UNIVERSITY OF PANNONIA

GEORGIKON FACULTY

DOCTOR OF PHILOSOPHY

(PhD)

THESIS

AHMAD MOUSAPOUR GORJI

KESZTHELY, HUNGARY

2011

UNIVERSITY OF PANNONIA
GEORGIKON FACULY

DOCTORAL SCHOOL OF CROP PRODUCTION AND HORTICULTURAL SCIENCES
PLANT BREEDING, GENETICS AND AGROBIOTECHNOLOGY PROGRAM

HEAD OF THE DOCTORAL SCHOOL
PROF. RICHARD GÁBORJÁNYI, DSC

INCREASING THE EFFICIENCY OF POTATO RESISTANCE BREEDING WITH CONVENTIONAL AND MOLECULAR GENETIC METHODS

DOCTOR OF PHILOSOPHY (PhD) THESIS
WRITTEN BY

AHMAD MOUSAPOUR GORJI

SUPERVISORS
DR. ZSOLT POLGÁR, PhD
AND
DR. JÁNOS TALLER, PhD

KESZTHELY, HUNGARY
2011

INCREASING EFFICIENCY OF POTATO RESISTANCE BREEDING WITH CONVENTIONAL AND MOLECULAR GENETIC METHODS

Written By

AHMAD MOUSAPOUR GORJI

Written at the University of Pannonia, Doctoral School of Crop Production and Horticultural Sciences, Plant Genetics and Biotechnology Program

Supervisors: Dr. Polgár Zsolt and Dr. János Taller

I propose for acceptance (yes /no)

Signature

The candidate has achieved……..% at the comprehensive exam,

I propose the thesis for acceptance as the reviewer:

Name of reviewer: …….yes /no

Name of reviewer: ……..yes /no

The candidate has achieved……..% at the public discussion.

Veszprém/Keszthely,

…..……..

Chairman of the Committee

Labeling of the PhD diploma ……..

……

President of the UCDH

Table of Contents

ABSTRACT

Increasing the efficiency of potato resistance breeding with traditional and molecular genetic methods

Potato is one of the most important food crops. In spite of it's over all wide range of adaptability, the yield of potato can be dramatically decreased by biotic and abiotic factors like pathogens, water and heat stress. To protect quantity and quality of the, yield utilization of natural resistance of the crop is essential. Current potato breeding combines traditional and modern molecular techniques to reach this goal. Here I report the results of our effort on this field.

Potato virus Y is one of the most yield decreasing pathogens of potato. Varieties carrying PVY resistance gene in multiplex format where alleles are originate from different *Solanum* species may have more durable resistance and can reduce the influence of this pathogen. To achieve this goal we successfully bred and identified one "triplex" breeding line (99.373) where the alleles of the extreme resistance gene of PVY (Ry) originates from three species (*S. stoloniferum, S. tub.* ssp. *andigenum* and *S. hougasii*) and two more "duplex" lines (99.384 and 98.433) were alleles were combined from *S. stoloniferum* and *S. tub.* ssp. *andigenum*. Results were achieved after artificial infection of the segregating populations of the tested lines with the Hungarian PVY^{NTN} isolate (D-10), DAS-ELISA test and proved statistically by Chi square test (X^2).

For the promotion of breeding to abiotic stresses, we applied an *in vitro* screening method to compare potato lines by measuring their root number and root length under different osmotic stress conditions. In this context, our aim was to identify QTLs playing role in osmotic stress response and to develop markers closely linked to them. For this reason the comparison of different marker identifying techniques (Intron targeting, SCoT, SCAR, SSR, ISSR and RAPD) and creation of a detailed genetic map was initiated on an F1 population of cv. White Lady and S440. Based on the reaction under field conditions, these parental genotypes differ in their reaction to natural heat, drought stress and resistance to PVY^{NTN}. As a result we identified 6 major QTLs which closely mapped in coupling to some molecular markers on different linkage groups. These markers were mapped on 13 linkage groups for White Lady and 14 for S440.

This study gave us a lot of information to estimate the reliability of techniques and usability of those markers in the fingerprinting of tetraploid potato genotypes and varieties. The results revealed that SCOT, ISSR and RAPD markers are capable to generate high number of polymorphic markers. The efficiency of SCOT for fingerprinting of varieties was proved to be higher than the others but it was relatively the same as ISSR and RAPD for fingerprinting of F1 population. During the work we developed a new marker technique named IT-SCoT where we could combine advantages of three marker techniques namely IT, SCoT and TRAP. Based on the obtained results, we believe that the development of gene-targeted markers which are located close to the candidate genes will be useful for further molecular studies in tetraploid potato.

KIVONAT

A burgonya rezisztencia-nemesítés hatékonyságának növelése hagyományos és molekuláris genetikai eszközökkel

A burgonya az egyik legjelentősebb élelmiszernövény. A rendkívül széles adaptációs képessége ellenére biotikus és abiotikus stressz faktorok, mint például különböző kórokozók vagy a szárazság és hő-stressz komolyan veszélyeztetik a növény termőképességét. A termés minőségi és mennyiségi biztosítása érdekében a természetesen előforduló rezisztenciaforrások hasznosítása fontos. E célból, a jelen programban a burgonya rezisztencia nemesítését célzó hagyományos és molekuláris genetikai kutatásokat folytattunk.

A burgonya Y vírus a termést leginkább veszélyeztető burgonya vírus. A különböző vad *Solanum* fajokból származó PVY rezisztenciagént hordozó multiplex fajtákkal jelentősen csökkenthető a vírus által okozott kár. E célból sikeresen azonosítottunk egy "triplex" vonalat (99.373), melyben az extrém rezisztenciát biztosító allél 3 különböző fajból származik (*S. stoloniferum, S. tub.* ssp. *andigenum* and *S. hougasii*), és két "duplex" nemesítési vonalat (99.384 és 98.433), melyekben a *S. stoloniferum* és *S. tub.* ssp. *andigenum* Ry génjei találhatók meg.

Az abiotikus stresszekkel szembeni nemesítés előmozdítására egy *in vitro* eljárásban teszteltük burgonya genotípusok gyökérszám alakulását és gyökérhossz növekedését különböző ozmotikus stressz körülmények között. Itt az ozmotikus stressz válaszban szerepet játszó QTL-ek és velük kapcsolt molekuláris markerek azonosítása volt a célunk. A vizsgálatokat két különböző stressz választ adó genotípus, a White Lady fajta és az S440 nemesítési vonal keresztezéséből származó F1 populáción hajtottuk végre. Markerezéshez a következő eljárásokat alkalmaztuk: Intron targeting, SCoT, SCAR, SSR, ISSR and RAPD. A markerekből kapcsoltsági térképet szerkesztettünk. Összesen 6 QTL-t azonosítottunk, míg a White Lady-ben 13, az S440-ben pedig 14 kapcsoltsági csoportot kaptunk.

A vizsgálatok során külön tanulmányban hasonlítottuk össze a SCoT, ISSR és RAPD eljárások hatékonyságát a tetraploid burgonyában. A SCoT eljárás hatékonyabbnak bizonyult a fajták összehasonlító vizsgálatában, mint a másik két módszer, azonban a genotípusok vizsgálatában nem találtunk jelentős különbséget a három módszer között.

Munkáink során az IT és SCoT primerek kombinálhatóságát és az együttesen alkalmazott IT-SCoT primerek polimorfizmus detektáló képességét teszteltük. Eredményeink arra utalnak, hogy ez az eljárás új lehetőségeket kínál a célgén környezetében lokalizálható markerek kifejlesztésére a tetraploid burgonyában.

ABSTRAKT

Steigerung der Effizienz der Kartoffel Resistenzzüchtung mit traditionellen und molekulargenetischen Methoden

Kartoffel ist eine der wichtigsten Nahrungspflanzen. Trotz seines breiten Spektrums an Anpassungsfähigkeit, kann die Ausbeute an Kartoffel dramatisch biotischen und abiotischen Faktoren wie Krankheitserreger, Wasser-und Hitzestress gefährdet werden. Zum Schutz die Quantität und die Qualität des Ertrages, Nützung der natürlichen Resistenz der Pflanzen ist unerlässlich. Aktuelle Kartoffelzüchtung kombiniert traditionelle und moderne molekulare Methoden, um dieses Ziel zu erreichen. Hierbei gebe ich Bericht über die Ergebnisse unserer Bemühungen auf diesem Gebiet.

Potato virus Y ist eine der Erträge verringert Krankheitserreger der Kartoffel. Sorten die PVY-Resistenz-Gen in Multiplex-Format haben, und bei dem Allele von verschiedenen Solanum Arten stammen, werden möglicherweise mehr dauerhafte Beständigkeit haben, und können den Einfluss dieser Erreger reduzieren. Für dieses Ziel wir haben erfolgreich gezüchtet und identifiziert eine "Triplex" Zuchtlinie (99,373), wo die Allele der extreme Resistenz-Gen von PVY (Ry) stammten ursprünglich aus drei Arten (*S. stoloniferum*, *S. tub.* ssp. *andigenum* und *S. hougasii*) sowie bei zwei weitere "Duplex"-Linien (99,384 und 98,433) wurden Allele aus *S. stoloniferum* und *S. tub.* ssp. *andigenum* kombiniert. Die Ergebnisse nach der künstlicher Infektion der Trennung Populationen der getesteten Linien mit dem ungarischen PVY^{NTN} (D-10), DAS-ELISA-Test die wurden statistisch durch Chi-Quadrat-Test (X2) erwiesen.

Entwickelnd der Zucht gegen abiotischen Stress haben wir in-vitro-Screening-Methode zur Kartoffel-Linien angewendet vergleichend ihrer Wurzelnummer und Wurzellänge unter verschiedenen osmotischen Stress-Bedingungen.

In diesem Zusammenhang unser Ziel war QTLs zu identifizieren die in osmotischer Stress-Reaktion eine wichtige Rolle spielen sowie in der Nähe stehenden Markers zu entwickeln. Aus diesem Grund Vergleichen den verschiedener Markertechniken (Intron-Targeting, SCOT, SCAR, SSR, IRSS und RAPD) und Erstellung einer detaillierten genetischen Verbindugsmappe wurde auf einem F1 Population von cv White Lady und S440 eingeleitet.

Aus den Markern wurde eine genetische Verbindugsmappe aufgestellt. Als Ergebnis 6 Haupt QTLs wurden identifiziert, und in White Lady wurden 13 und in S440 wurden 14 Verbindungsgruppen erwiesen. In den Experimenten wurde die Effizienz der verschiedenen Techniken wie SCOT, ISSR und RAPD in eigenen Aufsätzen in der tetraploiden Kartoffel verglichen. Die Effizienz des SCOT für das Fingerprinting von Sorten erwies sich höher zu sein als die andere, aber es gab kaum kein Verschiedenheit in den Genotypen, vergleichend die drei Methoden.

Während der Arbeit entwickelten wir eine neue Marker-Technik mit dem Namen IT-SCoT, wo die Vorzüge von drei Marker Techniken, nämlich IT, SCOT und TRAP kombiniert wurden. Unsere Ergebnisse zeigten, dass diese neue Technik für die Entwicklung von funktionellen Markern in der Nähe der Kandidatgene, in der tetraploiden Kartoffel nützlich sein kann.

ABBREVIATIONS

AAD - Arbitrarily Amplified Dominant
AFLP - Amplified Fragment Length Polymorphism
AMOVA - Analysis of Molecular Variance
AP-PCR - Arbitrarily Primed Polymerase Chain Reaction
ARF6 - Auxin Response Factor Family
BC - Backcross
BPB – Brome Phenol Blue
CAPS - Cleaved Amplified Polymorphic Sequence
cDNA – Complementary Deoxyribonucleic acid
CIA - Chloroform and Iso-amyl Alcohol
CPB - Colorado Potato Beetle
DAF - DNA Amplification Fingerprinting
DAS-ELISA - Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay
DD - Differential Display
DDRT-PCR - Differential Display Reverse Transcription PCR
DGGE-RFLP - Denaturing Gradient Gel Electrophoresis RFLP
DH – Double Haploid
DI - Diversity Index
DNA - Deoxyribonucleic acid
EBN - Endosperm Balance Number
EDTA - Ethylene Diamine Tetraacetic Acid
EM - Expectation-Maximization
EMR - Effective Multiplex Ratio
ER - Extreme Resistance
EST – Expressed Sequence Tag
F-SSCP - Fluorescence-based PCR-SSCP
HR - Hypersensitive Reaction
ISSR - Inter Simple Sequence Repeats
IT - Intron Targeting
IT-SCoT - Intron Targeting-Start Codon Targeted
LG – Linkage Group
LOD - Logarithm of Odds
MAS - Marker-Assisted Selection
MCMC - Markov Chain Monte Carlo
MI - Marker Index
MIM - Multiple Interval Mapping
MP-PCR - Microsatellite-primed PCR
mRNA – Messenger Ribonucleic acid
NCBI - National Center for Biotechnology Information
NIL - Near-Isogenic Lines
NJ – Neighbor Joining
OP-PCR - Oligo Primer-PCR
PAGE - Polyacrylamide Gel Electrophoresis
PCL - Plant Cells Lysis
PCoA - Principal Coordinate Analysis
PCR – Polymerase Chain Reaction
PIC - Polymorphic Information Content
PLRV - *Potato leafroll virus*
PMTV – *Potato mop-top virus*
PRRs - Pattern Recognition Receptors
PTNRD- Potato Tuber Necrotic Ring Spot Disease
PVA - *Potato virus A*
PVM - *Potato virus M*
PVP - Polyvinyl- Pyrrolidone
PVS - *Potato virus S*
PVY - *Potato virus Y*
QTL - Qualitative Trait Loci

RAD - Representational Difference Analysis
RAPD - Random Amplified Polymorphic DNA
RFLP - Restriction Fragment Length Polymorphism
RNA - Ribonucleic acid
RP - Resolving Power
RP-PCR - Random Primer-PCR
RT-PCR - Reverse Transcription PCR
SAGE - Serial Analysis of Gene Expression
SC - Self-Compatible
SI - Self-Incompatible
SCAR - Sequence Characterized Amplified Region
SCoT - Start Codon Targeted
SNP - Single Nucleotide Polymorphism
SODA - Small Oligo DNA Analysis
SRAP - Sequence-Related Amplified Polymorphism
SRS - Short Repeat Sequence
SSCP-PCR - Single Strand Conformation Polymorphism-PCR
SSCP-RFLP - Single Strand Conformation Polymorphism RFLP
SSH - Suppressive Subtraction Hybridization
SSLP - Simple Sequence Length Polymorphism
SSR - Simple Sequence Repeat
STS - Sequence Tagged Site
TBE - Tris-HCL, Boric Acid, EDTA
TE - Tris-HCL, EDTA
TPS - True Potato Seed
TRAP - Target Region Amplified Polymorphism
TRS - Tandem Repeat Sequence
TRV - *Tobacco rattle virus*
VIGS - Virus-Induced Gene Silencing
WL - White Lady

1. INTRODUCTION

The potato (*Solanum tuberosum*) is a New World crop that was unknown to the rest of the world until the 1500's (Hermanova et al., 2007). It is one of the world's most productive, nutritious, and tasty vegetables with 329.6 million tonne fresh weight of tubers produced in 2009 from 18.3 million hectares of land (http://faostat.fao.org). Over the last 50 years the registered planted area has decreased in developed countries and increased in developing countries mainly because of its high yield per unit area and nutritive value (Karim et al., 1997). The World's average yield is 18 t/ha currently, while the potential of the plant is above 100 t/ha.

Potatoes are grown in 149 countries from latitudes 65°N to 50°S and at altitudes from sea level to over 4500m, with a concentration of diversity in the Andes (Spooner and Van den Berg 1992; Hijmans., 2001). Wild tuber-bearing *Solanum* species are distributed from the southwestern USA (38°N) to central Argentina and adjacent Chile (41°S) (Hawkes, 1990; Spooner and Hijmans, 2001). The greatest diversity of wild potato species is currently found near Lake Titicaca on the border of what is now Peru and Bolivia (Hawkes, 1990; Simmonds, 1995; Glave, 2001). In contrast to other crop plants, there is a diverse pool of potato wild species because of their wide geographical distribution and great range of ecological adaptation which could be a source of traits for potato breeding – e.g. tolerance to biotic as well as abiotic stress factors (Hawkes, 1994; Frusciante et al., 2000; Watanabe, 2002; Hijmans et al., 2003). The species used in breeding programmes as donors of tolerance and resistance traits are particularly *S. demissum, S. acaule, S. chacoense, S. spegazinii, S. stoloniferum, S. vernei* (Caligari, 1992). In the present study we used *S. stoloniferum, S. tub.* ssp. *andigenum* and *S. hougasii* as a source of resistance to biotic stress.

A review of past showed that potato breeding in the modern sense began in 1807 in England when deliberate hybridization between different varieties was performed by artificial pollination (Knight, 1807; Bradshaw and Mackey, 1994). Priorities for modern agriculture are yield stabilization and at the same time a decrease in the use of fertilizers, pesticides and water (Epso, 2005). Potato cultivars with higher levels of disease and pest resistance where alleles are originated from different *Solanum* species are highly desirable, but of course further to these properties they must also retain the marketable yield and quality required for a modern cultivar to be successful (Bradshaw and Mackey, 1994; Gorji et al., 2011). Furthermore, to expand potatoes production in a wider range of environments, for longer growing seasons and to increase yield stability in terms of

quantity and quality under certain growing conditions resistance to abiotic stresses is important.

The evaluation of osmotic stress tolerance of potato genotypes (*Solanum* spp.) in conventional field trials is rather time consuming and labor intensive. Moreover the results are often confounded by many field and environmental variations (Ingram et al., 1994; Erusha et al., 2002; Iwama and Yamaguchi 2006; Georgieva et al., 2004). Simulation of osmotic stress under *in vitro* tissue culture conditions can minimize environmental variation due to defined nutrient media, controlled conditions and homogeneity of stress application. In 1993 in the study by Leport et al. it was suggested that the *in vitro* selection for stress tolerance will have a significant place in the strategy of establishing plant systems with optimal stress reaction and output in the future. Applying osmotic stress during the regeneration phase was found to be the most efficient for the selection for drought tolerance (Hsissou and Bouharmont, 1994). Gopal and Iwama (2007) investigated *in vitro* screening of potato against osmotic stress mediated through sorbitol and mannitol. Their results demonstrated that osmotic factors in culture media adversely affected plantlet growth, and genotypes differed for their responses. They concluded that *in vitro* screening of potato under specific and limited osmotic stress conditions might provide a system capable to differentiate genotypes effectively to determine their expected root mass production under field conditions.

Osmotic stress tolerance in plants is known to be a quantitatively inherited trait. It is generally under the control of small number of quantitative trait loci (QTLs). The identified advantageous marker alleles linked to QTLs could help to introduce new varieties having higher tolerance to osmotic stress by marker assisted selection of genotypes with higher phenotypic value (Bálint et al., 2008). A significant progress has been made recently in developing the theory of linkage analysis and quantitative trait locus mapping in autotetraploid species for a full-sib family derived from crossing of two parents (Luo et al. 2001; Hackett et al., 2001). Genetic or DNA based marker techniques such as RFLP (restriction fragment length polymorphism), RAPD (random amplified polymorphic DNA), SSR (simple sequence repeats), AFLP (amplified fragment length polymorphism) and ISSR (inter simple sequence repeats) are routinely being used in genetic and qualitative trait loci (QTL) mapping as well as in diagnostic genomic fingerprinting, population genetic studies, ecological, evolutionary, taxonomical, genetic studies of plant sciences, phylogenetic and systematic studies at various levels, using both distance- and parsimony-based approaches (Gupta et al., 1999; Bussell et al., 2005; Semagen et al., 2006; Mark et al., 2007; Agarwal et al., 2008). These techniques are well established and their advantages as well as

limitations have been realized.

Molecular markers provide numerous advantages over conventional phenotype based alternatives as they are stable and detectable in all tissues regardless of growth, and differentiation, development, or defense status of the cell are not confounded by the environment, pleiotropic and epistatic effects. In recent years, a new class of advanced techniques has been emerged, primarily derived from the combination of earlier basic techniques. Advanced marker techniques tend to amalgamate advantageous features of several basic techniques. The newer methods also incorporate modifications in the methodology of basic techniques to increase the sensitivity and resolution to detect genetic discontinuity and distinctiveness. The advanced marker techniques also utilize newer class of DNA elements such as retrotransposons, mitochondrial and chloroplast based microsatellites, thereby revealing genetic variation through increased genome coverage. Techniques such as RAPD and AFLP are also being applied to cDNA-based templates to study patterns of gene expression and uncover the genetic basis of biological responses.

Research objectives

The research objectives of the present study are the followings:

1) Development of hetero multiplex potato genotypes carrying PVY resistance genes (Ry).
2) Comparison of polymorphism detecting power of the ISSR, RAPD and SCOT markers in potato varieties as well in F1 genotypes.
3) Construction of genetic linkage map in tetraploid potato using different marker types.
4) Identification of gene-targeted markers linked to PVY ^{NTN} resistance gene (Ry_{sto}) which could be used in potato breeding programs.
5) Development of genetic markers which are linked to osmotic stress tolerance in tetraploid potato under *in vitro* conditions.
6) Development of new gene- targeted markers named IT-SCoT.
7) Partial isolation of genes which are induced by PVY^{NTN} infection.

2. LITERATURE REVIEW

The cultivated potato is an unusual crop that it has an extremely large secondary genepool consisting of related wild species that are tuber-bearing, albeit with small inedible tubers (Vreugdenhil et al., 2007). The taxonomy of the cultivated potato and its wild relatives has been the subject of study for many years. There are hundreds of species of *Solanum* and many infraspecific taxa around the world, only about 200 produce tubers. Eight of these are cultivated in some scale (Manjit et al., 2007). The taxa have been classified in series, with different authors recognizing different numbers of series, often with different circumscriptions. Correll, 1962 and Hawkes, 1990 documented 26 and 21 series, respectively. Some of the series contain only one or just a few species, indicating that their relationship to the other species is not clear. On the contrary, series such as *Piurana* and especially *Tuberosa* are large groups of species that may not be closely related to each other (Vreugdenhil et al., 2007).

There is a polyploid series from diploid (2n = 2x = 24) to hexaploid (2n = 6x = 72), in which nearly all of the diploid species are self-incompatible outbreeders and the tetraploids and hexaploids are mostly self-compatible allopolyploids that display disomic inheritance (Hawkes, 1990). The odd numbered polyploids, while mostly sterile, are able to maintain themselves vegetatively through the tubers. The cultivated potato, *Solanum tuberosum* L., is a tetraploid (2n = 4x = 48) species that displays tetrasomic inheritance and accommodated in the series *Tuberosa*, a rather large and variable group without clear diagnostic characters. The origin of the cultivated potatoes has been described as the result of successive hybridizations between diploid members of the brevicaule complex, accompanied by chromosome doubling leading to the tetraploid forms. The crop itself has been classified into seven cultivated species (*Solanum ajanhuiri, Solanum chaucha, Solanum curtilobum, Solanum juzepczukii, Solanum phureja, Solanum stenotomum* and *S. tuberosum* with two subspecies, *tuberosum* and *andigena*), showing several ploidy levels.

The place of origin of the group of tuber-bearing potato species has been suggested to be the Mexican/Central American area, where those species are found that are considered to be phylogenetically primitive (Vreugdenhil et al., 2007). The most obvious domestication originated in the Andes Mountains of South America, which in a sense, were named for potato agriculture ("Andes" is derived from a Quechua word for field terraces). Simmonds (1995) concluded that just a few closely related diploid species in the series *Tuberosa* (e.g., *Solanum brevicaule, S.*

leptophyes, and *S. canasense*) were domesticated in the Andes of southern Peru and northern Bolivia more than 7.000 years ago. Closely related wild species grow in diverse places from Chile through Central America and into North America as far as Utah. Correll (1962) says little or no domestication occurred in Central and North America because other foods were readily available. Spooner et al., (2005) provided molecular taxonomic evidence for a single domestication in the highlands of southern Peru from the northern group of members of the S. *brevicaule* complex of diploid species. This group contains species such as *S. canasense, S. multidissectum,* and *S. bukasovii,* some of which are not always clearly resolved and perhaps could be better reduced to a single species, *S. bukasovii*. Sukhotu and Hosaka (2006) also concluded from chloroplast data that species such as these were first domesticated in Peru with a later spread to Bolivia. The result of domestication was a diploid cultigen *S. tuberosum* group *Stenotomum* (Dodds, 1962) from which all of the other cultivated potatoes were derived.

