Dr. Çiğdem ULUBAŞ SERÇE in the Faculty of Agricultural Sciences and Technologies, Niğde Ömer Halisdemir University, Nigde, Turkey during September, 2013. During his PhD thesis research he worked on ‘‘Phenotypic and Genotypic characterization of *Potato Virus Y* (PVY) resistance in Potato varieties and crosses; study of transmission ability of some PVY strains by aphids’’.