2.1. Introduction of potato to Europe

There is still much debate over which group of potatoes was introduced into Europe at the end of the 16th century and subsequently to the rest of the world from the 17th century onward (Pandey and Kaushik, 2003). The large-scale cultivation of the potato began only in the beginning of the 19th century. Initially, potato was used as a medicinal plant and grown by pharmacists, particularly in Spain. The first record of cultivated potatoes outside of South America is their export in 1567 from Gran Canaria in the Canary Islands to Antwerp in Belgium (Hawkes and Francisco-Ortega, 1993). Further, they were first recorded in Spain in 1573 in the market archives of the Hospital de La Sangre in Seville (Hawkes and Francisco-Ortega, 1992). It was later introduced to other parts of Europe by merchants and kings, who encouraged the cultivation of this efficient plant to increase local agricultural production. Hawkes and Francisco-Ortega (1993) have argued that Andigena potatoes were introduced into the Canary Isles and from there to mainland Europe. Then spread northeastward across Europe as the growing of potatoes. An alternative theory is that after an European potato blight epidemic new genotypes were introduced, probably *S. tuberosum* ssp. *tuberosum* L., originating from Chile (Hawkes 1990). It seems safest to assume that the early introductions of cultivated potatoes to Europe came from both the Andes and Chile, but was few in number and hence captured only some of the biodiversity present in the cultivated potatoes of South America (Manjit et al., 2007).

2.2. Potato production in Hungary

Potato is an essential foodstuff in Hungary. The average consumption of potato is approximately 65 kg/year/capita. Out of that less than 10 % is consumed as processed food.

The potato production area is dramatically decreased during the last 15 years from 50.000 to 22.000 ha. After Hungary joined the EU, the seed potato production area also drastically decreased from 1500 ha to 250 ha. The total production reached 600.000 Mts in 2009. Out of that 5000 Mts was only seed potato (FAO, 2010). The total production was 1% of EU's total potato production and could just cover the needs of local market. The national average yield is about 25-27 Mts/ha. According to production quantity, Hungary is in the 50th position based on the FAO's report, while taking the view of production area Hungary is in the 59th place. Twenty percent of the total production area is covered by Hungarian varieties; those were mainly bred at Keszthely. The leading varieties are named as: Red Scarlet (NL), Laura (D), Kondor (NL), Desiree (NL), Cleopatra (NL), Agria (D), as well as Balatoni Rózsa (HU), Hópehely (HU), Góliát (HU) and Rioja (HU).

2.3. History of potato research at Keszthely, Hungary

Based on 200 years long tradition, modern potato research and breeding activities have been existed since 1950 at the Potato Research Centre, Keszthely. The Centre operates under a university system and is the only institution dedicated to potato research and breeding exclusively in Hungary. It is an appreciated centre of basic and applied research, breeding, extension and education of experts for potato. One of its major duties is the breeding of profitable potato varieties those suitable for Central European agro-ecological conditions due to their resistance against major potato pests, pathogens and extreme weather conditions. The research fields of the Centre starting from basic to applied are all dedicated toward this goal and try to cover all important issues of the potato sector.

From the sixties till the middle of eighties of the previous century the Centre operated a consistent, large resistance-breeding program utilizing several wild species germplasm. There were years when 1.5 – 2 million of seedlings were produced and screened by artificial infection with major potato pathogens and pests (viruses, nematodes and late blight) to incorporate resistance genes into cultivated genetic background. In the crossing program different accessions of *S. stoloniferum, S. acaule, S. tub.* ssp. *andigenum, S. vernei* and *S. hougasii* were most intensively

used directly or through species hybrids. From this enormous work the Centre recently released 11 varieties (Démon, Balatoni Rózsa, Katica, Lorett, Góliát, Rioja, Hópehely, White Lady, Vénusz Gold, Luca XL, Kánkán). These varieties due to their complex resistance, high yielding potential and outstanding consumption quality are unique in their kind. All the varieties show extreme resistance to the economically most important potato virus (PVY) and high field resistance to PLRV. Out of eleven 9 is resistant to common scab, potato wart and golden cyst nematodes, while two of them to potato late blight as well that makes those especially advised for organic production.

Recently advanced parental line screening methods, somatic hybridization, genetic modification and markers assisted selection techniques are involved into the breeding methodology of the Centre.

2.4. Genetic resource of wild potato species

Several issues regarding the conservation of potato genetic resources, including ownership, collection, classification and genetic erosion, have been discussed by Bamberg and del Rio (2005). Representative samples of many wild potato species have been collected and are maintained in genebanks around the world. In most cases, the accessions are increased by means of true seed that is generated in the genebank (*ex situ*). In general, this process has not altered the genetic diversity of the *ex situ* germplasm using the current standard techniques that are applied in most major genebanks (del Rio et al., 1997a). However, del Rio et al., (1997b) found significant genetic differences between gene-bank-conserved and re-collected *in situ* populations of several accessions and concluded that *in situ* preservation may be important for the backup of diversity already present in genebanks and for the preservation of new diversity that can be accessed in future re-collections.

2.5. Genetics of potato species

The number of ploidy levels of potato species, based on a haploid number of 12, ranges from diploid (2n = 24) to hexaploid (6n = 72), and includes triploids, tetraploids, and pentaploids (Watanabe, 2002). There is some evidence that polyploidy played an important role in the environmental differentiation and range expansion of wild potatoes (Hijmans et al., 2007). The

specific ploidy levels are in relation to the phenomenon of unreduced gametes. Next to the normal haploid gametes (n), several genotypes produce unreduced gametes (2n) as a result of meiotic anomalies (Carputo and Barone, 2005). The frequency of 2n pollen production varies from 2% up to 10% (Watanabe, 2002). Cultivated potatoes are tetrasomic tetraploids (4n = 48) but the majority (80%) of the wild species are diploid (Carputo and Barone, 2005). Hijmans et al., (2007) documented that 123 species have been found in diploid cytotypes and only 43 species in polyploids. Nearly all of the diploid species as well as tetraploid *S. tuberosum* subsp. *tuberosum* are outbreeders. The incompatibility is of a gametophytic, multi-allelic nature based on the occurrence of S alleles (Dodds, 1965). A dominant self-incompatibility inhibitor has been found in *S. chacoense* (Hosaka and Hanneman, 1998; De Jong and Rowe, 1971) and used in breeding. The tetraploids and hexaploids are mostly self-compatible allopolyploids that display disomic inheritance (Hawkes, 1990).

Some of the problems and complexities of working with a tetraploid genome were overcome after 1958 with the production of haploids (also called dihaploids) of *S. tuberosum* and genetic studies at the diploid level involving crosses with other diploid *Solanum* species (Hougas et al., 1958). The dihaploids were, however, usually male sterile, and most dihaploids and diploid species were self-incompatible. Furthermore most economically important traits displayed continuous variation, which required biometrical rather than Mendelian analysis. Hence in potato genetics it was not possible to achieve the same degree of sophistication as in the genetic analysis of crosses between truebreeding inbred lines that display disomic inheritance. Nevertheless, as in other crops, knowledge of quantitative genetics provided the bases for efficient conventional potato breeding, which is still the main route to produce new cultivars. The concepts of heritability, additive and non-additive genetic variation, genotype × environment interaction, and population improvement are all important in predicting and improving the response to selection and rate of progress. High quality mechanized fieldwork and computer based data capture and analysis is essential in this endeavour.

2.6. Potato breeding

2.6.1. Reproductivity

The reproductive biology of potato is ideal for creating and maintaining variation. Tuber-

bearing *Solanum* species have unique reproductive characteristics: a possibility of both vegetative and sexual reproductive strategy; production of gametes with unreduced chromosome number; existence of different ploidity levels and presence of an endosperm dosage system that regulates interploidy/interspecific crosses (Carputo and Barone, 2005). All these traits have been of great importance in breeding as well as in classification and evolutional studies.

2.6.2. Crossability

In general, potato species are insect-pollinated, cross-breeding species. The crossability of species has been determined through artificial pollinations across many years (Jansky, 2009). The results of crossability can be explained primarily but not exclusively in terms of endosperm balance number (EBN), which can be regarded as the effective rather than the actual ploidy of the species (Johnston et al., 1980; Johnston and Hanneman, 1980). This phenomenon has a great importance in breeding programs and in the potential of interploidy/interspecific crosses. The EBN is a number varying from 1 to 4, expressing the effective ploidy of *Solanum* species (Carputo and Barone, 2005). For normal development of the endosperm, after fertilization the maternal genome must be twice of the paternal genome (2:1). The EBN is independent of the ploidity level, and its behavior is additive. The EBN of cultivated *S. tuberosum* is 4, whereas the EBN of most of the wild species (either diploid or tetraploid) is 2. Several natural and artificial mechanisms are available to circumvent the EBN incompatibility. The natural occurrence of unreduced gametes makes it possible that species with lower EBN can be crossed with species with higher EBN. The artificial systems are the production of dihaploids or the polyploidisation. Despite the EBN system, potatoes of different groups can be combined by somatic fusion *in vitro* (Carputo and Barone, 2005). Today breeders can usually achieve sexual hybridization between *S. tuberosum* and its wild relatives by manipulation of ploidy with due regard to EBN (Ortiz, 1998, 2001; Jansky, 2006).

Unilateral incompatibility is known to occur when a self-incompatible (SI) species is pollinated by a self-compatible (SC) one so that *S. verrucosum* (SC female) × *S. phureja* (SI male) is successful, but the reciprocal cross fails (Hermsen, 1994; Jansky, 2006). Sometimes incompatible pollen can be helped to achieve fertilization through a second pollination with compatible pollen, a technique known as mentor pollination (Hermsen, 1994; Jansky, 2006). These phenomena have been reviewed by Camadro et al. (2004) in the context of how sympatric species maintain their integrity. From time to time potato breeders have unexpected successes and failures when attempting to overcome barriers to hybridization.

2.6.3. Outcrossing

Outcrossing is enforced in cultivated (and most wild) diploid species by a single S-locus, multiallelic, gametophytic self-incompatibility system (Dodds, 1965). Cross-pollination between field plots of *S. phureja* has been estimated to decline from 5.1% at 10 meters to 0.2% at 80 meters based on a pollen donor possessing a dominant marker (Schittenhelm and Hoekstra, 1995). This information is useful in planning isolation distances for natural true-seed multiplication of genebank accessions and cultivars propagated by this method. In self-compatible species (tetraploid *S. tuberosum*), 40% (range 21% to 74%) natural crosspollination was estimated to occur in ssp. *andigena* in the Andes (Brown, 1993) and 20% (range 14% to 30%) in an artificially constructed Andigena population (Glendinning, 1976). Outcrossing creates an abundance of diversity by recombining the variants of genes that arose by mutation. As a consequence, potatoes are highly heterozygous individuals that display inbreeding depression on selfing.

2.6.4. Artificial hybridizations

Today most cultivars come from deliberate artificial hybridizations. The aim is to generate genetic variation on which phenotypic selection process across a number of vegetative generations can be done till the identification of unique genotypes having potential to be released as new cultivars. For successful deliberate hybridization breeders usually encourage flowering by the periodic removal of daughter tubers, and sometimes by grafting young potato shoots onto tomato or other compatible solanaceous plants. Pollinations can also be done on flowers attached to stems that have been cut and placed in jars of water with an anti-bacterial agent to reduce contamination (Peloquin and Hougas, 1959). The floral characteristics of potatoes and methods of artificial hybridization and self-pollination have been described by Plaisted (1980). Details can also be found in Caligari (1992), Douches and Jastrzebski (1993), and in the textbook Breeding Field Crops by Poehlman and Sleper (1995).

2.6.5. Conventional breeding

In conventional breeding of potato the exploitation of genetic resources through modern technology is a critical component (Knight, 2003). Conventional breeding of new and improved potato cultivars is a long-term, dynamic, and complex process. It is essentially based on phenotypic selection, involving crosses between tetraploid varieties and advanced clones, and then

field evaluation and selection. The process takes approximately 10–12 years. The sequence of activities in a conventional breeding program usually involves: (1) establishment of objectives, (2) selection and cross of parents in accordance with the objectives, (3) selection of seedlings, and (4) evaluation of clones that may have commercial potential. Although breeding programs may differ on some details, the basic principles are virtually the same. High number of seedlings (50000 to 250000) needs to be grown and tested to identify a new and improved cultivar (Bradshaw, 2000; Bradshaw and Mackay, 1994; Caligari, 1992; Douches and Jastrzebski, 1993; Hoopes and Plaisted, 1987; Mackay, 2005; Tarn et al., 1992). Several authors have presented breeding schemes for the development of new cultivars (Ross, 1986; Rousselle-Bourgeois and Rousselle, 1996; Struik and Wiersema, 1999; Tarn et al., 1992). Programs for the identification of superior parents have been developed by Bradshaw and Mackay (1994), Brown and Dale (1998), Gopal (1998), and Tarn et al. (1992). Data processing programs for potato breeding programs have been developed by Tarn et al. (1992) and by Kozub et al. (2000).

Because of the close affinity between the cultivated potato and its wild relatives, it is relatively easy to incorporate related germplasm into cultivated forms (Peloquin et al., 1999). Many cultivars already contain one or more disease resistance genes that can be traced back to primitive cultivars or wild species (Ross, 1986). However the use of exotic germplasms is rather time consuming as several back crosses with cultivated parent and rigorous selection program are needed to get rid of undesired characters originated from the exotic parent while keeping the resistance. It requires a relatively long-term commitment without immediate payoff in terms of new cultivars (Pavek and Corsini, 2001; Plaisted and Hoopes, 1989; Tarn et al., 1992). Spooner et al. (2004) provide an extensive list of potential uses of wild species in breeding programs.

2.6.6. Selection of parents

Potato breeding traditionally involves crosses between pairs of parents with complementary phenotypic features. The parents will have genes introgressed from wild species and they may also be from complementary groups of germplasm to exploit yield heterosis (Bradshaw, 2009). The choice of parents is important because breeding can never simply be a number game. The number of possible bi-parental crosses increases from 4,950 in the case of 100 parents to 499,500 in the case of 1,000 parents and on to a staggering 49,995,000 in the case of 10,000 parents. Breeders can now complement phenotypic assessments of potential parents with a genotypic assessment of

diversity using molecular markers and hence capture allelic diversity in a smaller core set of parents. They can also use genetic distance based on molecular markers (Powell et al., 1991) to complement co-ancestry/pedigree analysis (Tarn et al., 1992; Gopal and Oyama, 2005) to avoid closely related parents yielding inbreeding depression and to ensure genetic variation for continued progress. Both analyses are required because clustering based on molecular markers can be different from clustering based on pedigree (Sun et al., 2003).

2.6.7. Breeding strategy

The key decisions that breeders need to make is what germplasm and breeding methods will be used, whether new cultivars will be propagated vegetatively or through true potato seed (TPS), whether or not new cultivars will be genetically modified and how to achieve durable disease and pest resistance. The breeding objectives must also include the demands of the export markets and evaluations of potential cultivars must include various trials in target countries (Struik and Wiersema, 1999). The objectives will vary from country to country, but all programs are likely to involve selection for higher yield, appropriate maturity and dormancy, tuber characteristics that affect quality and suitability for particular end uses, and resistance to abiotic and biotic stresses. If possible, they should also possess improved nutritional and health properties while appropriate tuber morphology, texture, adequate solids, low reducing sugar content, freedom from mechanical damage, bruising, and internal defects remain as important as they were in the last decades. Sound decisions require knowledge of the evolution of the modern crop, target environments and end uses for new cultivars, the reproductive biology of cultivated potatoes and their wild relatives, and the population structure of pathogens and the epidemiology of diseases. Genetic knowledge is also required which increased dramatically for the potato since the first molecular marker map appeared in 1988 (Bonierbale et al., 1988, 2003). From practical point of view objectives need to be translated into the improvements required over existing cultivars and into selection criteria that can be used by breeders. Details of each important traits and their inheritance can be found in the review of Bradshaw, 2007. Future breeding programs however must include new goals as efficient water and fertilizer usage as well.

2.6.7.1. Resistance to abiotic stresses

The genus *Solanum*, section Petota, offers a tremendously diverse gene pool that can be

utilized in potato breeding (Watanabe, 2002). The wild potatoes naturally developed in diverse conditions and are adapted to a wide range of environmental stresses (Pérez et al., 2000). To expand potato growing in a wider range of environments, for longer growing seasons and to increase of yield stability in terms of quantity and quality under a certain growing conditions resistance to abiotic stresses is important. These stresses include drought, heat, cold, mineral deficiency and salinity, with water stress being the most important one affecting potato production in most areas of the world (Vada, 1994). In setting breeding objectives, it is important to distinguish between drought avoidance (e.g., through early maturity), drought tolerance, and water-use efficiency. Compared to other species, potato is very sensitive to water-stress because of its shallower root system (Iwama and Yamaguchi, 2006) and has been classified as moderately salt-tolerant to moderately salt-sensitive (Maas, 1985). Improvement of root traits (root number and root length) is considered to be important for developing osmotic stress tolerant genotypes (Rossouw and Waghmarae, 1995; Iwama and Yamaguchi, 2006; Lahlou and Ledent, 2005). It has been shown that larger and deeper roots contribute to osmotic tolerance in many crops as well as potato (Schafleitner et al. 2007; Lahlou and Ledent, 2005). The accumulation of polyols (mannitol, sorbitol, inositol and their derivatives) is considered to be related to drought and salinity stress tolerance in many plant species (Peuke et al. 2002; Sakthivelu et al., 2008; Ehsanpour and Razavizadeh, 2005; Mohamed et al., 2000; Watanabe et al., 2000; Dobranszki et al., 2003).

2.6.7.2. Resistance to biotic stresses

Serious yield losses and reductions in quality can occur when potato plants and tubers are infected by fungal, bacterial, and viral diseases or damaged by insects, mites, and nematodes. Summary of the global distribution of potato diseases has been given by Hide and Lapwood (1992) and of potato pests by Evans et al. (1992).

Among viral disease, potyviruses are the most important. Potato viruses are either spherical (isometric), such as the *potato leafroll virus*, rod shaped or filamentous, such as potato viruses Y, X, A, S, M and the *Aucuba mosaic virus*. Most viruses require special vectors for distribution in crops, such as aphids, nematodes or fungi. Aphides are virus vector par excellence. They transmit viruses in potato crops both in a non-persistent manner (virus A and Y) and in a persistent manner (*Potato leafroll virus*) (van der Zaag et al., 1996; van der Zaag, 2007; Szajko et al., 2008).).

In contrast to other crop plants, there is a diverse pool of potato wild species, which could be a

source of traits for potato breeding – e.g. tolerance to biotic as well as abiotic stress factors (Frusciante et al., 2000; Watanabe, 2002; Hijmans et al., 2003). Because of tremendous diversity within wild species and even within accessions, fine screening is necessary to identify individual clones with resistance genes (Vreugdenhil et al. 2007). It has been demonstrated that some disease resistance (R) genes occupy orthologous region of the genomes of potato, tomato and pepper (Bradeen et al., 2008).

The hybridization of potato plants of extremely distant origin may introduce novel resistance genes into potato gene pool (Vreugdenhil et al., 2007). Colon et al (1993) used embryo rescue to introduce late-blight resistance genes into the potato from the solanaceous weed species *Solanum nigrum* and *Solanum villosum*. Valkonen et al. (1995) used embryo rescue to transfer the extreme resistance to PVY found in the non-tuber-bearing 2X, 1EBN species *Solanum brevidens* to the cultivated potato. Chavez et al. (1988) used bridging crosses, ploidy manipulations, and embryo rescue to transfer PLRV resistance from the non-tuber-bearing 2x, 1EBN species *Solanum tuberosum* to tuber-bearing species. The species used in breeding programs as donors of tolerance and resistance traits are particularly *S. demissum, S. acaule, S. chacoense, S. spegazinii, S. stoloniferum, S. vernei* (Caligari, 1992). The *Solanum stoloniferum* and *Solanum demissum* have been characterized as a main source of virus and Late-blight resistance genes, respectively (Hawkes, 1990).

2.6.7.2.1. Mechanisms of resistance

Like all other plants solanaceous plants are attacked by a wide range of pathogens and insects leading to significant crop losses (Strange and Scott, 2005). In response to these attackers, passive and active defense mechanisms have evolved. Active defense responses can be subdivided into adaptive and innate immunity. Adaptive immunity in plants appears to be restricted to antiviral defense responses depending on an RNAi like mechanism (Voinnet, 2005). The innate immune system is more general and responds to a wide variety of plant pathogens. Innate immunity relies on specialized receptors that can be roughly divided into two groups: the Pathogen or Pattern Recognition Receptors (PRRs) and the Resistance (R) proteins (Nürnberger et al., 2004; Zipfel and Felix, 2005). R proteins are encoded by large gene families, numbering several hundreds of genes per genome (Meyers et al., 2003). Resistance mediated by R proteins is often associated with the appearance of localized cell death at the infection site, a phenomenon called the hypersensitive

reaction (HR). HR is an efficient defense strategy in plants that restricts pathogen growth and can be activated during host as well as non-host interactions. HR involves programmed cell death and manifests itself in tissue collapse at the site of pathogen attack. This is distinct from the resistance response mediated by PRR receptors, as these generally do not induce an HR response upon pathogen recognition (Jones and Dangl, 2006).

2.6.7.2.2. Genetics of disease resistance

Disease resistance genetic studies are often based on tetraploid families, however, even major genes are difficult to identify at the tetraploid level due to complexities of tetrasomic segregation (Vreugdenhil et al., 2007). Solomon Blackburn and Barker (2001) listed 28 major genes/alleles responsible for virus resistance in potato. Some virus resistance genes may be found in closely linked clusters or they may be single genes that confer broad spectrum resistance. Barker and Solomon (1990) observed an approximately 1:1 segregation ratio for PLRV in a cross between a susceptible and resistant tetraploid clone. They suggest that a single dominant gene may confer resistance, but it was not possible to determine the genotypes of the parents. In contrast, when Brown and Thomas (1994) carried out inheritance studies at the diploid level, with the wild species *S. chacoense*, a single dominant resistance locus was identified and parental genotypes were determined based on offspring ratios. Barker (1997) suggested a single dominant resistance gene for PVA, PVX and PVY. It is interesting that there are several examples of two-gene resistance systems in potato. Singh et al. (2000) determined that resistance to PVA in potato cultivars due to two independent genes with complementary gene action. Vallejo et al. (1995) suggested that PVY resistance in a diploid *Phureja Stenotomum* Group population is controlled by complementary action of two dominant genes. Both genes must be present to confer resistance. They also found that two dominant genes control resistance to PVX in *Phureja-Stenotomum* Group hybrids. However, this system exhibits duplicate dominant epistasis, as only one of the two genes is necessary for resistance. Similary, Kriel et al (1995) found complementary gene action is responsible for resistance to ring rot in *S. acaule*. However, genetic screens using virus-induced gene silencing (VIGS) have identified a large number of genes required to induce HR, only subsets (± 10-20%) of these are required for disease resistance (Ooijen, 2007).

2.6.7.2.3. Multiplex resistances

Breeding strategies can be designed to develop genotypes having resistance genes against more then one pathogen or pest (Multiplex resistance I.), or to develop genotypes where alleles of a certain resistance genes can originate from one (Multiplex II/a.) or even from several different wild potato species (Multiplex II/b = Heteromultiplex). Hybrids containing large proportion of wild germplasm may express multiple resistances because wild *Solanum* relatives are rich in disease resistance genes. Jansky and Rouse (2003) identified resistance to several diseases in populations of diploid interspecific hybrids. Chen et al. (2003) identified wild species genotypes with multiple resistances to late blight, Colorado Potato Beetle (*CPB*), and blackleg (*E. carotovora).* Similarly, De Maine et al. (1993) argue that Phureja Group is a valuable source of multiple disease resistance genes. Incorporation of disease resistance genes from difference source of wild potato could release clones or parental lines carrying resistance genes in multiplex state. Solomon-Blackburn and Barker (1993) created clones with strong PLRV resistance by combining genes that limit virus multiplication with those for resistance to infection. Colon et al. (1995) combined minor genes for late-blight resistance from four wild *Solanum* species with diploid Tuberosum Group clones. Murphy et al. (1999) used conventional hybridization between two tetraploid breeding clones, each with different disease resistance traits, to create a clone with resistance to several diseases. Resistance genes frequently encode resistance to some but not all the isolates of a certain pathogen. To create more durable or wider range of resistance genotypes having resistance genes from different sources is advantageous. Mendoza et al. (1996) created parental lines having the Ry gene of *S. tuberosum* ssp. *andigena* in triplex format. Polgár et al. (2002) developed duplex breeding lines where alleles of a resistance gene to PVY originate from *S. stoloniferum, S. hugasii* and *S. tuberosum* ssp. *andigena.*

2.6.7.2.4. Advantage of multiplex resistance

By the use of breeding lines having a resistance gene in duplex, triplex or even quadruplex state as parents, the ratio of resistant genotypes in their progenies from a cross with a susceptible parent can be dramatically increased. Consequently the selection process for the combination of resistance with quality traits can be more effective. In multiplex genotypes if the alleles originates from different sources (eg. different species) the achieved resistance can be more durable compared to genotypes were the resistance is based on one particular allele. Spitters and Ward

(1988) found that resistance to potato cyst nematodes was more durable in clones with two resistance genes instead of one.

2.7. Potato viruses

The worldwide distributed potato viruses are the *Polerovirus Potato leafroll virus* (PLRV), the *potyviruses* PVY and PVA, the *Potexvirus* PVX and the *Carlaviruses* PVM and PVS. The PLRV is probably the most damaging and widespread viruses, while recently the importance of PVY is dramatically increased worldwide due to the appearance of a new tuber necrotic strain PVY^{NTN}. PVY is aphid-transmitted in a non-persistent manner, and hence it is harder to control with aphicides (De Bokx and van der Want, 1987). *Potato virus Y* is the typical member of the genus *Potyvirus* (*Potyviridae* family), containing 128 approved and 89 tentative species (Waterworth and Hadidi, 1998; Rajamaki et al., 2004; Fauquet et al., 2005., Shukla et al., 1994).

2.7.1. Genetics of PVY resistance

In potato, there are two main types of resistance to PVY, extreme resistance (ER) and hypersensitive reaction (HR). Both hypersensitive reaction and extreme resistance (*Ry*) are common type of single gene resistance to PVY (Vreugdenhil et al. 2007; Barker and Harrison, 1984; Ross, 1986; Valkonen et al, 1996). The Ry genes for ER confer extremely high level of protection against different strains of PVY (Ross, 1986; Valkonen et al., 1996). The HR to PVY is strain specific in potato. Hypersensitivity to PVY^0 and/or PVY^N was described in wild *Solanum* species (Valkonen, 1997; Ruiz de Galarreta et al., 1998; Solomon- Blackburn and Barker, 2001). HR was also observed in cultivated potato, however, only after infection with the ordinary strain of PVY (Jones, 1990; Valkonen et al., 1998; Sorri et al., 1999). Potato cultivars expressing HR to PVY^N infection were not reported so far (Valkonen, 2007). The first HR gene, Ny_{tbr}, causing necrotic response to PVY^0 infection in potato mapped on potato chromosome IV (Celebi-Toprak et al., 2002). Szajko et al. (2008) reported the first potato HR gene, which induces necrotic response and restriction of common and necrotic variants of PVY. The gene, designated as Ny-1, was mapped on potato chromosome IX.

Several wild relatives of cultivated potato have been identified as potential source of PVY resistance for breeding programmes:

Solanum stoniferum, S. chacoense, S. tuberosum ssp. *andigena, S. tuberosun* ssp. *tuberosom, S.*

acaule, S. chacachense, S. acroscopicum, S. ambosium, S. arnezii, S. doddsii, S. fernandezianum, S. megistacrolobum, S. plustre, S. polyadenium, S. polytrichon, S. sparsipilum, S. sucrense, S. tarnii, S. trifidum. (Munoz et al., 1975; Horvath and Wolf, 1991; Valkonen et al., 1992; Singh et al., 1994; Bősze et al., 1996; Valkonen, 1997; Takács et al., 1999 ; Flis et al., 2005; Song et al., 2005).

2.7.2. PVY^{NTN}

PVY^{NTN} is a subgroup of PVY^{N} causing potato tuber necrotic ring spot disease (PTNRD) (Beczner et al. 1984). Variants of PVY^{N} designated as PVY^{NTN} infection can induce a rapid and severe systemic veinal necrosis and severely damaged tubers that cannot be marketed or stored (Beczner et al. 1984; Szajko et al., 2008). The symptoms are different from those of two soil-borne viruses: the corky ringspot symptoms caused by the nematode transmitted *tobacco rattle virus* (TRV) and the sprain symptoms caused by potato mop-top virus (PMTV) that is transmitted by the powdery scab pathogen, *Spongospora subterranea* (Jeffries, 1998). Symptom development following PVY^{NTN} infection depends on many factors, all of which are not yet fully elucidated (Le Romancer et al., 1994; Browning et al., 2004). The biological and genetic variability of PVY has been recently reviewed (Glais et al., 2002), but until now, it is unclear which genomic region of PVY is responsible for the tuber necrosis symptoms. Thus, specific detection of PVY^{NTN} using molecular methods is not yet possible. The place where PVY^{NTN} and other strains of PVY are found summarized in Table 1.

Table 1. Strains of PVY and their occurrence in potato

Strain	Common Name(s)	Occurrence in potato
PVY^{o}	Common strain	Worldwide
PVY^{n}	Tobacco Veinal Necrosis Strain	Europe, USSR, Africa, South America (1950's South America), North America (1990 Canada), Japan, Taiwan {PVYn management plan established due to the finding in Canada}, Montana 1999, other states 2000
	Tobacco Necrotic Strain	
PVY^{ntn}	Potato Tuber Ringspot Disease	Whole Europe, North America: California and Pacific Northwest 2000, South- Africa (2000)
	Tuber Necrotic Strain	
	Tuber Necrotic Ringspot Disease	
PVY^{c}	Stipple Streak Strain	Australia, India, UK, and some parts of Europe

2.8. Molecular breeding

The objectives of a plant breeder can be realized through conventional breeding complemented with various biotechnology developments (e.g. Damude and Kinney, 2008; Xu et al., 2009). With the development of molecular tools plant breeding is becoming quicker, easier, more effective and more efficient (Phillips, 2006). Plant breeders will be well equipped with innovative approaches to identify and/or create genetic variation, to define the genetic feature of the genes related to the variation (position, function and relationship with other genes and environments), to understand the structure of breeding populations, to introduce novel alleles or allele combinations into specific cultivars or hybrids, and to select the best individuals with desirable genetic features which enable them to adapt to a wide range of environments.

With the development of DNA-based molecular markers, the extensive genetic mapping of chromosomes became readily possible for several species. The genomes are usually highly similar at the gene order level and this similarity allows the prediction of gene locations among species (Xu et al., 2005). Differences between species of plants are generally not due to novel genes, but to novel allelic specifications and interactions (Xu, 2010).

Molecular genetic markers have been widely employed to identify cryptic and novel genetic variation among cultivars and related species and used to increase the efficiency of selection for agronomic traits and to pyramid genes from different genetic backgrounds. However for heterosis, molecular basis is not understood but it is used as the basis for many seed-producing industries. Genomics and particularly transcriptomics are now being used to identify the heterotic genes responsible for increasing crop yields. Comprehensive quantitative trait locus-based phenotyping (phenomics) combined with genome-wide expression analysis, should help to identify the loci controlling heterotic phenotypes and thus improve the understanding of the role of heterosis in evolution and domestication of crop plants (Lippman and Zamir, 2007), and finally to make it possible to predict hybrid performance.

Genetic modification of crops today involves the interfacing of molecular biology, cell and tissue culture, and genetics breeding. The transfer of genes by cellular and molecular means will increase the available gene pool and lead to second generation biotechnology plant products such as those with a modified oil, protein, vitamin, or micronutrient content or those that have been engineered to produce compounds that can be used as vaccines or anticarcinogens. While all these new innovations have been useful, practical plant breeding continues to be based on hybridization

and selection with little change in the basic procedures (Wollenweber et al., 2005; Xu and Crouch, 2008).

2.8.1. Genetic markers

The concept of genetic markers is not a new one; Gregor Mendel used phenotype-based genetic markers in his experiment in the nineteenth century. Later, phenotype-based genetic markers for Drosophila led to the establishment of the theory of genetic linkage. The limitations of phenotype based genetic markers led to the development of more general and useful direct DNA based markers that became known as molecular markers. With the development of molecular biology, genetic variation can now be identified at the molecular level based on changes in the DNA and their effect on the phenotype instead of visual selection. Molecular changes can be identified by many techniques that have been used to label and amplify DNA and to highlight the DNA variation among individuals.

. Genetic markers are biological features that are determined by allelic forms and can be used as experimental probes or tags to keep track of an individual, a tissue, cell, nucleus, chromosome or gene. In classical genetics, genetic polymorphism represents allelic variation. In modern genetics, genetic polymorphism is the relative difference at any genetic locus across a genome. Genetic markers can be used to facilitate studies of inheritance and variation. Desirable genetic markers should meet the following criteria: (i) high level of genetic polymorphism; (ii) co-dominance (so that heterozygotes can be distinguished from homozygotes); (iii) provide adequate resolution of genetic differences; (iv) clear distinct allele features (so that different alleles can be identified easily); (v) even distribution on the entire genome; (vi) generate multiple, independent and reliable markers; (vii) neutral selection (without pleiotropic effect); (viii) simple, quick and easy detection (so that the whole process can be automated); (ix) low cost of marker development and genotyping; (x) high reproducibility (so that the data can be accumulated and shared between laboratories), (xi) need small amounts of tissue and DNA samples to generate and (xii) have linkage to distinct phenotypes. Most molecular markers belong to the so-called anonymous DNA marker type and generally measure apparently neutral DNA variation. Suitable DNA markers should represent genetic polymorphism at the DNA level and should be expressed consistently across tissues, organs, developmental stages and environments; their number should be almost unlimited; there should be a high level of natural polymorphism; and they should be neutral with

no effect on the expression of the target trait. Finally, most DNA markers are co-dominant or can be converted into co-dominant markers.

In contrast to co-dominant markers, dominant markers are generally based on the feature, that there is no need to have any preliminary sequence information from the analyzed organism. However some markers with the need of preliminary information for their development can also be dominant and vice versa. It depends on the feature of genome at the concrete location. Moreover, dominant markers are generated all over the whole genome sampling multiple loci at one time, providing a high and robust resolution analysis. These methods generate a relatively large number of markers per sample in a technically easy and cost effective way. However, arbitrarily amplified dominant (AAD) markers have been criticized by their negative features that are: i) homoplasy, the co-migration of same size fragments originating from independent loci among different analyzed samples; ii) non-homology, co-migrating bands are paralogous (originate from different positions in different individuals) instead of being ortologous (originate from the same genomic location); iii) nested priming, amplicons result from overlapping fragments; iv) heteroduplex formation, products are also generated from alternate allelic sequences and/or from similar duplicated loci; v) collision, the occurrence of two or more equally sized, but different fragments of an individual; vi) non-independence, a band is counted more than once, due to co-dominant nature or nested priming; vii) artefactual segregation distortions, caused by loci miss-scoring, undetected co-dominance or poor gel resolution (Gort et al., 2009; Bussell et al., 2005; Simmons et al., 2007).

2.8.2. Basic molecular marker techniques

Basic marker techniques can be classified into two categories: (i) non-PCR-based techniques or hybridization based techniques and (ii) PCR-based techniques.

2.8.3. PCR-based techniques

After the invention of polymerase chain reaction (PCR) technology (Mullis and Faloona, 1987), a large number of approaches for generation of molecular markers based on PCR were developed, primarily due to its apparent simplicity and high probability of success. PCR-based techniques can further be subdivided into two subcategories: (i) arbitrarily primed PCR-based techniques or non-sequence specific techniques and (ii) sequence targeted PCR-based techniques.

Sequence-based techniques can further be classified into four subcategories: (i) Repeat sequence-based markers; (ii) mRNA-based markers; (iii) DNA-based markers; (iv) Single nucleotide polymorphism-based markers. The major molecular marker technologies that are currently available listed in Table 2. Only a selection type of markers which utilized in the present study will be discussed. There are several comprehensive reviews that cover all the important DNA markers, e.g. Reiter (2001), Avise (2004), Mohler and Schwarz (2005) and Falque and Santoni (2007). Further information regarding the application of DNA markers in genetics and breeding can be found in Lörz and Wenzel (2009).

Table 2. List of DNA markers.

Hybridization based markers	Restriction fragment length polymorphism (RFLP) Single strand conformation polymorphic RFLP (SSCP-RFLP) Denaturing gradient gel electrophoresis RFLP (DGGE-RFLP)
PCR-based markers	Randomly amplified polymorphic DNA (RAPD) Intron targeting (IT) Start codon targeted (SCoT) Sequence tagged site (STS) Sequence characterized amplified region (SCAR) Random primer-PCR (RP-PCR) Arbitrary primer-PCR (AP-PCR) Oligo primer-PCR (OP-PCR) Single strand conformation polymorphism-PCR (SSCP-PCR) Small oligo DNA analysis (SODA) DNA amplification fingerprinting (DAF) Amplified fragment length polymorphism (AFLP) Sequence-related amplified polymorphism (SRAP) Target region amplified polymorphism (TRAP) Insertion/deletion polymorphism (Indel)
Repeat sequence-based markers	Satellite DNA (repeat unit containing several hundred to thousand base pairs (bp)). Microsatellite DNA (repeat unit containing 2–5 bp). Minisatellite DNA (repeat unit containing more than 5 bp). Simple sequence repeat (SSR) or simple sequence length polymorphism (SSLP). Short repeat sequence (SRS). Tandem repeat sequence (TRS).

Table 2 (*continued*)

mRNA-based markers	Differential display (DD) Reverse transcription PCR (RT-PCR) Differential display reverse transcription PCR (DDRT-PCR) Representational difference analysis (RDA) Sequence tagged sites (STS) Serial analysis of gene expression (SAGE)
Single nucleotide polymorphism based markers	Single nucleotide polymorphism (SNP)

2.8.3.1. Intron targeting structure and method

An efficient strategy to generate gene-specific markers for mapping in plants is the Intron Targeting (IT) method. IT primer pairs are complementary to the sequences of the exons flanking the targeted intron. Since the targeted intron sequence is generally less conserved than the exons, the amplified product may display polymorphism due to length/nucleotide variation among introns in the alleles of the gene. On the other hand, the higher level of sequence conservation in the exons ensures that all alleles can be effectively amplified. If a single targeted intron is too short, primers may be designed to match exons flanking two introns and an internal exon, thereby fostering the detection of length polymorphism. EST-specific primers allow the amplification of genomic DNA across intron regions producing the PCR products that exhibit size or presence/absence polymorphisms. The basic assumption for this strategy is that introns contain more DNA polymorphisms than exons: non-coding regions (introns) evolve much faster than the coding regions (exons) (Small et al., 2004). Therefore, intron-targeting strategy of primer design is expected to yield higher polymorphism frequency (and therefore more efficiency) than other EST–PCR-based conventional strategies. The prerequisite of the method is that the genomic region harboring the gene is sequenced and mRNA, assembled EST consensus or at least EST sequences also exist. The genomic DNA and the EST sequence of a gene in a model organism and the EST sequence from the studied plant can also be sufficient.

ESTs are generated from single-pass sequencing of randomly picked cDNA clones (Adams et al., 1991). The EST approach and subsequent gene-expression profiling (cDNA microarrays) have proven to efficiently identify genes and analyze their expression during different developmental

stages, or under various environmental stresses (Fowler and Thomashow, 2002; Milla et al., 2002; Bhalerao et al., 2003; Dubos and Plomion, 2003; Dhanaraj et al., 2004; Wei et al., 2005). ESTs are also useful for providing markers for genome mapping (Boguski and Schuler, 1995; Hudson et al., 1995; Picoult- Newberg et al., 1999; Eujayl et al., 2002): since they target specific genes, EST-derived markers are particularly useful for QTL analysis, single locus mapping and QTL mapping (if they are used as candisdates for the QTL loci).

The first significant potato EST project was reported by Crookshanks et al. (2001), who analyzed 6077 ESTs, of which 2254 were full length, from a mature tuber cDNA library made from field-grown potatoes (*S. tuberosum* var. Kuras). Ronning et al. (2003) report the sequencing of 61940 ESTs from a wide range of diverse potato tissues, both below and above ground, and including pathogen-challenged material.

2.8.3.2. Start Codon Targeted (SCoT)

In recent years, many new alternative and promising marker techniques have been developed in line with the rapid growth of genomic research (Gupta and Rustgi, 2004). Due to the tremendous growth in public biological databases, the development of functional markers that are located in or near the candidate genes have become considerably easy (Andersen and Lubberstedt, 2003). With initiating a trend away from random DNA markers towards gene-targeted markers, a novel marker system called SCoT (Collard and Mackill, 2009) was developed based on the short conserved region flanking the ATG start codon in plant genes that is conserved for all genes. This method uses single 18-mer primers in single primer polymerase chain reaction (PCR) and an annealing temperature of 50°C. At least 2 min is necessary for extension time because of distance in base pairs between primer binding sites of the template. PCR amplicons are resolved using standard agarose gel electrophoresis. This method was validated in rice using a genetically diverse set of genotypes and a backcross population. Reproducibility test using duplicate samples and conducting PCR on different days revealed that SCoT markers are generally reproducible, and it is suggested that primer length and annealing temperature are not the sole factors determining reproducibility. They are dominant markers like RAPDs and could be used for genetic analysis, quantitative trait loci (QTL) mapping and bulk segregation analysis (Collard and Mackill, 2009). However, it is feasible that some SCoT markers would be co-dominant due to insertion-deletion mutations; these would be the minority like co-dominant RAPDs (Davis et al., 1995). In principle,

SCoT is similar to RAPD and ISSR because the same single primer is used as the forward and reverse primer (Collard and Mackill, 2009; Gupta et al., 1994). However, PCR amplification using SCoT primers targets gene regions surrounding the ATG initiation codon on both DNA strands. Due to the basis of SCoT primer design, it is expected that SCoT markers to be distributed within gene regions that contain genes on both plus and minus DNA strands. It is also possible that pseudogenes and transposable elements may be used as primer binding sites by SCoT polymorphism technique.

.

2.8.3.3. Single Strand Conformation Polymorphism (SSCP)

Single strand conformation polymorphism is the mobility shift analysis of single-stranded DNA sequences on neutral polyacrylamide gel electrophoresis, to detect polymorphisms produced by differential folding of single-stranded DNA due to subtle differences in sequence (often a single base pair). SSCP is one of the easiest methods for detecting an SNP (Orita et al., 1989; Dean et al., 1990). Since this method can detect an SNP in only small DNA fragments (100 bp - 400 bp) many primer pairs are therefore necessary for the screening of a point mutation in large genes of more than 2 kb by the polymerase chain reaction (PCR)-SSCP. SSCP is most effective for DNA with a relatively high GC content. SSCP analysis of DNA with intermediate GC content will be facilitated by cooling the gel to 4°C during electrophoresis. The maximum size of DNA used for SSCP analysis is directly proportional to its GC content. With low GC content, the maximum DNA fragment size is less than 200 bp.

In the absence of a complementary strand, the single strand experiences intra strand base pairing, resulting in loops and folds, that gives it a unique 3D structure which can be considerably altered due to single base change resulting in differential mobility (Orita et al., 1989). The SSCP analysis proves to be a powerful tool for assessing the complexity of PCR products as the two DNA strands from the same PCR product (Hayashi, 1992) often run separately on SSCP gels, thereby providing two opportunities to score a polymorphism and secondly, resolving internal sequence polymorphisms in some PCR products from identical places in the two parental genomes. The PCR-based SSCP analysis is a rapid and sensitive technique for detection of various mutations, including single nucleotide substitutions, insertions and deletions, in PCR-amplified DNA fragments (Hayashi, 1993). Thus, it is a powerful technique for gene analysis particularly for detection of point mutations (Fukuoka et al., 1994). The technique shares similarity to RFLPs as it

can also decipher the allelic variants of inherited and genetic traits. However, unlike RFLP analysis, SSCP analysis can detect DNA polymorphisms and mutations at multiple places in DNA fragments. The SSCP gels have been used to increase throughput and reliability of scoring during mapping by PCR fingerprinting in plants (Li et al., 2005). Fluorescence-based PCR-SSCP (F-SSCP) is an adapted version of SSCP analysis involving amplification of the target sequence using fluorescent primers (Makino et al., 1992). The major disadvantage of the technique is that the development of SSCP markers is labor intensive and costly and cannot be automated.

2.8.3.4. Inter-Simple Sequence Repeat (ISSR)

Inter-simple sequence repeat is a PCR technique that uses repeat-anchored or non-anchored primers to amplify DNA sequences between two inverted SSR (Zietkiewicz et al., 1994). They are arbitrary multiloci markers produced by PCR amplification with a microsatellite primer. Amplification in the presence of non-anchored primers also has been called microsatellite-primed PCR, or MP-PCR, (Meyer et al., 1993). Such amplification does not require genome sequence information and leads to multilocus and highly polymorphous patterns (Zietkiewicz et al., 1994; Tsumara et al., 1996; Nagaoka et al., 1997). ISSR markers do not require a prior knowledge of the SSR targets sequences, are universal, easy to handle, highly reproducible due to their primer length and to the high stringency achieved by the annealing temperature and were found to provide highly polymorphic fingerprints (Zietkiewicz et al., 1994; Kojima et al., 1998; Bornet and Branchard, 2001). ISSR is also a 'quick and dirty' method with enough resolution to distinguish genotypes within a relatively narrow range of genetic diversity. It is cheap and simple (fingerprints can be generated with simple agarose gel electrophoresis) and therefore can be used for routine variety identification. ISSR markers will be useful for genetic diversity and study of interspecific and intraspecific relationships in plant breeding (Bornet and Branchard, 2001). They have been successfully used for the assessment of genetic diversity in corn and bean (Kantety et al., 1995; Galván et al., 2001), for cultivar identification in oilseed rape and potatoes (Charters et al., 1996; Bornet et al., 2002), for mapping of plant chromosomes (Kojima et al., 1998) and for linkage to a specific gene (Akagi et al., 1996).

2.8.3.5. Random Amplified Polymorphic DNA (RAPD)

The basis of RAPD technique is differential PCR amplification of genomic DNA. It deduces

DNA polymorphisms produced by ‘‘rearrangements or deletions at or between oligonucleotide primer binding sites in the genome’’ using short random oligonucleotide sequences (mostly ten bases long with 50-80% GC) (Williams et al., 1991). As the approach requires no prior knowledge of the genome that is being analyzed, it can be employed across species using universal primers. The major drawback of the method is that the profiling is dependent on the reaction conditions so may vary within two different laboratories and as several discrete loci in the genome are amplified by each primer, profiles are not able to distinguish heterozygous from homozygous individuals (Bardakci, 2001). Due to the speed and efficiency of RAPD analysis, high-density genetic mapping in many plant species such as alfalfa (Kiss et al., 1993), faba bean (Torress et al., 1993) and apple (Hemmat et al., 1994) was developed in a relatively short time. The RAPD analysis of NILs (near-isogenic lines) has been successful in identifying markers linked to disease resistance genes in tomato (*Lycopersicon* sp.) (Martin et al., 1991), lettuce (*Lactuca* sp.) (Paran et al., 1991) and common bean (*Phaseolus vulgaris*) (Adam-Blondon et al., 1994). Arbitrarily primed polymerase chain reaction (AP-PCR) and DNA amplification fingerprinting (DAF) techniques are independently developed methodologies, which are variants of RAPD. For AP-PCR (Welsh and McClelland, 1990) a single primer (about 10–15 nucleotides long) is used. The technique involves amplification for initial two PCR cycles at low stringency. Thereafter the remaining cycles are carried out at higher stringency by increasing the annealing temperature). This variant of RAPD was not very popular as it involved autoradiography but it has been simplified as fragments can now be fractionated using agarose gel electrophoresis. The DAF technique involves usage of single arbitrary primers shorter than ten nucleotides for amplification (Caetano-Anolles and Bassam, 1993) and the amplicons are analysed using polyacrylamide gel along with silver staining.

2.8.3.6. Application of primer pair

The use of primer pairs would be possible to obtain additional amplified bands compared to single arbitrary primers. Theoretically, four times more bands would be expected from a single amplification reaction using two primers, than by using each primer individually in separate reactions (Williams et al., 1993). Such applications would also enable the appearance of novel amplified products which are absent from single primer reactions, as it was previously suggested in the case of RAPD by Williams et al., (1993). The simultaneous use of different primers in a single reaction would also enable the development of several new combinations from the same primer

sets and thus reducing research costs.

2.9. Molecular Markers, Maps and Population Genetics of Potato

2.9.1. Molecular markers, fingerprinting

DNA-based genetic markers essentially detect point mutations, insertions, deletions or inversions in allelic DNA fragments, which can differentiate the individuals of the same species. These DNA polymorphisms are usually selectively neutral and hence a transient phase of molecular evolution, in which they are maintained in a species by mutational input and random extinction. DNA-based markers show Mendelian inheritance, are available in unlimited numbers and most of them are phenotypically neutral. DNA fingerprinting for cultivar or variety identification using DNA-based genetic markers has become an important tool for genetic identification in plant breeding and germplasm management (Jondle, 1992; Smith, 1998). One of the most important decisions for DNA fingerprinting is the marker system and technique to be used. Prevost and Wilkinson (1999) concluded that ISSR-PCR gives a quick, reliable and highly informative system for DNA fingerprinting of potato. Demeke et al. (1993) used RAPD markers to identify potato cultivars. They discovered that RAPD technique is suitable for the detection of polymorphism, regardless of tissue or environment factors and is a highly useful method to distinguish and identify potato cultivars and clonal variation of cultivars. Sosinski and Douches (1996) also used RAPD technique to fingerprint North American potato cultivars. Their result showed that all cultivars were discriminated with as few as 10 primers and could distinguish the Russet Burbank from a white-skinned clone by one band. Menendez et al. (2002) proved that AFLP markers are very well suited to quickly generate a large number of segregating DNA markers for fingerprinting and linkage analysis. AFLPs are the markers of choice for linkage map construction in segregating populations derived from crossing tetraploid parents (Meyer et al., 1998) and when aiming at a high-density map (Isidore et al., 2003) to aid map-based cloning and physical mapping. Another marker type which is useful for fingerprinting of potato is simple sequence repeat (SSR). SSR markers are highly informative and helpful for fingerprinting and linkage studies in potato because potato genotypes are highly heterozygous for multiple SSR alleles (Provan et al., 1996; Milbourne et al., 1998). All four possible genotype classes in diploid F1 progeny may be identified, similar as found for RFLP markers. Under very good circumstances

of amplification, separation and visualization, the dosage of co-dominant SSR alleles can be assessed in heterozygous tetraploid potato cultivars by band intensity. McGregor et al. (2000) compared RAPD, ISSR, AFLP and SSR markers for fingerprinting in tetraploid potato. Their results showed that all the techniques could individually identify all the cultivars, but the mean number of bands generated per primer (or primer pair) for each cultivar was different.

2.9.2. Genetic linkage map

Genetic linkage maps are essential to identify either low copy number loci affecting certain phenotypes and QTL as well and moreover are valuable in basic genetic studies or in applied breeding programs, especially for the identification and selection of genotypes with specific combinations of favorable traits. To develop a linkage map by classical genetic markers like morphological traits, several segregating populations are required, as only a limited number of loci segregates in each population. With the initiation of molecular markers, unlimited amounts of segregating loci became available from a single cross. For outbreeding species like potato, heterozygous parents are used to obtain segregating populations and mapping can be carried out in the F1 progeny of a single cross.

2.9.3. First-generation genetic linkage maps of potato

Despite the fact that the modern cultivated potato is autotetraploid, the vast majority of molecular marker-based genetic linkage mapping in potato to date has been carried out at the diploid level in order to circumvent the complexities associated with tetrasomic inheritance. The first published molecular-marker-based genetic linkage maps of potato were based on restriction fragment length polymorphisms (RFLPs). Bonierbale et al. (1988) took advantage of the synteny between the genomes of tomato and potato by employing tomato-derived cDNA and gDNA probes that had been used previously to construct an RFLP-based map in tomato (Bernatzky and Tanksley, 1986).

One of the early illustrations of the utility of PCR-based markers for map construction in potato was the use by Van Eck et al. (1995) of the highly multiplex amplified fragment length polymorphism (AFLP) technique (Vos et al., 1995) to add a further 264 markers, using only six AFLP primer combinations, to the maps constructed by Jacobs et al. (1995). Subsequently, Rouppe van de Voort et al. (1997) demonstrated that AFLP-based maps produced in fairly unrelated

diploid *S. tuberosum* genotypes may be aligned relative to each other by virtue of the fact that AFLP markers of the same size (electrophoretic mobility) produced by the same primer combination in different genotypes are generally allelic and will map to homologous positions. Microsatellite- or simple sequence repeat (SSR)-based markers combine the convenience of a PCR-based assay with the co-dominant and locus-specific nature of RFLPs and have become the system of choice for linkage mapping in most well-characterized plant species. Milbourne et al. (1998) characterized SSR loci in potato, from a mixture of database searches, cDNA libraries and selectively enriched small insert libraries. To date, the utility of SSR-based markers in potato has been limited by the relatively low numbers that have been mapped.

2.9.4. Second-generation linkage map in tetraploid potato

Originally, most molecular-marker-based linkage analyses were performed in populations derived from the F1 generations of crosses between two inbred (homozygous) diploid parents (e.g. BCs, F2, RILs and dihaploids). This is due to the fact that many agronomically important crop species are fully self-fertile and that in such populations linkage analysis is simplified because only two alleles segregate per locus and the linkage phase (coupling or repulsion) of all markers is known. Linkage analysis is more complicated in populations derived from the progeny of crosses between non-inbred parents of an outbreeding species. Markers vary in the number of segregating alleles, one or both parents may be heterozygous at a locus, and frequently the linkage phase of the markers is unknown (Maliepaard et al., 1997). This is the case in diploid potato crosses, which have been used to avoid the complicating factor of tetrasomic inheritance in most mapping studies to date. Diploid potatoes are highly heterozygous and generally self-incompatible, precluding the possibility of obtaining inbred lines. Formulae for the calculation of recombination fractions and methods for the construction of genetic linkage maps in crosses between heterozygous parents have been presented by Ritter et al. (1990) and Ritter and Salamini (1996). In addition, most computer packages used to construct genetic linkage maps can now deal with segregation data obtained from crosses between heterozygous parents for both dominant and codominant markers, eg. Join Map (Stam and Van Ooijen, 1995). However, most potato breeding is carried out at the tetraploid level, and much variation for traits of interest to breeders is present in cultivars and advanced tetraploid breeding lines. Thus, the ability to perform linkage and QTL analysis at the tetraploid level in potato would be extremely useful in a practical breeding context.

The autotetraploid nature of potato implies the random pairing of four homologous chromosomes at meiosis. This, in conjunction with its high level of heterozygosity, results in a large number of possible allelic combinations at a single locus. In the most extreme case, with four independent alleles contributed by each parent, 36 possible genotypic classes can be found in the progeny. It is the computational difficulty in resolving this number of possible genotypic classes, and the subsequent problem of following recombination events between two such loci (as well as the occurrence of quadrivalents, and double reduction), that has discouraged linkage analysis at this ploidy level.

2.10. Quantitative trait loci (QTL)

QTL mapping is the statistical study of the alleles that occur at a locus and the phenotypes (physical forms or traits) that they produce. Because most traits of interest are governed by more than one gene, defining and studying the entire set of genes related to a trait gives hope to understand real effect of the genotype of an individual. Early QTL studies were based on manipulations of whole chromosomes, including substitution of one chromosome from one inbred line into another. The approach was refined to apply to small segments of chromosomes, delineated first by morphological markers (Thoday, 1961). The earliest QTL mapping studies in tetraploid potato (Bradshaw et al., 1998; Meyer et al., 1998) were based on testing for associations at single markers using regression-based approaches. However, unless there is sufficiently dense genome coverage by markers, or unless the marker and QTL are tightly linked in coupling, single-marker analysis will still result in a low resolution of QTL location.

As large numbers of molecular markers become available and thus the whole genome mapping of quantitative traits become feasible, Xu (1997) described how multiple quantitative trait loci (QTL), either clustered together or dispersed in different chromosomes, can first be dissected by molecular marker-assisted QTL mapping and selection and then pyramided into one genetic background, either by marker-assisted selection (MAS) or transformation of cloned multiple QTL..

2.10.1. Marker-based QTL mapping

Before the discovery of molecular markers, marker-based analysis utilized the data from single markers (e.g. Sax, 1923). Here, only one or a few markers could be analysed in an experiment because the number of markers available at that time was limited. Further, most of them were

morphological or biochemical markers making it impossible to construct a complete linkage map by using single or even multiple populations. With the development of high density molecular maps, it became apparent that simple (one-locus) marker-based analysis alone could not fully utilize the genetic information harboured in complete linkage maps for QTL mapping. To fully exploit the potential of complete linkage maps to locate QTL more efficiently and accurately, many QTL mapping approaches have been developed using multiple markers simultaneously. The following references are highly recommended for a full coverage of QTL mapping statistics: Thoday (1961); Soller and Beckmann, 1990; Xu and Zhu (1994); Lynch and Walsh (1998); Liu (1998); Sorensen and Gianola (2002) and Wu and Casella (2007).

2.10.2. Interval modeling mapping

A marker close to a QTL of small effect will give the same signal as a marker some distance from a QTL of large effect. Lander and Botstein (1989) developed a QTL mapping method known as interval mapping, by using two flanking markers, which overcomes the three disadvantages of analysis of variance at marker loci. The method makes use of a genetic map, like analysis of variance and assumes the presence of a single QTL between two marker loci. Van Ooijen (1992) described this method in more detail to make it more understandable, while Xu et al. (1995) extended the statistics issues to DH populations. Interval mapping is currently the most popular approach for QTL mapping in experimental crosses. Bradshaw et al. (2008) applied interval mapping of quantitative trait loci for 16 yields, agronomic and quality traits in potato and totally identified 39 QTLs.

Most of the single-QTL methods can be extended to multiple QTL by conditioning additional marker loci and using conditional probabilities for multi-locus genotypes. This approach has been used to develop explicit models for two or three linked QTL (e.g. Knapp, 1991; Haley and Knott, 1992; Martinez and Curnow, 1992; Kearsey and Hyne, 1994; Wu and Li (1994, 1996); Hyne and Kearsey, 1995; Jansen, 1996; Satagopan et al., 1996).

2.10.3. Multiple interval mapping

Genetic mapping approaches involving multiple QTL have been developed. In general, there are three different approaches: (i) maximum likelihood using EM include multiple interval mapping (MIM) (Kao and Zeng, 1997) and sequential testing to search model space; (ii) multiple

imputation (Sen and Churchill, 2001) uses Bayesian log posterior odds and sequential testing and pairwise plots to search; and (iii) Markov chain Monte Carlo (MCMC) (Satagopan et al., 1996) employs Markov chain sampling to search model space. MIM based on Kao and Zeng (1997) and Kao et al. (1999) is a multiple-QTL oriented method combining QTL mapping analysis with the analysis of genetic architecture of quantitative traits through a search algorithm to search for number, positions, effects and interaction of significant QTL. Using markers for simultaneous multiple QTL analysis was suggested first by Lander and Botstein (1989), although the idea was pursued only with a very limited scope. Bayesian statistics via MCMC for mapping QTL is also based on multiple QTL, particularly when it is combined with a reversible-jump process. MIM consists of four components:

-an evaluation procedure to analyse the likelihood of the data given a genetic model (number, position and epistasis of QTL);

-a search strategy to select the best genetic model (among those sampled) in the parameter space;

-an estimation procedure to estimate all parameters of interest in the genetic architecture of quantitative traits (number, positions, effects and epistasis of QTL; genetic variances and covariances explained by QTL effects) given the selected genetic model; and

-a prediction procedure to estimate or predict the genotypic values of individuals based on the selected genetic model and estimated genetic parameter values for MAS.

2.11. Resistance factors mapped in potato

Numerous genes conferring resistance to viruses, nematodes, bacteria, fungi, oomycetes, and insects have been mapped in potato. Genes for extreme resistance to PVX and PVY originating from at least four different potato species have been placed on the potato molecular map. Some of PVY resistance genes that mapped in potato so far are as follows:

Ry_{sto}

Ry_{sto}^{a} and Ry_{sto} (designated also as Ry-f_{sto}) were mapped on chromosome XI and XII respectively (Brigneti et al., 1997; Flis et al., 2005; Song et al., 2005; Valkonen et al., 2007; Cernak et al., 2008). Ry_{sto}^{a} reside in the resistance gene hotspot on the long arm of chromosome XI (Brigneti et al., 1997; Hamalainen et al., 1998 and 2000) in the same region, where Ry_{adg} was

mapped (Gebhardt and Valkonen, 2001). Recently, however, the Ry_{adg} linked markers used in the *S. stoloniferum* mapping experiments did not confirm the position of Ry_{sto}^{a} on potato chromosome XI (Valkonen et al., 2007). GP58 and STMOOO3d were recommended as anchor markers for Ry_{sto}^{a} and Ry_{sto}, respectively (Brigneti et al., 1997; Song et al., 2005).

Ry-f_{sto}

The gene Ry-f_{sto} has been obtained from *Solanum stoniferum* a species of wild potato originating in Mexico (Hawkes, 1990) and mapped to chromosome XII (linkage group XII)(Hawkes, 1990., Flis et al., 2005; Song et al., 2005). It is known as an extreme resistance gene and conferring resistance to potato virus Y (Witek et al., 2006). Ry-f_{sto} and Ry_{sto} genes that are located on chromosome XII might be identical (Vreugdenhil et al., 2007). GP122 is used as an anchor marker for Ry-f_{sto} (Flis et al., 2005).

Ry_{adg}

The Ry_{adg} encoding resistance to PVY in potato is inherited monogenically with a dominant fashion showing no strain specific resistance (Ross, 1958; Munoz et al., 1975). Ry_{adg} derived from *S. tuberosum* ssp. *andigena* growing in South America (Munoz et al., 1975; Plaisted and Hoopes, 1989) mapped on long arm of potato chromosomes XI (Hämäläinen et al., 1997; Brigneti et al., 1997). The Ry_{adg} gene is located in the known resistance gene cluster containing S_{enl}, R_{mcl}, Na_{adg}, etc. (Gebhardt and Valkonen, 2001). GP58 proved as an anchor marker for Ry_{adg} (Hamalainen et al., 1997).

Ny_{tbr}

Loci that control limited necrotic response (hypersensitivity) may lead to resistance against the virus and will limit the spread of the virus to another plants are designated as N genes according to the convention for potato (Valkonen et al., 1996). S. t*uberosum* ssp. *tuberosum* native to Chile is used as a source of resistance (Spooner and Bamberg, 1994). Ny_{tbr} is monogenic dominant and mapped to chromosome IV between the TG316 and TG208 markers at LOD=2.72. It is a dominant hypersensitivity gene against PVY^{o}. This location does not correspond to any other mapped resistance genes in potato (Celebi-Toprak et al., 2002). The closet marker linked to Ny_{tbr} is TG506 (map distance 3.4 cM). On the other side of Ny_{tbr} the closet marker is Tg208 with a map distance of 12.4 cM (Celebi-Toprak et al., 2002).

Ry_{chc}

The gene Ry_{chc} was localized on chromosome IX and originates from *S. chacoense* (Sato et al., 2006; Asama et al., 1982). Ry_{chc} was mapped to the most distal end of the chromosome IX where the recovery of recombinant genotypes is extremely reduced. The location of Ry_{chc} is different from those of the other extreme resistance genes to PVY, but possibly resides in resistance gene cluster (Sato et al., 2006). Ry_{chc} is inherited in a dominant, monogenic fashion. CT220 is known as an anchor marker for Ry_{chc} (Hosaka et al., 2001). Although, the exact map distance between Ct220 and Ry_{chc} is unknown due to the unexpectedly suppressed recombination frequency (Sato et al., 2006). The extreme resistance gene Ry_{chc} also mapped recently on potato chromosome IX in Japanese cv. Konafubuki and showed tight linkage with the marker TG421 (Sato et al., 2006)

Ny-1

Ny-1 is the first gene that confers HP in potato plants to common and necrotic strains of PVY. Expression of HP was temperature–dependent in cv. Rywal (*Solanum tuberosum ssp*. *tuberosum*, 2n=4x=48).The locus *Ny-1* mapped on the short arm of potato chromosome IX. Screening of F1 individuals using $SC895_{1139}$ (SCAR marker) indicated a close linkage (0.5 cM) between the marker and locus *Ny-1* (Szajko et al., 2008).

2.12. Markers assisted breeding

Selection of individuals with desirable traits from a breeding population can be based on phenotype, genotype, or a combination of both. Phenotypic selection is more efficient for a trait with high heritability because it uses the sources of variation of all the loci, while markers can use only those loci to which they are linked (Charcosset and Gallais, 2003). Marker assisted selection (MAS) will be more effective than phenotypic selection when the proportion of additive variance accounted for by the marker loci is greater than the heritability of the trait (Dudley, 1993). Computer simulation shows that MAS can be more efficient than selection based only on a phenotype if the heritability of the trait is between 0.05 and 0.5 and the markers are close to the loci of interest (Moreau et al., 1998).

In marker-assisted breeding the plant breeder takes advantage of the association between agronomic traits and allelic variants of genetic, mostly molecular, markers. In the case of limited number of alleles affect the phenotype and they have major effects on the phenotype, such as a

single gene-based disease resistance, the assessment of association is straightforward: mapping a monogenic trait goes along with the mapping of markers. . In this case, breeders depend on a direct relationship between genotype and phenotype to monitor the presence of the desired alleles in the populations to be studied. For quantitative traits, however, a reliable assessment of trait–marker association requires large scale field experiments as well as statistical techniques, known as quantitative trait loci (QTL) mapping. Once marker–trait associations have been reliably assessed, the breeder is able to monitor the transmission of trait genes via closely linked markers.

Compared to phenotypic assays, as summarized from Xu (2002), Peleman and van der Voort (2003), Xu (2003) and Xu and Crouch (2008), DNA markers offer great advantages to accelerate the cultivar development time as a result of the following.

1. Increased reliability: error margins on the measurement of phenotypes tend to be significantly larger than those of genotyping scores based on DNA markers.

2. Increased efficiency: DNA markers can be scored at seedling stage or even based on seed before germination. By selecting at the seedling stage or based on seed DNA, considerable amounts of time and space can be saved.

3. Reducing costs: there are ample traits where the determination of the phenotype costs more than the performance of genotyping using a PCR assay or hybridization. Every plant that can be rejected before planting, particularly for those with the seed that is big enough for single seed-based DNA extraction, will in such settings save a considerable amount of money (Xu, 2010).

2.12.1. Components of marker-assisted selection

Key issues in successful deployment of molecular markers in MAS are the follows summarized based on the studies by Xu (2003) and Mohler and Singrün (2004):

1. Markers should co-segregate or map as close as possible to the target gene (e.g. less than 2 cM), in order to have low recombination frequency between the target gene and the marker. Accuracy of MAS will be improved if, rather than a single marker, two markers flanking the target gene are used. Ideally, gene-based markers that are developed from the sequence of the target gene, or functional markers that reveal functional differences associated with the target gene, are more preferred as segregation between the marker and the target gene will no longer exist or will be reduced to a minimum.

2. For unlimited use in MAS, markers should display polymorphism between genotypes that

have and do not have the target gene.

3. Cost-effective, simple and high-throughput markers are required to ensure genotyping power needed for the rapid screening of large populations. Hybridization-based non-PCR markers that can reveal difference from DNA samples directly would be more preferred. In addition, marker-assisted background selection depends on molecular markers that are well characterized and distributed over the whole genome. It is most desired to use gene-based markers for both marker-assisted foreground and background selection. In this case, a core set of markers can be established for both purposes so the same markers can be used for foreground selection in some crosses but background selection in others. As summarized by Xu (2003), there are five key components that are required for efficient MAS, including: (i) suitable genetic markers and their characterization; (ii) high-density molecular maps; (iii) established marker–trait associations for traits of interest; (iv) high-throughput genotyping systems; and (v) functional data analysis and delivery.

2.12.2. Marker characterization

It is not enough to just have thousands of genetic markers in hand. To use molecular markers efficiently, they have to be characterized for many features, including: number of alleles; polymorphism information content (PIC); allelic difference (e.g. allele sizes and their range); allele feature (e.g. haplotypes) in standard or control cultivars; signal strength under specific genotyping conditions; background or noise signal; PCR or hybridization conditions; chromosome location (flanking markers and genetic distances); and information required for multiplexing.

Characterization of molecular markers helps to identify markers close to the genes of interest and to evaluate germplasm and breeding materials. A core set of molecular markers should be characterized for each plant species and these markers should be evenly distributed on all chromosomes and suited for multiplexing. Many crop plants have now established core-set markers and have been used for evaluation of germplasm accessions, construction of heterotic pools and MAS (see Xu (2003) for an example in rice). Many efforts have been made to characterize array-based markers and optimize genotyping systems.

2.12.3. Validation of marker–trait associations

QTL markers identified using a single mapping population may not be automatically used

directly in unrelated populations without marker validation and/or fine mapping (Nicholas, 2006; Knoll and Ejeta,2008). The marker–trait association must be validated, in representative parental lines, breeding populations and phenotypic extremes before it can be used for routine MAS, particularly for QTL with relatively small effects. In some instances, markers will lose their selective power during this validation step. In these cases, it is necessary to identify new markers (through fine mapping or candidate gene analysis) around the target locus in order to find marker–trait associations that are shared across different breeding populations.

For more precise genotypic selection of complex traits such as the minor-gene controlled abiotic stress tolerance, more closely linked markers, preferably gene-based markers, or even better, functional nucleotide polymorphic markers (Rockman and Wray, 2002; Andersen and Lübberstedt, 2003; Dwivedi et al., 2007), need to be developed. This should be combined with precise phenotyping in order to maximize the power of detection and minimize the chance of false negatives.

2.13. Subtraction Suppressive Hybridization (SSH)

Suppression subtractive hybridization (Diatchenko et al., 1996) is a technique that uses PCR to quickly compare the expression of mRNA from different samples and show the relative difference in the concentration of these molecules. It is a powerful technique that enables researchers to compare two populations of mRNA and obtain clones of genes that are expressed in one population but not in the other. This technology has the advantage over microarray technology in being an ‘open’ technology, with the possibility of discovering ‘new’ transcripts. It can be used to enrich for differentially expressed genes. Subtracted cDNA libraries are hybridization and PCR based and result in normalization of the sample. They can be combined with full length cDNA libraries. Faivre-Rampant et al. (2004) have used the SSH approach to make a cDNA library enriched for 385 different genes that are up-regulated in the potato tuber apical bud on dormancy release. One of these cDNAs was identified as encoding a member of the auxin response factor family (ARF6), and the expression pattern of this gene was determined byISH.

The principle of the technique is shown in Fig. 1: (i) prepare cDNAs from two stages/ conditions; (ii) separately digest tester (from the same source as sample to be tested) and driver cDNA (from a normal sample) to obtain shorter fragments; (iii) divide tester cDNA into two portions and ligate each to a different adaptor, while driver cDNA has no adaptors; (iv) hybridization kinetics lead to

equalization and enrichment of differentially expressed sequences among single strand tester molecules; and (v) ultimately generate templates for PCR amplification from differentially expressed sequences. As a result, only differentially expressed sequences are amplified exponentially.

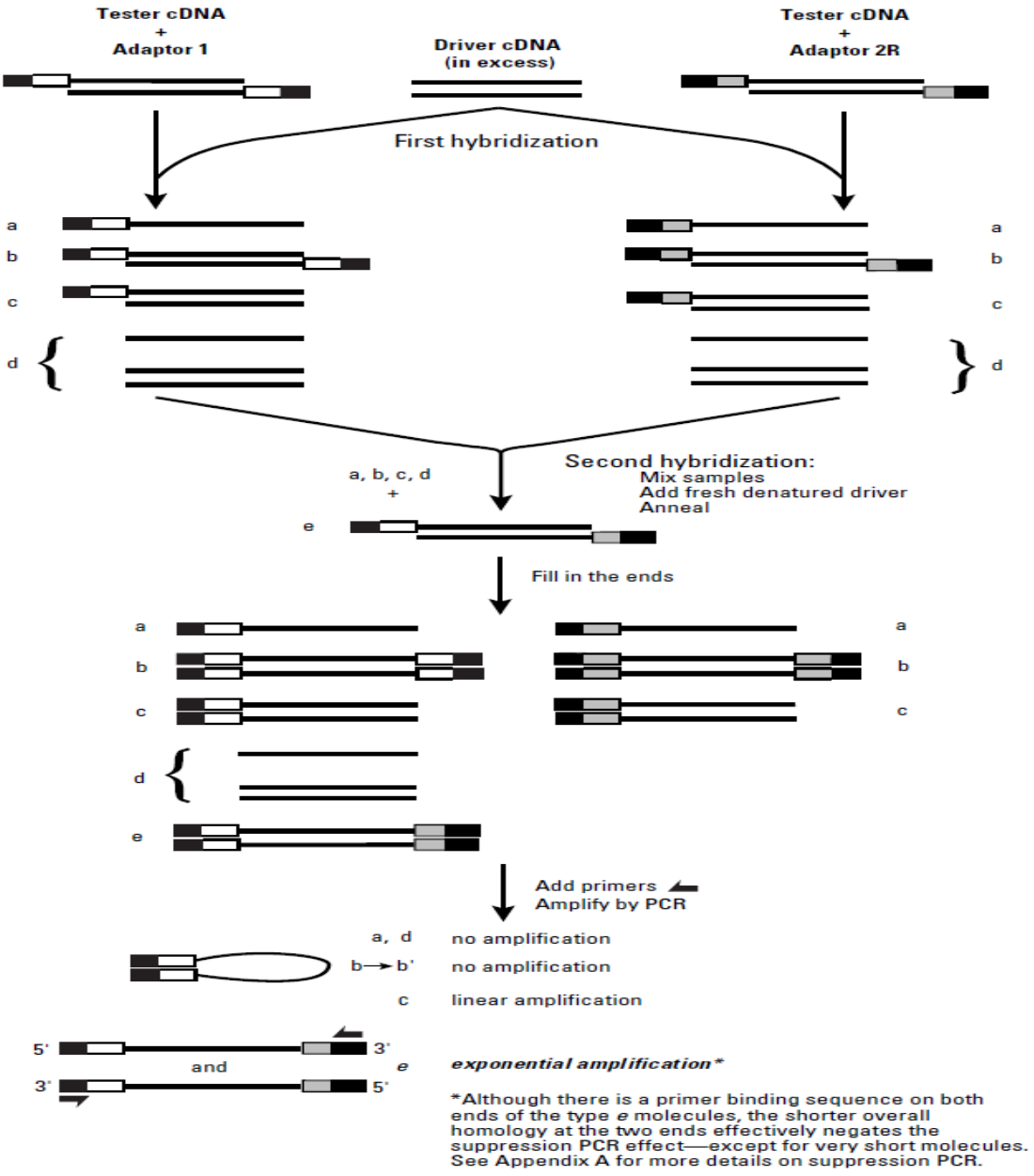


Fig.1. Suppression subtractive hybridization (SSH) (Clontech catalog No: 637401)

3. MATERIALS AND METHODS

3.1. Plant material

Twenty four potato varieties (Table 3) and randomly selected 85 genotypes from a tetraploid F_1 potato population were used in this study. All the F_1 genotypes were developed in the Potato Research Center (University of Pannonia, Keszthely, Hungary) from a cross between White Lady as female and S440 as male parent. Based on the reaction under field conditions, these parental genotypes differ in their reaction to natural heat, drought stress and resistance to PVY^{NTN}. WL is resistance to PVY^{NTN} and tolerant to natural heat and drought stress. In contrast, S440 is susceptible to PVY^{NTN} and showed low tolerance to natural heat and drought stress.

Table 3. Potato cultivars used in this study and their country of origin

Cultivar	Cultivar	Cultivar	Cultivar
Gülbaba (HU)	Sante (NL)	Lorett (HU)	Panda (G)
Somogyi kifli (HU)	Snowden (USA)	S440 (USA)	Lvovjanka (RU)
Atlantic (USA)	Franzi (G)	WL (HU)	Kennebec (USA)
Desiree (NL)	Agria (G)	Irga (P)	Linzer delicate (AU)
Shepody (CA)	Rioja (HU)	Kondor (NL)	Saturna (NL)
Swiss (USA)	Katica (HU)	Cleopatra (NL)	Vénusz Gold (HU)

(G = Germany, HU = Hungary, NL = Netherlands, CA = Canada, P = Poland, AU = Austria, RU = Russia and USA = United States of America)

3.2. Hetero multiplex study

Seven PVY resistant breeding lines (line 96.353., 97.557., 97.559., 97.560., 98.433., 99.373., 99.384), bred by the Potato Research Center, carrying the Ry gene (originated from *S. stoloniferum* (*S.sto), S. hougasii (S.hou)* and. S. a*ndigena* (*S.and))* potentially in hetero multiplex state based on their pedigree data were crossed with a PVY susceptible line, S440. In the resistance test about 200 genotypes of each family were evaluated in three replications after mechanical inoculation with the Hungarian PVY^{NTN} isolate D-10. Virus level was determined by DAS-ELISA after 4 weeks of

infection. The copy number of Ry genes was deduced from the ratio of resistant and susceptible plants. Chi square test (X^2) was used to prove the fitness of observed segregation ratios to predicted ones.

3.3. Assessment of virus infection using the classical double sandwich ELISA technique

During the first step of incubation the surface of the microtiter plate is coated with the antigen specific antibody. During the second incubation step, the antigen bound to the fixed antibody forming the antibody-antigen complex. During the third incubation step the antibody-antigen complex reacted with the AP-labeled antibody forming the double antibody sandwich. The positive as well as negative controls added to the plate. To determine the healthy background, the fresh extract of healthy plant (White Lady) also added to the plate. The enzymatic reaction monitored at 405 nm, after 2 hours.

3.4. Osmotic stress assay

To discriminate stress sensitive and tolerant parents *in vitro* osmotic stress conditions were optimized. At first 40, three leaf stage shoot tips of WL and S440 were grown on basic MS media and cultured individually in glass tubes containing 10 ml basic MS media supplemented with different concentration of mannitol (0 as control, 0.15 mol/dm^3, 0.2 mol/dm^3 and 0.3 mol/dm^3, 10 plants/concentration) at 16/8 hours illumination and 20^{o}C. The study was carried out as a factorial experiment based on a completely randomized design, 10 replications and two factors. After 10 days data were recorded for root number and root length. In the second experiment osmotic stress tolerance of 85 F_1 genotypes from the cross of WL and S440 was tested under the same conditions but only at the selected 0.3M concentration of mannitol where significant difference was detected between the two parents in the first experiment.

3.5. Genomic DNA isolation

Genomic DNA was extracted from 80 mg of leaf and stem tissue of *in vitro* plants using the modified procedure (Gorji et al. unpublished) of Walbot and Warren (1988) as follows:

3.5.1. Lysis of plant cells and protein denaturation

1. Grind 50-100mg plant tissue in liquid nitrogen using a mortar and pestle or in 1.5 ml

microfuge tubes with minimal volume of quartz sand using a pestle.

2. Add 1ml PCL solution (100mM Tris-HCL (pH 8), 50mM EDTA (pH 8), 1M NaCl and 1% PVP (polyvinyl-pyrrolidone) and 80µl 10% SDS to grounded material.
3. Vortex and shake the tubes for a few times. Incubate at 60^{o}C for 45 minutes. During incubation samples are gently mixed (2-3 times).
4. Centrifuge at 10000 rpm for 5 min at room temperature.
5. Transfer the supernatant (600µl) to fresh tubes and add 500 µl isopropanol and 150 µl protein precipitation solutions (7.5M ammonium-acetate). Samples are mixed by gentle inversion and incubated at -20^{o}C for at least 30 minutes. The incubation should not exceed 12 hours.
6. Centrifuge at 15000 rpm for 10 minutes.

3.5.2. Purification:

7. Discard supernatant and resolve the DNA pellet in 600 µl TE (10mM Tris-HCL, pH 8; 1mM EDTA, pH 8) buffer.
8. Add 500 µl CIA (chloroform and isoamyl alcohol (24:1)) and mix by vortexing (10 min with 90rpm).
9. Centrifuge at 15000 rpm for 5 minutes.
10. Pipette 400µl from the upper phase to a new 1.5 Eppendorf tube (carefully).
11. Add 1ml 99.5% ethanol and 50µl 3M sodium-acetate then gently inversion the tubes.
12. Centrifuge at 15000 rpm for 10 minutes
13. Discard the liquid phase and resolve the pellet in 400 µl 70% ethanol.
14. Centrifuge at 15000 rpm for 5 minutes.
15. Discard supernatant and dry pellet completely (upside down of the tubes on paper- towel).
16. Dissolve the pellet in 100-200 µl TE buffer

3.6. RNA isolation

We used RNAzol®RT to isolate mRNA and micro RNA in separate fractions. RNAzol®RT separates RNA from other molecules in a single-step based on the interaction of phenol and guanidine with cellular components. No chloroform-induced phase separation is necessary to obtain pure RNA. A biological sample is homogenized or lysed in RNAzol®RT. DNA, proteins,

polysaccharides and other molecules are precipitated from the homogenate/lysate by the addition of water and removed by centrifugation. The pure RNA is isolated from the resulting supernatant by alcohol precipitation, followed by washing and solubilization. Protocol for isolation of mRNA and micro RNA fraction are as follows:

1. Homogenization - 1 ml RNAzol®RT + up to 100 mg tissue.
2. DNA/protein precipitation - homogenate + 0.4 ml water, wait 5 - 15 min, 12,000 g x 15 min.
3. mRNA precipitation - 1 ml supernatant + 0.4 ml of 75% ethanol, wait 10 min, 12,000 g x 8 min.
4. mRNA washes - 0.4 ml 75% ethanol, 8,000 g x 1 - 3 min; wash twice.
5. RNA solubilization – water.
6. Micro RNA precipitation - post mRNA supernatant (Step 3) + 0.8 vol. isopropanol, wait 30 min, 12,000 g x 15 min.
7. Micro RNA washes - 0.4 ml 70% isopropanol, 8,000 g x 3 min, wash twice.
8. RNA solubilization – water.

3.7. Molecular marker assays

IT, IT-SCoT, SSCP, SCoT, SCAR, SSR, ISSR and RAPD primers were used to discovery of polymorphic fragments using an Eppendorf Mastercycler ep 384 (Eppendorf, Germany) and a Robocycler (Stratagene, USA). List of primers and their sequences summarized in Table 8 and 10 (appendix). Details of each method briefly are as follows:

3.7.1. Intron targeting (IT)

3.7.1.1. Identification of expressed sequence tags

Seven hundred *S. tuberosum* EST sequences with unidentified exon-intron structures and 200 potato gene sequences with known resistance mechanisms and metabolic pathways were obtained from the National Center for Biotechnology Information (NCBI) dbEST and used to query the NCBI databases using blastx, blastn, or tblastx. In the case of gene sequences we selected single- and low-copy genes as primary targets. In cases in which the exon-intron structures of the genes were known, flanking primers were designed for the exons to amplify across the intercalated introns. Gene sequences with unknown exon-intron structures and potato ESTs with high similarity to genes discovered in other organisms (principally tomato and *Arabidopsis thaliana*) were objected to further analysis. Analyses were conducted against public domain sequences available

at NCBI in August 2010 to find the putative exon and intron sequences. This was achieved by aligning the spliced transcript sequence with its parent genomic sequence to identify correct exon-intron junctions. We preferred a product size within the range of 200 – 1200 bp and used filtering parameters accordingly to select suitable exons. After locating the precise positions of the introns, oligonucleotide primers were designed from predicted exon sequences using the primer designer program PRIMER 3 (http://frodo.wi.mit.edu/primer3/input.htm).

3.7.1.2. IT analysis

PCR was conducted in a final reaction volume of 12 µL. PCR reaction mixture contained 40 ng DNA as template, 1.2 µl from each 10 µM 12-mer primer, 1.2 µl 2 mM dNTP (Fermentas, Lithuania), 1.5 µl 10x PCR buffer (1 mM Tris-HCl, pH 8.8 at 25°C, 1.5 mM $MgCl_2$, 50 mM KCl and 0.1% Triton X-100) and 0.29U of DyNazyme II (Finnzymes, Finland) polymerase. The reactions were performed according to the following profile: 3min at 94°C, followed by 35 cycles at 94°C for 1 min, 51°C for 1min and 72°C for 1 min. The final extension step was at 72°C for 10 min. Amplified PCR products were mixed with 5 µl BPB dye (99.5% de-ionized formamide, 10 mM EDTA pH 8, 0.05% bromophenol-blue, xylene-cyanol dye solution, 1 µl steril H_2O) and separated in 1.5% agarose gels (Promega, USA) in 0.5x TBA (Tris-HCl, Boric acid, EDTA). After electrophoresis, amplified bands were visualized by ethidium-bromide staining and documented with a GenGenius Bio Imaging System (Syngene, UK).

3.7.2. IT-SCoT markers

IT-SCoT markers were developed in our lab to combine advantages of three marker techniques namely IT, SCoT (Start Codon Targeted) and TRAP (Target Region Amplified polymorphism). Sequences of forty potato genes with known resistance mechanisms, metabolic pathways and chromosome location were obtained as mentioned for IT primers. After identification of putative exon and intron sequences, primer pairs were designed based on the short conserved region flanking the ATG start codon in exon part of potato genes where one or maximum two introns could be targeted. This technique is termed as SCoT when primers are used as single, and IT when primer pair is used, and TRAP when single primers are used with other random primers such as ISSR. PCR reaction mixture, amplification conditions, analysis and documentation of amplified products were carried out as mentioned for IT and SCoT primers.

3.7.3. Single Strand Conformation Polymorphism (SSCP)

The amplified single and monomorphic bands of forty IT primers were subjected to single strand conformation polymorphism (SSCP) analysis. PCR reaction mixture and amplification conditions were carried out as mentioned for IT primers. Four µl of PCR products mixed with 10 µl dye solution containing 95% formamide, 10 mm NaOH and 0.05% of bromophenol blue. Amplified PCR products were denatured at 95°C for 5 min and immediately chilled on ice for 3 min. In order to separate single stranded DNAs, 5 µl denatured samples were loaded onto a vertical 0.5 mm x 28.5 cm x 20cm nondenaturing polyacrylamide gel. Electrophoresis was performed in 1x TBE buffer at room temperature for 10–12 hrs. DNA fragments were visualized by modified silver staining method of Bassam et al. (1991) (Gorji et al. unpublished) summarized in Table 4.

Table 4. Ultrasensitive Silver Staining

Step	Reagent	Time required[a]
Fixation	Fixer Solution[1] (7.5% acetic acid)	25-30 minutes
Wash (3x)	Deionized water	3 minutes each
Silver impregnation	Silver solution[2] (1.5 g/L AgNo3 & 600µL formaldehyde	25 minute
Rinse	Deionized water	5-20 seconds
Image development	Developer solution[3] (30-40g/L Na2Co3 & 800µL formaldehyde & 900µg/L sodium thiosulfate[4])	5-15 minutes
Stop	Fixer Solution (7.5% acetic acid, use at 4°C)	1-2 minutes

[a] Treatment times vary for gels having larger dimensions. More time is required when staining larger gels.

[1] The fixing solution can be stored at room temperature.

[2] The silver/ formaldehyde solution is made up freshly as required from a silver stock solution that can be stored shielded from light for a relatively long period. Add formaldehyde to the silver solution about 5-10 minutes before using.

[3] The developer solution is made up freshly, and generally used at 4-8°C. Sodium carbonate

solution can be prepared in bulk and is relatively stable. Sodium thiosulfate added from a stock solution prepared fresh on a daily basis. Formaldehyde must be stored at room temperature since cold storage will inactivate it. Both formaldehyde and sodium thiosulfate must be added immediately before use.

[4] To achieve 900µg/L sodium thiosulfate add 450 or 225 µL/L of a 0.1 or 0.2 g/50ml stock solution respectively

Note: Silver staining is better doing in dark room

3.7.4. SCOT analysis

Fifteen pairs of random combinations of 12 single SCOT primers without any initial screening were used for fingerprinting. The use of primer pairs was preferred to a single primer as they produced more polymorphic bands in our previous experiments. The sequences and their combinations are listed in Table 8. PCR amplification was carried out in a total reaction solution of 12 µl in 384-well plates using Eppendorf Mastercycler ep 384. PCR reaction mixtures contained 40 ng DNA template, 1.2 µl from each 10 µM 12-mer primer, 1.2 µl 2 mM dNTP (Fermentas, Lithuania), 1.5 µl 10 x PCR buffer (1 mM Tris-HCl, pH 8.8 at 25°C, 1.5 mM $MgCl_2$, 50 mM KCl and 0.1% Triton X-100) and 0.29U of DynaZyme II (Finnzymes, Finland) polymerase. Amplified PCR products were mixed with 5 µl Bromophenol blue dye (99.5 % de-ionized formamide, 10 mM EDTA pH 8, 0.05% bromophenol-blue, xylene-cyanol dye solution, 1 µl pure sterile water) and separated in 1.5% agarose gels (Promega, USA) in 0.5x TBA (Tris-HCl, Boric acid, EDTA). Standard PCR cycling parameters were used: an initial denaturation step at 94°C for 4 min, followed by 35 cycles of 30s at 94°C, 1 min at 50°C and 2 min at 72°C. The cycles were followed by 5 min at 72°C for final extensions. After electrophoresis, amplified bands were visualized by ethidium-bromide staining, and documented with GenGenius Bio Imaging System (Syngene, UK).

3.7.5. SCAR analysis

PCR reaction mixture, analysis and documentation of amplified products were carried out as mentioned for IT primers. Amplification conditions were 1 min initial denaturation step at 94°C, followed by 35 cycles of 30 sec at 94°C, 1 min at 54°C, and 1 min at 72°C. The reactions were completed by a final extension step of 10 min at 72°C. Primers and detections were explained by Cernak et al. (2008).

3.7.6. SSR analysis

The simple sequence repeat (SSR) markers (Milbourne et al., 1998) localized on potato chromosome XI and XII were tested in this study. The PCR reactions were performed in 25 µL reaction mixtures in a Robocycler (Stratagene, USA) with 96-well microtiter plates containing the following components: 50 ng DNA, 0.5 µL 10 mM dNTP (Fermentas, Lithuania), 2.5 µL 10x Taq buffer (Fermentas), 2 µL 25mM $MgCl_2$ (Fermentas), 1µl from each 10 µM primer, 0.5 u Taq DNA polymerase (Fermentas). PCR was carried out by initially denaturing template DNA at 94 °C for 4 min, followed by 35 cycles at 94°C for 30 s, 51°C for 30 s, and 72°C for 1 min. The final extension step was at 72°C for 5 min. PCR products were separated in 1.5% agarose gel (Promega, USA) in 0.5x TBE buffer and were stained with ethidium-bromide.

3.7.7. ISSR analysis

Inter-simple sequence repeat assay was conducted using 15 single primers. Primer sequences are listed in Table 8. PCR reactions were carried out as described in SCOT analysis with the exception of primer volume (2.4 µl). The thermocycler program for PCR was set to 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 1 min at 50°C and 2 min at 72°C. The final extension at 72°C was hold for 5 min. The annealing temperature for UBS835, UBS841, UBS842 and UBS844 primers was 55°C. All PCR amplification products were separated in 1.5% agarose gel in TBE, stained with ethidium-bromide and documented with a GenGenius Bio Imaging System (Syngene, UK).

3.7.8. RAPD analysis

To choose appropriate RAPD primers, various combinations of 50 RAPD primers were tested in an initial screening using two parents and 6 selected genotypes of WL × S440 cross population (3 resistant and 3 susceptible to *Potato Potyvirus Y*). After the screening procedure, fifteen primer combinations (Table 8) were randomly selected from suitable combinations for further analysis. Each sample was amplified twice to verify reproducibility. Total volume and composition of reaction mixtures were the same as described in the case of SCOT analysis. The PCR profile was as follows: 4 min predenaturation at 94°C, followed by 35 cycles of 30 s denaturation at 94°C, 1 min annealing at 37°C and 2 min extension at 72°C. The cycles were followed by 5 min final extensions at 72°C. Detection of PCR products was the same like in the case of SCOT analysis.

3.8. Construction of linkage map

Preliminary cluster analysis was done on each parent for the markers identified as simplex, using the simple matching coefficient. These identified markers located on the same chromosome. All simplex, duplex and multiallelic markers were then analyzed by group average cluster analysis to partition them into LGs (Luo et al., 2001). Markers were analysed on the two parents separately. For each LG, recombination frequencies and LOD scores between every pair of markers were calculated for all possible phases using the Expectation-Maximization algorithm, as described by Luo et al. (2001). A simulated annealing algorithm (Hackett et al., 2003) was used to identify the order with the minimum value of the weighted least squares criterion (Stam, 1993) and to calculate map distances between the markers. Permutation test (Churchill and Doerge, 1994) was used to establish a 99% threshold for declaring a simplex to double-simplex linkage. TetraploidMap software was used to analyse data (Hackett and Luo, 2003).

3.9. Suppression subtractive hybridization

The RNA preparation and handling, first-strand cDNA synthesis, second-strand cDNA synthesis, *Rsa*I digestion, adaptor ligation, first hybridization, second hybridization and PCR amplification were performed based on PCR-Select^{TM} cDNA subtraction protocol (PCR-select™ cDNA subtraction Kit user manual, Cat. No. 637401) with minor modifications. CloneJET™ PCR cloning kit was used for cloning. Transformation of cloned cDNA into bacterial cells was carried out based on procedure suggested by Bioline (www.bioline.com)

3.10. Data analysis

Amplified products were scored as present (1) or absent (0) to form a binary matrix. Ambiguous bands were discarded and just distinct and clear bands were scored. It was presumed that co-migrating fragments had been amplified from analogous loci. Jaccard's similarity matrix, Shannon's information index based on *Log2,* variance (after Bowman et al., 1969), AMOVA and principal coordinate analysis (PCoA) were computed using FAMD 1.23β (Schluter and Harris 2006) program. Frequencies per data matrix for *I* was computed as follow: *p* (*i*) = presences (*i*)/ presences (data matrix). Distance Matrices were subjected to Neighbor-Joining methods to

generate a dendrogram. A Strict Consensus tree was calculated in order to estimate the structural stability of clusters and to evaluate the reliability of trees bootstrap analysis of the data was carried out with 2000 replication using Splits tree4 (Huson and Bryant, 2006). The band informativeness (I_b) estimated as I_b=1-(2x0.5 - p|) (Prevost and Wilkinson, 1999), where p is the proportion of the varieties or genotypes containing the band. The resolving power of the primer (Rp) measured in accordance with Rp= $\sum I_b$. Polymorphic Information Content (*PIC*) was calculated according to, $PIC= 1-p^2 -q^2$ (Ghislain et al., 1999) where *p* is frequency of present band and *q* is frequency of absent band. Marker Index (*MI*) was computed as *EMR* x *DI*, where *EMR* (Effective Multiplex Ratio) was the number of polymorphic markers generated per assay and *DI* (Diversity Index) was the average PIC value. QTL analysis, detection of homologous chromosomes and permutation test were performed using TetraploidMap software (Hackett and Luo, 2003). Statistical analysis was carried out using the SPS V11.5 and SAS system V8.

4. RESULTS

4.1. Hetero multiplex analysis

The purpose of this experiment was to determine the number of Ry genes in different breeding lines, which theoretically should carry the genes in multiplex state (duplex, triplex or quadruplex). These Ry genes originated from different wild species and were introgressed into the breeding lines to enhance the ratio of resistant offspring against PVY^{NTN} during the breeding process.

The obtained data from ELISA test were analyzed using X^2 test. The results showed that genotypes 99.384 (origin of Ry is *S. stoloniferum*. and *S. andigenum*) and 98.433 (*S.sto*. *S.hou*. or *S.and*.) are duplexes while 96.353, 97.560 and 97.559 are carry the resistance gene in simplex stage (Table 5). Genotype 99.373 was proved to be triplex at p=0.05 level (X^2 0.05= 5.08). At this level a χ^2 of 5.99 or greater would be needed to consider the result significant. Hence, we concluded that the calculated X^2 of 5.08 can originate from sampling error, mechanical mixing of seeds or natural mutation. For 97.557, there was a significant difference between the observed and expected ratios even for simplex, duplex or triplex stages. We assumed that it could be simplex genotype but to confirm it more progeny test are needed.

Table 5. Segregation ratio of potato breeding lines for PVY resistance in test crosses

Tested parental lines	Plants tested No.	Healthy plants No.	Infected plants No.	Chi ^{2}	Multiplex state
99.373	246	212	34	5.08	**Triplex**
99.384	241	192	49	3.47	**Duplex**
98.433	200	157	43	1.19	**Duplex**
96.353	200	129	71	5.55	Simplex
97.560	200	127	73	6.60	Simplex
97.559	200	109	91	0.76	Simplex
97.557	200	136	64	17.78	Unknown

4.2. Osmotic stress tolerance of potato genotypes and identifying the major QTLs

Identification of quantitative trait loci responsible to stress tolerance could help to develop new tolerant potato cultivars through markers-assisted selection. The objective of the current research was to identify and map loci which may play a major role in the control of osmotic stress tolerance in tetraploid potato under *in vitro* vegetative growth conditions. To achieve this goal the following procedures were performed:

4.2.1. Investigation of parents

To identify suitable concentration of mannitol for discrimination of susceptible and tolerant genotypes, the reaction of WL and S440 at different mannitol concentrations was tested based on factorial analysis. The results proved that there is a significant difference between WL and S440 for root number ($\alpha = 0.05$) under the examined conditions of osmotic stress. The average root number of WL (6.98/plant) was superior to the S440 (5.93/plant). WL had the same root number irrespective to applied mannitol concentration while the root number of S440 gradually decreased with increasing of osmotic stress. However, this decrease was significant for 0.3 M mannitol only (Table 6). The root number for S440 was insignificantly higher than for WL under the 0.0 mol/dm3 mannitol concentration (control condition), but it was lower at each level of osmotic stress indicating its higher susceptibility. The results also revealed that difference between WL and S440 was only significant under severe osmotic stress (Table 6).

The average length of root of the two parents did not differ from each other significantly at any of the tested mannitol concentrations. However, the increasing mannitol concentration decreased the root length for both parents significantly. The highest decrease was detected at 0.3M mannitol level (Table 6). The result also showed that in each level of osmotic stress the root length of S440 was insignificantly higher than that of WL (Table 6).

Table 6. Mean comparison of root number and root length of White Lady and S440 ($\alpha = 0.05$).

Treatment	Root number (No)	Root length (cm)
Parents (factor A)		
WL	6.98 a	2.72 a
S440	5.93 b	2.95 a
Mannitol concentration (mol/dm^3) (factor B)		
0.0 (control)	7.5 a	4.20 a
0.15	6.4 ab	2.92 b
0.20	6.3 ab	2.37 c
0.30	5.6 b	1.84 d
Interaction between parents and mannitol concentration (factor AxB)		
S440×0.0 (control)	7.7 a	4.43 a
WL×0.0 (control)	7.3 ab	3.97 a
WL×0.15	6.6 ab	2.83 bc
WL×0.20	7.0 ab	2.27 cd
WL×0.30	7.0 ab	1.80 d
S440×0.15	6.2 ab	3.01 b
S440×0.20	5.6 bc	2.46 bcd
S440×0.30	4.2 c	1.86 d

Means with the same letter are not significantly different

4.2.2. Investigation of F_1 genotypes

The reaction of 85 F1 progeny was tested on the selected mannitol concentration. Collected data for root number and root length were statistically analyzed based on Completely Random Design (CRD). The variance analysis indicated that the effect of osmotic stress on the root number was highly significant at genotype level ($p<0.0001$). WL and genotype 457, 103 and 460 produced significantly more roots than the others did (Fig. 2). Altogether 15 genotypes showed greater values than the parent S440 but the difference was only significant for the genotype 457, 103 and 460.

For root length, the result showed that significant differences exist between genotypes ($p<0.0001$). Thirteen and nineteen genotypes had longer roots than S440 and WL, respectively. However, it was only significant for genotype 448 (Fig. 2). In general, we found that genotypes

with high root number usually had longer roots (Fig 2). Pearson correlation coefficient revealed that there is a positive and significant correlation between root number and root length (r=0.66). Analysis of regression for curve estimation between root number and root length showed that observed data fit to quadratic trend (Fig. 3).

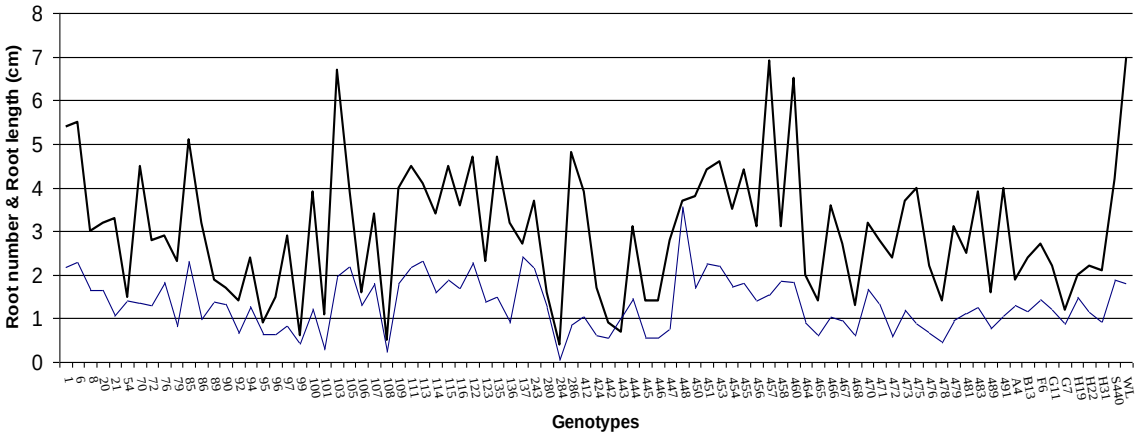


Fig 2. Illustration of root number and root length of F_1 genotypes. Root number upper ***thick line,*** root length lower *thin line*.

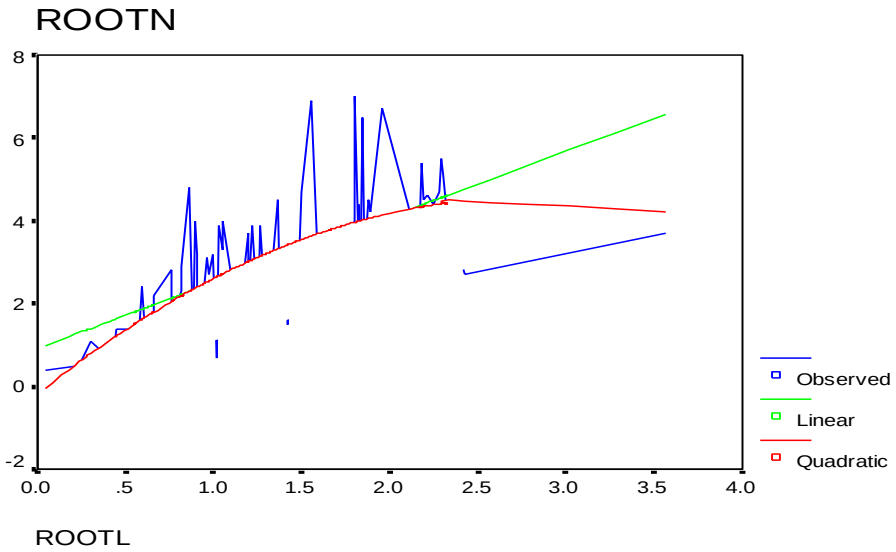


Fig 3. Curve estimation between root number and root length using White Lady, S440 and 85 F_1 genotypes. The regression lines for linear and quadratic trend are described by the equations: RN=0.923+ 1.587x and RN= -0.181+3.4x-0.61x^2, where RN is root number and x is root length (r^2 = 0.60)

4.3. Comparison of molecular techniques for detection of polymorphism

Three molecular markering techniques: SCOT, ISSR and RAPD markers were compared for their polymorphism detecting power in potato varieties as well in an F1 population of tetraploid potato genotypes.

The obtained data of computed Shannon's index, diversity index and marker index for SCOT, ISSR and RAPD markers are listed in Table 7. The oligonucleotide sequences of SCOT, ISSR and RAPD primers and the resulted multiple band patterns for genotypes and varieties are summarized in Table 8 and 9 respectively.

Table 7. Data of Shannon's Index, Diversity Index and Marker Index computed for SCOT, ISSR and RAPD markers

Marker	Shannon's Index		Diversity Index		Marker Index	
	varieties	genotypes	varieties	genotypes	varieties	genotypes
SCOT	6.70	4.47	0.40	0.18	47.60	4.71
ISSR	5.88	4.40	0.34	0.21	23.46	4.41
RAPD	5.32	4.35	0.28	0.24	14.00	5.00

Table 8. Characteristics of SCOT, ISSR and RAPD banding profiles produced in tetraploid potato genotypes: (PIC) Polymorphic information content, (Rp) Resolving power.

Primer code	Sequence (5´-3')	Total No. of bands	No. of scorable bands	No. of poly. bands	% of poly. bands	PIC values	RP
SCOT primers							
1	CAACAATGGCTACCACCA						
2	CAACAATGGCTACCACCC						
3	CAACAATGGCTACCACCG						
4	CAACAATGGCTACCACCT						
5	CAACAATGGCTACCACGA						
11	AAGCAATGGCTACCACCA						
12	ACGACATGGCGACCAACG						
13	ACGACATGGCGACCATCG						
14	ACGACATGGCGACCACGC						
16	ACCATGGCTACCACCGAC						
33	CCATGGCTACCACCGCAG						
36	GCAACAATGGCTACCACC						
SCOT (primer pairs)							
S01-02	Combination of primer 1 & 2	13	9	0	0	-	-
S01-36	Combination of primer 1 & 36	9	8	1	13	0.124	0.76
S02-03	Combination of primer 2 & 3	11	10	0	0	-	-
S04-05	Combination of primer 4 & 5	9	8	1	13	0.102	0.99
S04-11	Combination of primer 4 & 11	10	7	1	14	0.203	0.97
S04-12	Combination of primer 4 & 12	14	9	3	33	0.251	2.18
S04-16	Combination of primer 4 & 16	10	9	1	11	0.128	0.69
S04-36	Combination of primer 4 & 36	12	10	3	30	0.309	2.92
S05-11	Combination of primer 5 & 11	15	9	4	44	0.225	3.31
S11-16	Combination of primer 11 & 16	14	9	2	22	0.108	1.84
S13-14	Combination of primer 13 & 14	13	8	2	25	0.324	1.56
S13-16	Combination of primer 13 & 16	12	10	4	40	0.198	2.97
S14-16	Combination of primer 14 & 16	12	4	1	25	0.219	0.94
S16-36	Combination of primer 16 &36	11	10	2	20	0.090	1.33
S33-36	Combination of primer 33 & 36	14	10	1	10	0.074	0.92
	Total	179	130	26	-	-	21.38
	Average	11.9	8.7	1.7	20	0.181	

Table 8. (*continued*)

Primer code	Sequence (5´-3')	Total No. of bands	No. of scorable bands	No. of poly. bands	% of poly. bands	PIC values	RP
ISSR primers [a]							
Issr9	$(AC)_8$ YA	9	6	2	33	0.278	1.98
IssrL1	$(AG)_8$ C	9	8	0	0	-	-
IssrL3	$(AC)_8$ YG	11	9	4	44	0.339	3.84
IssrT2	DDCCACCACCAC CACCA	11	9	0	0	-	-
UBC807	$(AG)_8$ T	10	9	1	11	0.092	0.87
UBC808	$(AG)_8$ C	11	10	0	0	-	-
UBC809	$(AG)_8$ G	9	8	0	0	-	-
UBC810	$(GA)_8$ T	10	7	5	71	0.437	4.23
UBC812	$(GA)_8$ A	6	5	2	40	0.320	1.84
UBC813	$(CTC)_5$TT	12	9	1	11	0.099	0.97
UBC822	$(TC)_8$ A	8	5	1	20	0.167	0.99
UBC835	$(AG)_8$ YC	10	8	1	13	0.082	0.69
UBC841	$(GA)_8$ YC	12	8	1	13	0.133	0.85
UBC842	$(AG)_8$ YG	10	7	1	14	0.137	0.97
UBC844	$(CT)_8$ RG	9	7	2	29	0.228	1.61
	Total	147	115	21	-	-	18.83
	Average	8.6	6.8	1.4	20	0.210	
RAPD primer pairs							
R01-91	GTCAGTGCGGCA-GGCATGACCTGT	7	4	0	0	-	-
R05-52	AGGCATCCTGAG-AGATGCAGCCAG	13	8	1	13	0.138	0.80
R05-57	GGCTCGCATCTA-AGATGCAGCCAG	15	7	3	43	0.340	2.05
R05-77	CCCACTAGACTC-AGATGCAGCCAG	13	12	2	17	0.167	1.79
R06-67	CATACGGGCTAC- ACATGCCGTGAC	12	9	2	22	0.202	1.65
R06-77	CCCACTAGACTC- ACATGCCGTGAC	12	7	2	29	0.247	1.95
R08-79	GTCGTAGCGGAT-AATGCGGGAGTC	14	9	2	22	0.135	1.31
R15-59	CCCCCGTTAGAA-AGATGCGGGGTA	13	5	2	40	0.352	1.43
R15-87	GTGACCGAGTCG-AGATGCGGGGTA	12	8	3	38	0.298	2.69
R18-33	GTCATGCGACGA -CTGTCATGCCGA	12	9	2	22	0.201	1.79
R35-91	GTCAGTGCGGCA -GGCTTATGCCGT	13	9	1	11	0.126	0.78
R41-97	CGCGAATTCC-GGACCCTTACTG	13	10	0	0	-	-
R43-90	ACTCCAGCCAGG-CCATCGGAGGTC	11	7	1	14	0.137	0.97
R44-57	GGCTCGCATCTA-CCGTCCATCCAC	13	5	0	0	-	-
R45-67	CATACGGGCTAC-GTAAGGCGCATC	12	5	2	40	0.273	1.66
	Total	185	114	23	-	-	18.87
	Average	12.3	7.6	1.5	21	0.218	

[a] Y= (C,T); D= (A,G,T); R=(A,G)

Table 9. Characteristics of SCOT, ISSR and RAPD banding profiles produced in varieties of tetraploid potato: (PIC) Polymorphic information content, (Rp) Resolving power.

Primer code	Total No. of bands	No. of scorable bands	No. of poly. bands	% of poly. bands	PIC values	RP
Combination of SCOT primers[b]						
S01-02	13	11	5	0	0.38	3.83
S01-36	12	10	6	13	0.43	3.92
S02-03	14	13	9	0	0.43	6.08
S04-05	11	10	3	13	0.29	2.50
S04-11	12	10	5	14	0.45	2.75
S04-12	19	18	15	33	0.50	9.75
S04-16	14	13	10	11	0.49	5.33
S04-36	12	11	4	30	0.37	1.58
S05-11	13	11	7	44	0.26	2.42
S11-16	16	15	13	22	0.49	8.83
S13-14	13	12	9	25	0.42	4.83
S13-16	14	13	10	40	0.47	5.58
S14-16	17	16	13	25	0.49	8.92
S16-36	13	12	5	20	0.30	2.42
S33-36	16	12	5	10	0.29	2.50
Total	209	187	119	-	-	71.25
Average	13.9	12.5	7.9	61	0.4	
ISSR primers [*,b]						
Issr9	11	10	7	70	0.39	3.83
IssrL1	9	8	2	25	0.15	1.41
IssrL3	13	12	5	42	0.36	3.83
IssrT2	11	10	0	-	-	-
UBC807	14	13	11	85	0.49	8.42
UBC808	11	10	3	30	0.15	1.67
UBC809	6	6	0	-	-	-
UBC810	12	10	4	40	0.30	2.25
UBC812	12	11	8	73	0.45	5.33
UBC813	9	7	2	29	0.20	1.67
UBC822	6	4	3	75	0.34	1.75
UBC835	10	9	5	56	0.38	3.33
UBC841	13	12	8	67	0.46	5.75
UBC842	12	11	6	55	0.42	3.25
UBC844	10	9	5	56	0.40	4.12
Total	159	142	69	-	-	46.62
Average	10.6	9.5	4.6	47	0.34	

[*] Y= (C,T); D= (A,G,T); R=(A,G)

[b] Sequence of primers are same as that mentioned in Table 8.

Table 9. (*continued*)

Primer code	Total No. of bands	No. of scorable bands	No. of poly. bands	% of poly. bands	PIC values	RP
RAPD primer pairs [b]						
R01-91	8	7			-	
R05-52	11	10	1	10	0.06	0.67
R05-57	13	11	5	45	0.33	1.92
R05-77	11	10	3	30	0.19	1.50
R06-67	12	11	5	45	0.29	2.3
R06-77	11	8	1	13	0.14	0.83
R08-79	13	9	4	44	0.36	2.25
R15-59	12	10	4	40	0.30	3.42
R15-87	12	10	8	80	0.50	4.50
R18-33	14	13	8	62	0.43	5.00
R35-91	12	8	2	25	0.13	1.08
R41-91	12	11		0	-	-
R43-90	11	9	2	22	0.22	1.58
R44-57	12	9			-	
R45-67	16	15	7	47	0.36	5.58
Total	180	151	50		-	30.63
Average	12.0	10.1	3.3	31	0.28	

[b] Sequence of primers are same as that mentioned in Table 8.

4.3.1. SCOT analysis

The PCR amplification using SCOT primer pairs resulted in generation of reproducible amplification products. Fifteen primer pairs amplified 130 clear and scorable bands for the genotypes and 187 for the varieties. Effective multiplex ratio for genotypes and varieties was 26 and 119, respectively. The average number of scorable bands revealed by each primer pair was 8.7 for genotypes and 12.5 for varieties. Average numbers of polymorphic band per primer pair for genotypes and varieties was 1.7 and 7.9, respectively. The mean of percentage of polymorphism for each primer pair of genotypes was 20 and it was 61 for varieties. Out of SCOT primers, primer pairs S04-12,

S05-11, S11-16 and S13-14 showed more than one allele at a given locus. Diversity index and marker index for genotypes were 0.181 and 4.710 and for varieties it was 0.4 and 47.6, respectively. The Rp of the SCOT for genotypes (21.38) was less than that of varieties (71.25). The maximum Rp (3.31) was belonging to primer pair S05-11 of genotypes and primer pair S04-12 (9.75) of varieties. The band informativeness of genotypes was high and generally more than that of varieties (data not shown) but the number of polymorphic bands produced by each primer for the genotypes was less than that of the varieties. The same results were obtained with ISSR and RAPD markers. Shannon's information index computed to identify genetic diversity between genotypes and varieties. The results of genotypes and varieties were 4.470 and 6.704, respectively. The AMOVA was carried out to estimate population differentiation directly from molecular data and test hypothesis about such differentiation. The result showed that variation within population (94.9%) was more than among population (5.1%). In order to estimate structural stability of clusters and reliability of trees, bootstrap analysis was conducted with 2000 replications after constructing the Consensus Tree using NJ genetic distances based on the Dice coefficient. The analysis grouped genotypes and varieties into 9 and 7 main clusters, respectively. The SCOT marker technique identified all cultivars and 85 genotypes out of 87. For varieties, Phylogram comprises varieties Snowden, Atlantic, Swiss and S440 derived from USA in cluster D, Desiree, Cleopatra and Kondor from Netherlands in cluster F, Katica and Rioja from Hungary and Panda and Franzi from Germany in cluster G (Fig. 4). To create a predictive model based on uncorrelated variables, related to the original correlated variables and comparing to clustering analysis, we performed principal coordinate analyses (PCoA). The result of PCoA was comparable to the cluster analysis (Fig. 5). The first three most informative principle component explained 55.04% of the total variation.

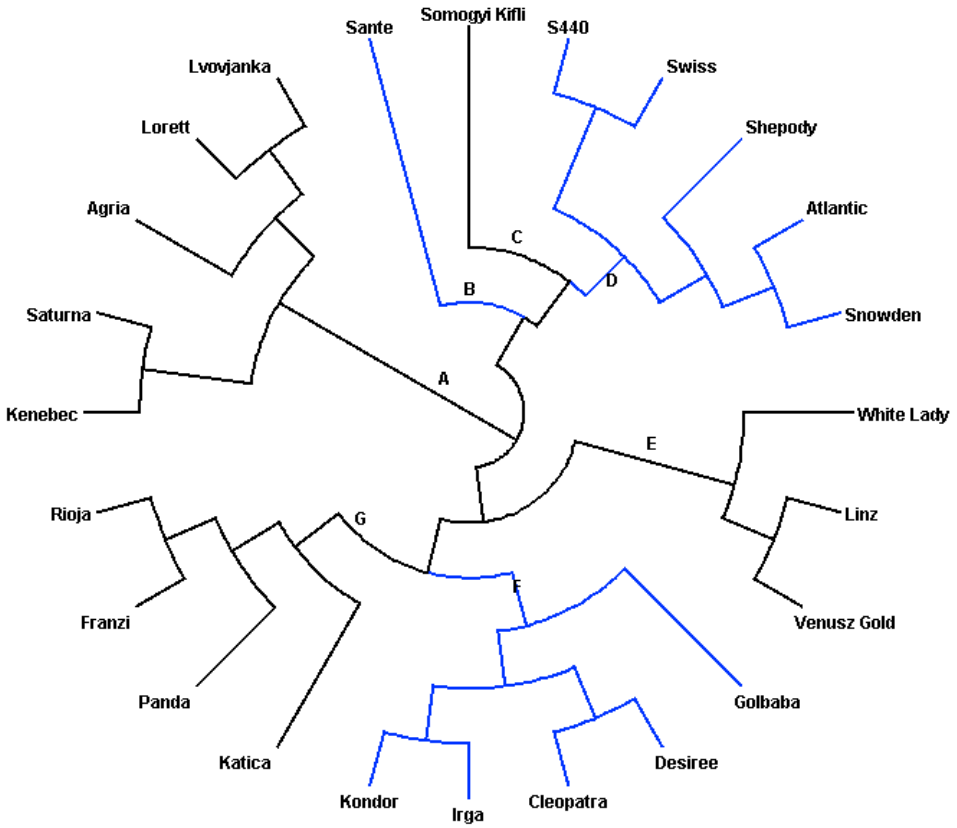


Fig.4. Circular phylogram of consensus tree, using the genetic distance of NJ, based on SCOT markers; letters indicate clusters.

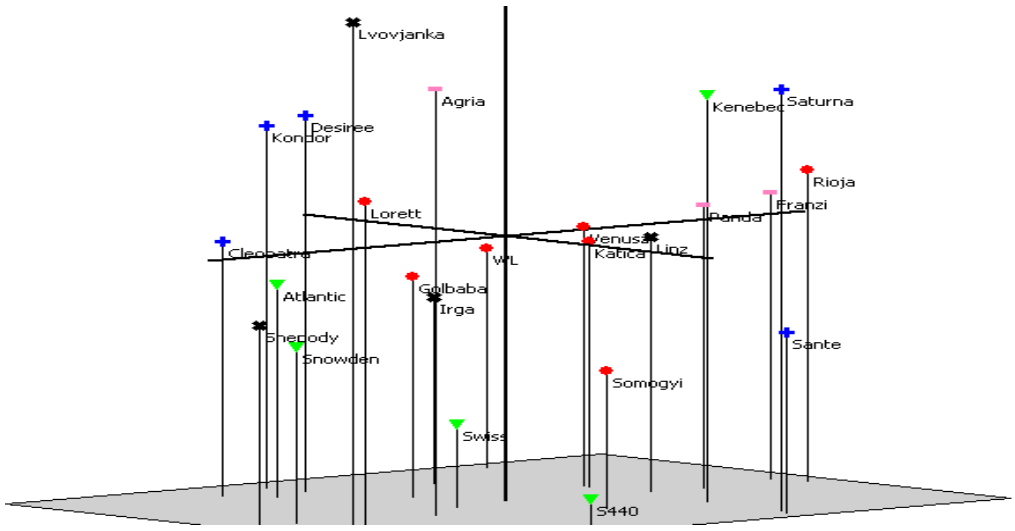


Fig. 5. Three-dimensional plot of principal coordinate analysis of 24 varieties using SCOT analysis. WL: White Lady. The symbol represents origin of cultivars, (where, circle= Hungary, Triangle = USA, Cross up = Netherlands, Cross side = Canada, Poland, Russia and Australia, Rectangle = Germany).

4.3.2. ISSR analysis

ISSR primers produced different numbers of DNA fragments, depending upon their simple sequence repeat motifs. ISSR9, ISSRL3, UBC810 showed polymorphic bands which were alleles of a single locus. For the analyzed genotypes, the 15 primers produced 147 fragments of which 115 were scorable and 21 were polymorphic. For varieties, they produced 159 fragments of which 142 were scorable and 69 were polymorphic. The mean number of scorable bands for genotypes and varieties was 6.8 and 9.5, respectively. PIC calculated for ISSRs scaled from 0.08 to 0.44 in the genotypes and from 0.15 to 0.49 in the varieties. The average percentage polymorphism of each primer for genotypes and varieties was 20 and 47, respectively. Diversity index and marker index for genotypes were 0.21 and 4.41 whereas it was 0.34 and 23.46 for varieties. For genotypes and varieties, the Rp of the ISSR was 18.83 and 46.64, respectively. The result of AMOVA exposed that variation within population and among population was 96.78% and 3.22% respectively. Overall Shannon's index for genotypes and varieties was 4.40 and 5.88, respectively. Construction of tree and bootstrap analysis was performed as in the case of SCOT markers. The results displayed that genotypes and varieties segregated into 10 and 8 clusters, respectively (Fig. 6). The ISSR marker technique identified 85 genotypes out of 87 and 22 cultivars out of 24. The result of principal coordinate analysis was comparable to the cluster analysis (Fig 7). The first three most informative principle components explained 56.24% of the total variation.

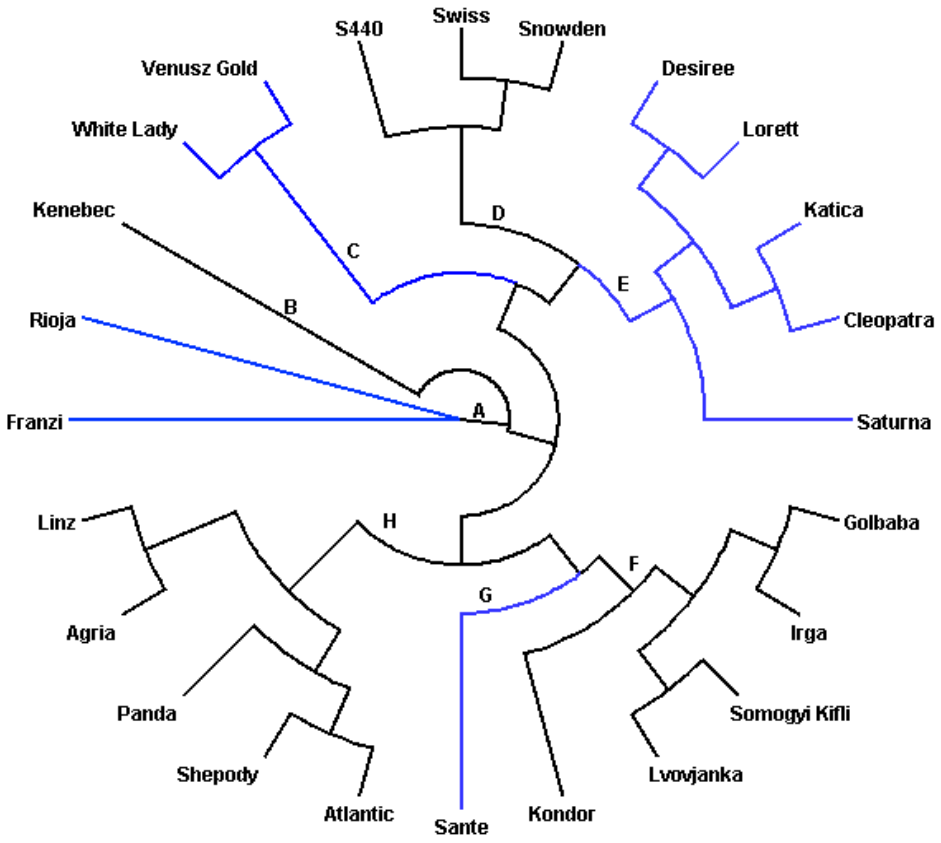


Fig.6. Circular phylogram of consensus tree, using the genetic distance of NJ, based on ISSR markers; letters indicate clusters.

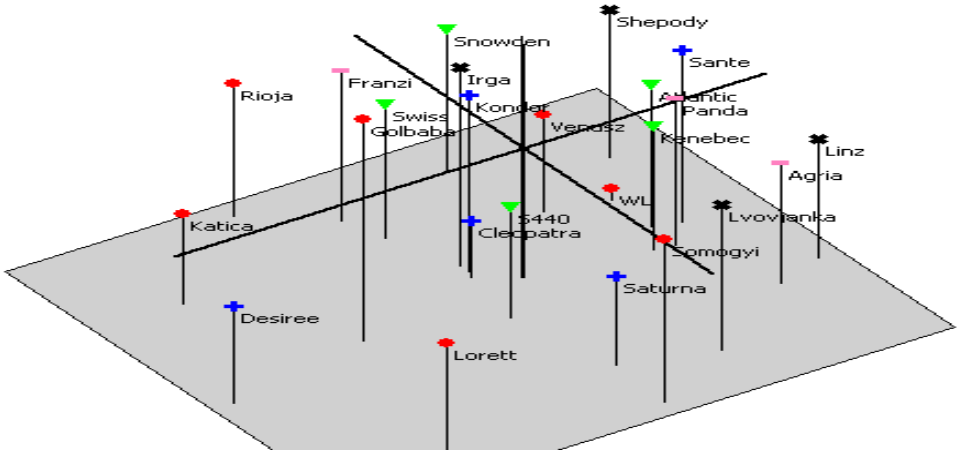


Fig.7. Three-dimensional plot of principal coordinate analysis of 24 varieties using ISSR analysis. WL: White Lady. The symbol represents origin of cultivars, (where, circle= Hungary, Triangle = USA, Cross up = Netherlands, Cross side = Canada, Poland, Russia and Australia, Rectangle = Germany).

4.3.3. RAPD analysis and markers comparison

PCR amplification of DNA, using 15 pairs of RAPD primers yielded 185 DNA fragments, of which 114 were scorable and could be scored in all genotypes. Out of all RAPD primer combinations only combination of primer 57 with primer 5 (R05-57) resulted amplified fragments that showed more than one allele for a given locus. Compared to SCOT primers, RAPDs generally produced less polymorphic and scorable bands per primer pair. For genotypes, the number of polymorphic and scorable bands per primer pair produced by RAPD primers was more than by ISSR primers. The number of polymorphic bands per primer pair produced with ISSRs was higher than by RAPDs using the varieties. PIC value ranged from 0.13 to 0.35, with diversity index of 0.22 for genotypes and from 0.06 to 50, with diversity index of 0.28 for varieties. The marker index for genotypes and varieties was 5.00 and 14, respectively. The Rp of the RAPD for genotypes and varieties was 18.87 and 30.63, respectively. Shannon's index for genotypes using RAPD markers was 4.35 and for varieties it was 5.32. For varieties, the result of AMOVA revealed that variation within groups (95.09%) was more than among groups (4.91%). In order to estimate the genetic distance among genotypes and varieties, the similarity matrix was computed with Jaccard's method. The results for SCOT, ISSR and RAPD discovered a high level of genetic diversity within the 87 genotypes (0.04 to 0.94) and varieties (0.1 to 0.79). The rate of genetic diversity among genotypes, based on SCOT, ISSR and RAPD markers was nearly equal. The similarity of varieties that were assessed in this study was generally low and less than that of the genotypes. The rate of genetic diversity among varieties, based on ISSR and RAPD markers was nearly equal and differed from SCOT markers (data not shown). Consensus tree with bootstrap analysis based on RAPD markers using NJ genetic distances showed diversity within the analyzed genotypes and varieties and grouped them into 12 and 10 clusters, respectively. The RAPD marker technique could identify 85 genotypes out of 87 and 22 cultivars out of 24 (Fig 8). The result of principal coordinate analysis was comparable to the cluster analysis (Fig 9). The first three most informative principle component explained 45.06% of the total variation. Comparison of phylograms created using SCOT, ISSR and RAPD markers demonstrated that only SCOT technique could distinguish all cultivars. ISSR and RAPD techniques can independently identify each cultivar except Rioja and Franzi. The

clustering pattern obtained with each type of markers showed some common groups and clustered some of the varieties according to the location where they were released or according to their relationship. White Lady and Vénusz Gold successfully grouped into the same cluster based on all marker techniques (WL is the female parent of Vénusz Gold). Desiree and Cleopatra were also effectively included into the same group based on SCOT and ISSR data (Desiree is one of the parents of Cleopatra). S440 and Swiss of USA origin and Gülbaba and Irga of Hungarian and Polish origin incorporated into the same group based on all markers. S440, Swiss and Snowden from USA clusters in the same group based on SCOT and ISSR data while variety Sante forms independent clusters in SCOT-based and ISSR-based ones. Panda and Franzi originating from Germany were integrated into the same group based on SCOT data. Somogyi Kifli (HU) and Lvovjanka (RU) were classified in the same small cluster based on ISSR and RAPD data.

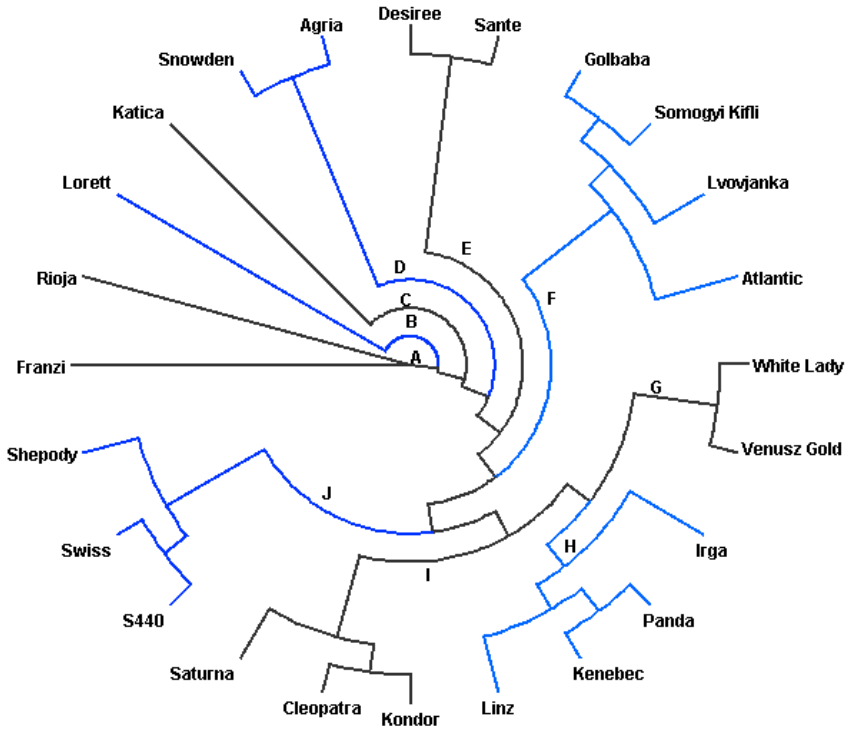


Fig.8. Circular phylogram of consensus tree, using the genetic distance of NJ, based on RAPD markers; letters indicate clusters.

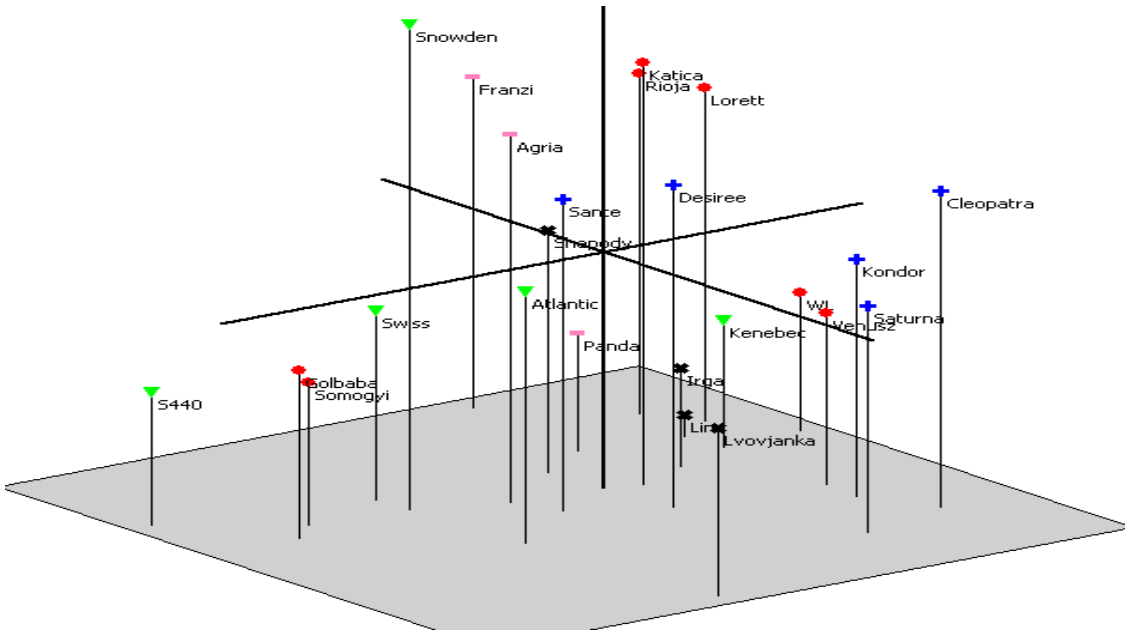


Fig.9. Three-dimensional plot of principal coordinate analysis of 24 varieties using RAPD analysis. WL: White Lady. The symbol represents origin of cultivars, (where, circle= Hungary, Triangle = USA, Cross up = Netherlands, Cross side = Canada, Poland, Russia and Australia, Rectangle = Germany).

4.4. Development of intron targeting primers

In this experiment we developed two-hundred-twenty intron targeting primer using potato expressed sequence tags (EST) and NCBI database records to detect polymorphism.

Out of 220 IT primers 120 showed polymorphism in primary screening of tetraploid potatoes. The primers which showed polymorphism in primary screening were checked for polymorphism in the segregating population and the results were used to construct a linkage map. The sequence, annealing temperature and GC percentage of the IT primers are listed in Table 10 (see appendix).

4.5. Development of IT-SCoT primers

Coupled with the rapid growth of genomics research, there has been a trend away from random DNA markers towards gene-targeted markers. Hence, we developed a novel gene-targeting marker system called IT-SCoT.

Preliminary analysis of IT-SCoT primers in tetraploid potato showed that 50 % of primers produced polymorphic bands when used as IT primers (primer pair). The rate of polymorphism was significantly higher (99%) when primers are used as SCoT primers (single primer). The number of polymorphic bands which were scorable varies from 1 to 5. The profiles produced by them were also different (Fig. 10). These primers were capable to amplify from tomato, eggplant, sunflower and common ragweed (*Ambrosia artemisiifolia*) genomic DNA. This project is under development and to asses of the potential of this approach is under testing.

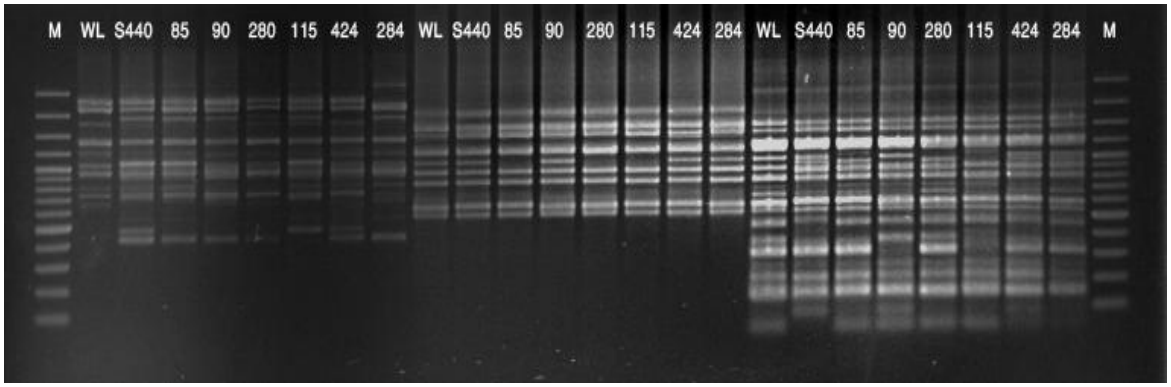


Fig. 10. IT-SCoT patterns generated by primers IT-SCoT57F (first 8 samples), IT-SCoT57R and IT-SCoT57 (primer pair, last 8 samples). Lane M, 100-bp ladder; WL: White Lady (female parent); S440 (male parent). Numbers represent F_1 genotypes of tetraploid potato.

4.6. Construction of a potato genetic linkage map

The basis for constructing genetic linkage maps for the 12 potato chromosomes is either an F1 or a backcross (BC) progeny of partially heterozygous parents, which segregates for a sufficient number of DNA polymorphisms. In this study, mapping was carried out using an F1 progeny. An acceptable map for each parent was separately achieved when most of the markers scored were arranged in a number of linkage groups which correspond to the number of chromosomes per genome complement. The obtained results for the parents White Lady and S440 are as follow:

4.6.1. White Lady

Eighty-nine markers were used to construct a partial linkage map of WL. Out of these, 65 segregated in a 1:1 ratio ($P<0.001$) and 24 segregated in a 5:1 ratio with $P<0.01$. Single linkage clustering and average linkage clustering were used to establish the linkage groups. The result of single linkage clustering discovered 12 groups at the 0.93 similarity. The average linkage clustering formed 12 LGs at the 0.31 similarity. Hence a linkage map was primarily built on distribution of 89 (15 ITs, 2 SSRs, 8 SCoTs, 5 ISSRs, 1 SCAR and 58 RAPDs) markers into 12 groups with 2 to 20 markers. Any marker that showed poor fit in the ordering was moved to another group or to a new group or was excluded. IT and SSR markers were used for the identification of different LGs corresponding to each chromosomes. Finally, we could identify 3 LGs corresponding to 3 chromosomes.

4.6.1.1. Chromosomes identified by IT and SSR markers

Chromosome VII

This linkage group (LG) has an IT marker (f79) which was amplified with a primer pair that was designed based on the EST sequence having full similarity with chromosome VII of potato. F79 was found to be a simplex marker and linked in repulsion with the duplex marker f52 and simplex marker f99. The map is shown in Fig. 11(i).

Chromosome XI

An anchor-IT marker f78 (generated in our study) beside four other IT markers and a RAPD marker were found in this linkage group. The primers for identification of f78 were designed based on the Ry1In gene sequence that shows high similarity with chromosome XI of potato. As f78 was only an anchor marker and allelic variation of that was not clear, the designation of it as chromosome XI is tentative until further mapping data are obtained. F78 is a simplex marker and is linked in coupling rearrangement with the duplex RAPD marker f42 and in repulsion with 2 simplex markers (f84 and f89) and 2 duplex markers (f83 and f87). F83, f84, f87 and f89 were also IT markers but the chromosomal position of them could not be determined by the similarity analysis. The

map is shown in Fig. 11(e).

Chromosome XII

This LG includes 10 markers, of which 5 were IT, 1 SSR, 1 SCAR, and 3 RAPD. The Ry_{sto} gene was previously also located in this group (Cernak et al, 2008). F23 (Cat260) is an anchor marker for chromosome XII and mapped in the same location with f24 (IT) and f25 (SCAR) markers. F23 is a simplex marker and linked in coupling with 7 simplex markers (f28, f37, f80, f26, f24, f25 and f27) and a duplex marker f77 (RAPD). F23 also linked in repulsion with the duplex marker f58 (RAPD). The LOD score of f23 with other markers in this group was usually high. The maximum LOD was in association with f24 and f25 (22.58) and the minimum of that was associated to f58 (0.41). The range of recombination frequentcy between f23 with other markers was from 0 (f24 and f25) to 0.31 (f58). F26 (STM0003) is also an anchor marker for chromosome XII (Milbourne et al. 1998) and mapped at 6 cM apart from f23 and was 5 cM distant from Ry_{sto} on the other side. F26 is also a simplex marker and linked in coupling with 7 simplex markers (f28, f37, f80, f23, f24, f25 and f27) and a duplex marker f77. F26 Also linked in repulsion with the duplex marker f58. The recombination frequency ranged from 0.0029 (f77) to 0.29 (f28) (data not shown). The map is shown in Fig. 11(g).

4.6.2. S440

A total of 111 markers were used to construct the genetic linkage map of S440. Among them 95 were found to be simplex while 16 were duplex marker. The result of single linkage clustering revealed 12 co-segregation groups at the 0.96 similarity. The average linkage clustering formed 12 linkage groups at the 0.33 similarity. Hence we firstly created a linkage map with 12 linkage groups, each containing 3 to 23 markers. Two groups were subsequently divided after more detailed assessments of recombination frequencies inside the groups. Out of 14 LGs we recognized 3 linkage groups corresponding to two chromosomes (IX and XII) based on the IT markers. The map is shown in Fig. 11.

4.6.2.1. Chromosomes identified by IT markers

Chromosome IX

A simplex (m100) and a duplex (m101) IT marker with high similarity with chromosome IX mapped in different LGs. We assume that these two LGs represent chromosome IX and marked with IXa and IXb until additional marker data will be obtained (Fig. 11a and b). The m101 is a duplex marker and is linked in coupling with two duplex (m48 and m129) and two simplex markers (m54 and m58). The m57 (simplex marker) was also linked in repulsion with m101. Recombination frequency of m101 with other markers in this LG was low and ranged from 0.0006 to 0.132. All markers linked with m101 were RAPDs except m129 that was an ISSR marker. The m100 is a simplex marker and linked in repulsion with 7 other simplex markers (m24, m82, m49, m61, m27, m16 and m45), of which m16 and m24 are ISSR and the remaining are RAPD markers. Recombination frequency of m100 with other markers ranged from 0.03 to 0.24.

Chromosomes XII

The simplex IT marker m97 identifies and aligns the S440 map of chromosome XII (Fig. 11c). One duplex and 6 simplex markers are linked in repulsion with m97. Four simplex markers are linked in coupling with m97 too. The recombination frequency of m97 with other markers in this group ranged from 0.21 to 0.44. We estimate that the designation of these as chromosome XII is tentative (because of its RF) until further mapping data are obtained.

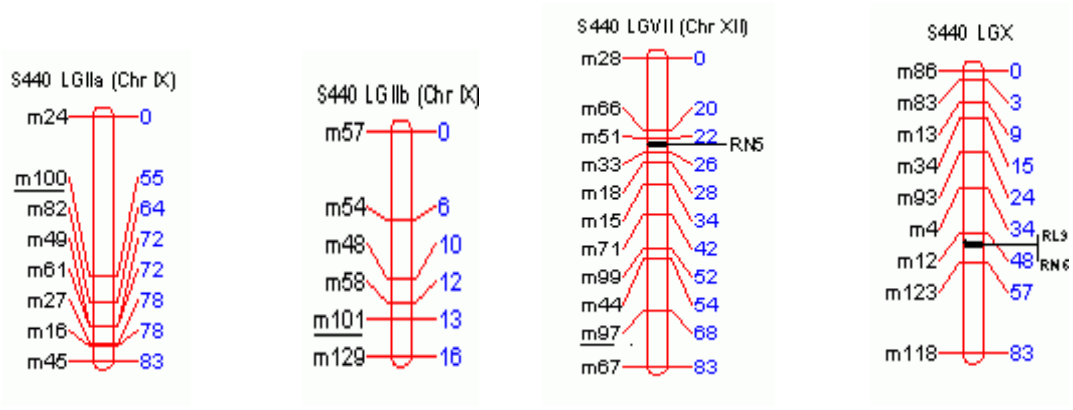
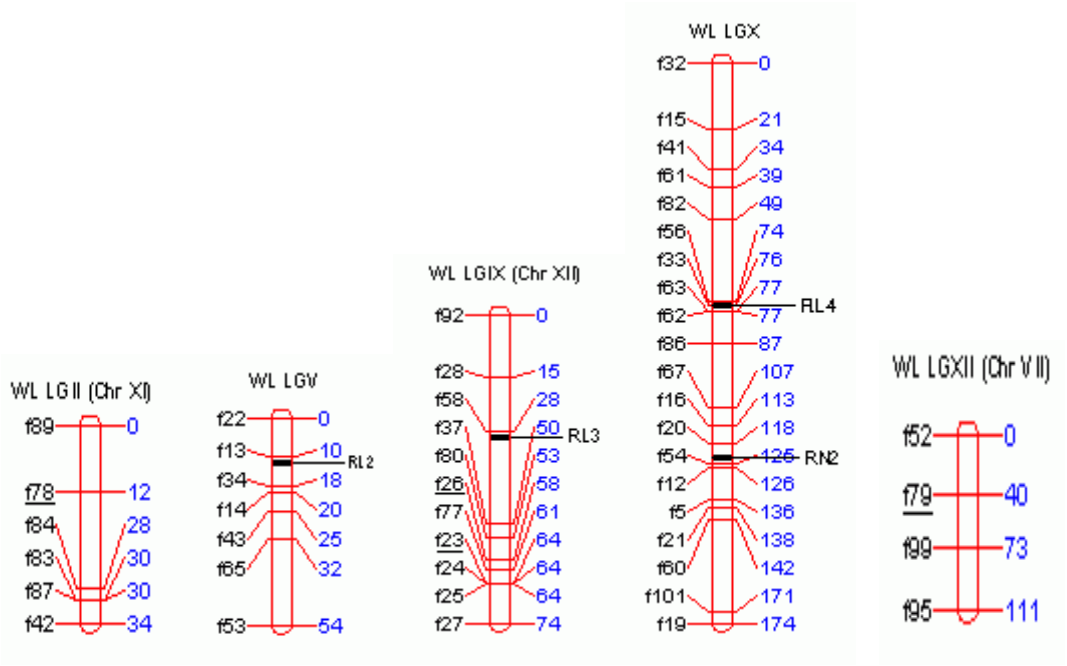


Fig 11. (a) (b) (c) (d)



(e) (f) (g) (h) (i)

Fig. 11. Linkage Groups and the corresponding chromosomes containing the identified QTLs (highlighted, RN = root number, RL = root length). The two parents are shown separately but the four homologous chromosomes are merged into a single map. Underline showing the anchor markers, WL: White Lady, a LGIIa (Chromosome IX), b LGIIb (Chromosome IX), c LGVII (Chromosome XII), d LGX, e LGII (Chromosome XI), f LGV, g LGIX (Chromosome XII), h LGX, i LGXII (chromosome VII)

4.7. QTL mapping

Multiple quantitative trait loci (QTL), either clustered together or dispersed on different chromosomes, can first be separated or dissected by molecular marker-assisted

QTL mapping and selection and then pyramided into one genetic background, either by marker-assisted selection (MAS) or transformation of cloned multiple QTL, to create transgressive progeny in plant breeding. In the presented study, our aim was to identify and map major QTLs affecting the osmotic stress tolerance in tetraploid potato during the vegetative growth under *in vitro* conditions. Osmotic stress tolerance was characterized by root length and root number. The results relevant to each trait are as follows:

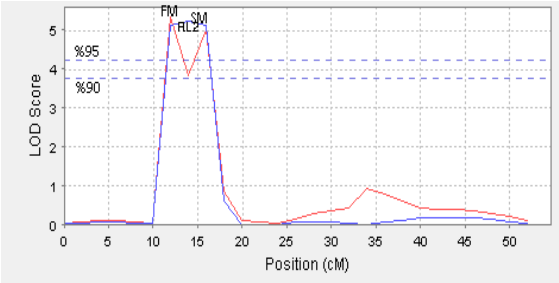
4.7.1. Root length

Nine QTLs with LOD>2 were identified for root length. Out of that 6 were for WL and 3 for S440. The results of permutation test confirmed 3 of those as major QTLs (Fig. 12). All these QTLs have two copies of dominant alleles for increasing resistance to osmotic stress. Out of them RL2 and RL3 are in linkage groups V and IX of WL and RL9 on linkage group X of S440. The linkage group IX was identified as chromosome XII using anchor markers as mentioned above. The QTLs on linkage group V of WL and X of S440 were closely linked in coupling to simplex ISSR marker f13 (2 cM) and SCoT marker m12 (4 cM) and individually explained 64.9 and 54.8% of phenotypic variance with maximum LOD 5.34 and 5.1, respectively. The QTL on chromosome XII of WL was closely linked in coupling to RAPD marker f58 (2 cM) and individually explained 52.3% of the phenotypic variance with maximum LOD 4.8. It is also closely linked to a QTL (RN1) of root number (<0.3 cM) that was not recognized as a major QTL based on permutation test but individually explained 29.74 % of phenotypic variance with LOD 2.23. The locations of the QTLs are shown in Figure 11. The positions, LOD scores and percentage phenotypic variance explained for the full model of six QTL genotypes is shown in Table 11.

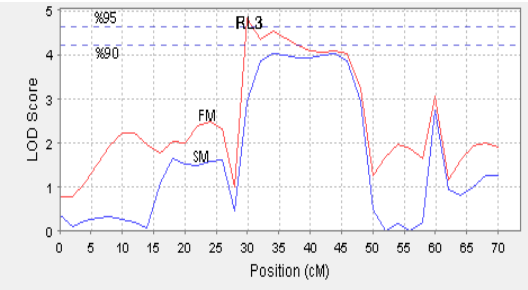
4.7.2. Root number

Five QTLs with LOD>2 were detected for root number. Out of those 3 were identified in White Lady and 2 in S440. Three of the QTLs were identified as major QTLs based on the permutation test results (Fig 12). Two of the major QTLs (RN5 and RN6) were present in S440 and one (RN2) in WL. The QTL of WL is in linkage group X and is closely linked in coupling to duplex RAPD markers f54 (3 cM) and f20 (4 cM) on

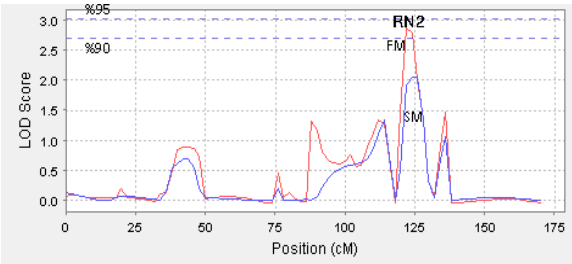
the opposite side and confer resistance to osmotic stress. It has two copies of dominant alleles on a chromosome in linkage group X and mapped in 46 cM of a QTL of root length (RL4) which explained 54.5% of the phenotypic variance with maximum LOD of 3.92 (Fig. 11h). The maximum LOD and variance explained of the QTL for root number in that linkage group were 2.87 and 19.19 %, respectively (Table 11). There are two copies of dominant alleles on chromosomes for RN5 and RN6 in linkage groups VII and X of S440 which responses to osmotic stress. The first one narrowly linked in coupling to simplex RAPD markers m33 (2 cM) and m51 (2 cM) on the opposite side. It individually explained 26.8% of phenotypic variance with maximum LOD 2.4. It is also mapped closely (2 cM) to a QTL (RL8) for root length which explained 38.4% of phenotypic variance with LOD 2.65 (Table 9). The QTL RN6 in linkage group X individually explained 43.1% of phenotypic variance (LOD 3.14) and closely (3 cM) linked in coupling to the simplex ISSR marker m123. The QTL RN6 linked to a major QTL of root length (RL9) that explained 54.76 % of variance with maximum LOD 5.1. Linkage between QTLs RN6 of root number and QTL RL9 of root length and mapping in the same location on the sam chromosome infer that they could in fact be the same allele as the two traits are associated. However, there are inadequate bridging markers to determine whether the QTLs in linkage group X are the same locus in the two parents.



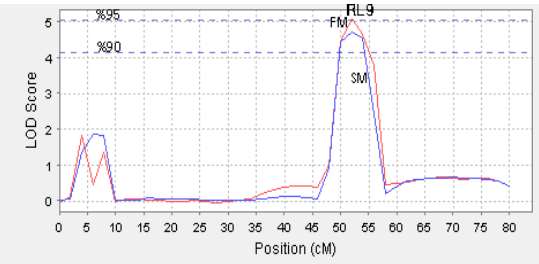
a. LG V of WL



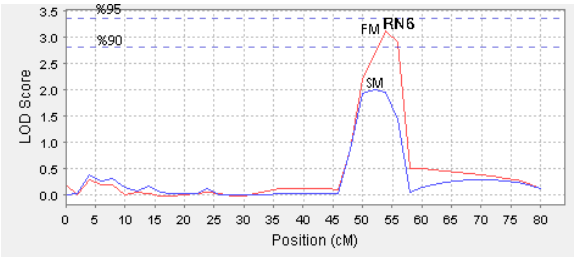
b. LG IX of WL

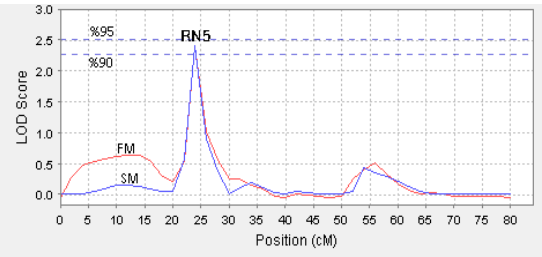


c. LG X of WL



d. LG X of S440





e. LG X of S440 f. LG VII of S440

Fig. 12. Results of permutation test for major QTLs. a, b and d shows the LOD profile for root length. c, e and f shows the LOD profile for root number. 90% and 95% thresholds are shown as horizontal lines in the upper panel. FM: profile of full model, SM: profile of simple model. LG: linkage group, WL: White Lady.

Table 11. Estimated parameters from QTL analysis.

Trait	Parent	LG	Position cM	Lod	R^2	Simplex marker Highest mean (SE)	Simplex marker Lowest mean (SE)
RL1	WL	IV	62	2	29.5	H14 9.82 (1.32)	H2/3 4.31 (0.330)
RL2	**WL**	**V**	**12**	**5.34**	**64.86**	**H14 10.53 (0.551)**	**H2/3 3.74 (0.249)**
RL3	**WL**	**IX**	**30**	**4.82**	**52.3**	**H14 11.00 (0.713)**	**H2/3 3.99 (0.273)**
RL4	WL	X	76	3.92	54.45	H14 10.47 (0.647)	H2/3 3.89 (0.274)
RL5	WL	XI	62	2.55	18.24	-H1 5.93 (444)	+H1 3.32 (0.546)
RL6	WL	XII	10	2.75	41.14	H1410.82 (0.946)	H2/3 4.24 (0.313)
RL7	S440	V	56	3.1	28.45	H14 11.91 (1.432)	H2/3 4.54 (0.336)
RL8	S440	VII	22	2.65	38.41	H14 11.33 (1.405)	H2/3 4.47 (0.328)
RL9	**S440**	**X**	**52**	**5.1**	**54.76**	**H14 10.45 (0.650)**	**H2/3 3.89 (0.277)**
RN1	WL	IX	30.26	2.23	29.74	H14 5.85 (0.550)	H2/3 2.77 (0.147)
RN2	**WL**	**X**	**122**	**2.87**	**19.19**	**H12 3.50 (0.228)**	**H3/4 2.49 (0.218)**
RN3	WL	XII	6	2	30.20	H14 5.77(0.520)	H2/3 2.75 (0.146)
RN5	**S440**	**VII**	**24**	**2.4**	**26.79**	**H34 4.1 (0.238)**	**H1/2 2.39 (0.171)**
RN6	**S440**	**X**	**52**	**3.14**	**43.1**	**H14 5.1 (0.364)**	**H2/3 2.63 (0.147)**

LG linkage group and R^2 = % phenotypic variance explained, +H1 means the presence of homologous chromosome 1 (Q), –H1 means the absence of homologue 1 (q), H2/3 means the presence of H2 and/or H3 (Q–) and H14 means the presence of H1 and H4 (qq), WL: White Lady, major QTLs are shown in *bold.*

4.8. Assessment of markers which closely mapped to QTLs

Comparison of molecular data for markers which closely mapped to major QTLs and result of *in vitro* assessment using 15 tolerant and 15 susceptible genotypes revealed that marker m12 was able to verify 55% of in vitro results. The rate for f13, f20, f58, m51 and f54 were 55, 50, 45, 42 and 40%, respectively.

4.9. Construction of subtractive cDNA library

For the isolation of genes which are induced in *Solanum tuberosum* cv. 'White Lady' by inoculation with PVY^{NTN} the suppression subtractive hybridization method was chosen. The cDNA from both tester and driver samples were isolated, subtractive hybridization was carried out and the hybrid sequences were removed. Consequently, the remaining unhybridized cDNAs represented differentially expressed clones in the tester which cDNAs are absent from the driver, i.e. these represent gene fragments which were induced by the infection. The experimental subtracted samples appeared as a smear from 0.2-3kb, with 10 distinct bands ranged from 0.2-1.25kb. The banding pattern of unsubtracted cDNA ligated with both adaptors (unsubtracted tester control) was different from the banding pattern of experimental subtracted DNA samples (Fig. 13).

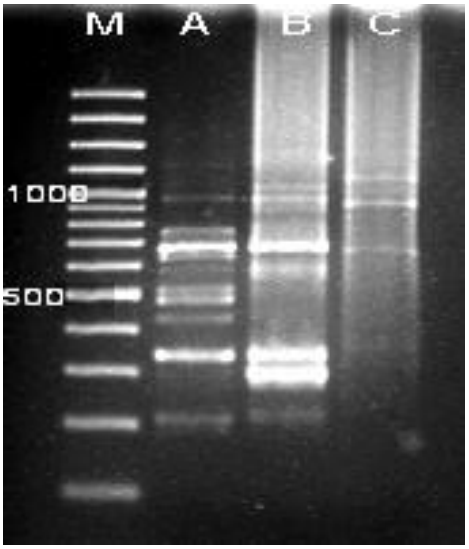


Fig.13. The results of experimental secondary PCR. M: DNA ladder (100bp). A: Secondary PCR products of subtracted tester cDNA. B: Secondary PCR products of unsubtracted driver cDNA. C: Secondary PCR products of unsubtracted cDNA ligated with both adaptors (unsubtracted tester control).

The fragments obtained by PCR were cloned into the pJET1.2/blunt cloning vector based on sticky-end cloning method and the plasmids were transferred into DH5*a* competent cells supplied by Bioline (Bio-85025). Around 500 transformed colonies were isolated and a cDNA library was generated representing ESTs from White Lady (Fig. 14). These colonies were cultured on LB medium (stock culture) and stored at -70ºC until further analysis. From the transformed colonies, 150 contained fragments with different size (150-1150bp), which were isolated for sequencing and similarity analysis.

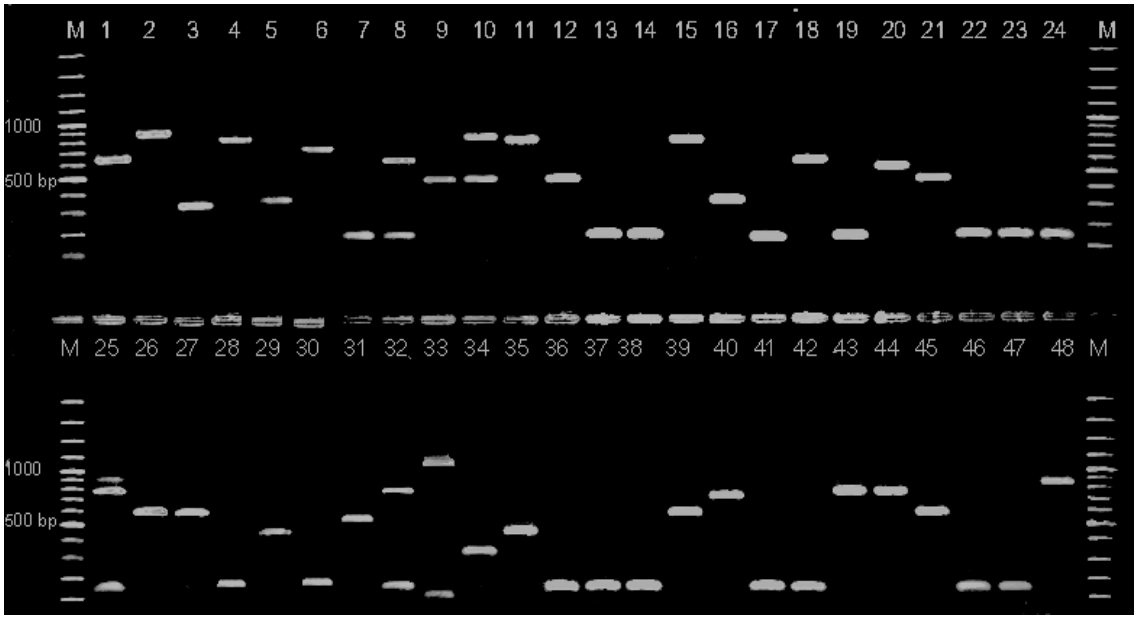


Fig. 14. The results of colony PCR. M: DNA ladder (100bp). Lane 1-48: different EST clones, which have been obtained after subtractive hybridization.

5. DISCUSSION

5.1. Hetero multiplex analysis

Incorporation of disease resistance genes from different source of wild potato species contributes to the production of clones or parental lines carrying resistance genes in hetero multiplex state. Breeding efforts were made to develop parental lines where alleles are originated from different wild potato species and the PVY resistance gene is in a hetero multiplex state, The objective of the this study was the identification of parental lines potentially carrying the alleles of Ry gene against PVY in duplex and triplex state where the alleles are originating from *S. stoloniferum* (4X), *S. tuberosum* ssp. *andigena* (4X) and *S. hougasii* (6X). As a result, we identified one triplex and two duplex genotypes where the extreme resistance genes of the three species are combined. These advanced lines could effectively be used in breeding programs focusing on combination of PVY resistance with quality traits of virus sensitive varieties by increasing the ratio of PVY resistant genotypes in progenies. Moreover the combination of different sources of Ry genes can lead to the development of more durable resistance against the rather changeable pathogen, PVY. Spitters and Ward (1988) found that resistance to potato cyst nematodes was more durable in clones with two resistance genes instead of one. Polgar et al. (2002) developed different hetero duplex lines for PVY resistance. Mendoza and Jayasinghe (1993) as well as Mendoza et al. (1996) developed lines carrying Ry gene originating from *S. tuberosum*, ssp. *andigena* in the duplex and triplex state, but according to our knowledge there is no report on the production and characterization of Ry hetero-triplex advanced genotype.

5.2. Osmotic stress

Osmotic stresses are among the major abiotic stress factors affecting significantly the success of plant production. Evaluation of osmotic stress tolerance/susceptibility is an essential step in the process of plant improvement. In the present study, we evaluated the osmotic stress response of two potato genotypes and their 85 F_1 progenies (cv. WL,

female parent and S440, male parent) under *in vitro* condition. The results showed that there is a difference between the parents for root number and root length when different concentrations of mannitol were used. Parent S440 was proved to be more susceptible to osmotic stress than WL. This phenomenon is in agreement with field behavior of parents where WL reacts with less tuber defects and lower yield decrease to drought and heat stresses. The concentration of 0.3M mannitol was found to be appropriate for the discrimination of sensitive and tolerant genotypes to osmotic stress based on factorial analysis. For root number, Dobranszki et al. (2003) previously reported a difference between sensitive and tolerant potato cultivars under in vitro and osmotic stress conditions. Our result revealed that root length is more susceptible to osmotic stress than root number. In our study there was no significant difference between WL and S440 for root length at different levels of mannitol. As the root number of S440 was comparable to WL under normal conditions and was significantly less at the maximum concentration of mannitol, we assume that a higher concentration of mannitol (above 0.3M) may discriminate WL and S440 based on root length as well. Root mass should be increased in tolerant potatoes under osmotic stress. Larger and deeper roots have been shown to contribute to drought tolerance in potato and many other crops (Schafleitner et al., 2007; Lahlou and Ledent, 2005). Maruyama et al. (2008) declared that the root-bending ratio of lettuce (*Lactuca sativa* L.), which is an indicator of growth sustainability under stress (Howden and Cobbett, 1992; Wu et al., 1996), was not affected at mannitol concentrations up to 5.0% (~ 280 mM), but declined with increasing mannitol concentrations ranging from 5.0% to 12.0%.

The result of F_1 genotypes showed that significant difference (<0.0001) exists between genotypes for root number and root length at 0.3M mannitol concentration. Transgressive segregation for root length was found in the present population and only one genotype (448) produced significantly longer roots than the parents. Lilley et al. (1996) previously reported a transgressive segregation in rice under osmotic stress condition.

5.3. Comparison of marker techniques

The total number of amplified fragments per primer-template combinations depends on the size of the template genome, primer sequence, PCR conditions, competition between potential amplicons and base-mismatching between primer and template (Bussell et al., 2005; Williams et al., 1993; Smith and Williams, 1994; Hallden et al., 1996). In the present study, the average number of scorable bands produced per primer for genotypes using SCOTs, ISSRs and RAPDs were less than those produced for varieties. The average size of SCOT fragments was larger than that of ISSR and RAPD product and the average size of RAPD fragments was smaller than that of ISSRs. Some of the SCOT, ISSR and RAPD primers resulted in polymorphic bands which were amplified from different alleles of a given locus. As the shared lack of PCR products represent the inverses of the shared presences for all individuals, we anticipate that they were different alleles of one locus and should not be scored as different characters. Scoring of different alleles of a given locus was previously reported by Albert (2005); Strong and Lipscomb (1999). They stated that amplification of different alleles of a given locus is a problem for dominant markers when plants having three or more alleles per locus.

The proportion of polymorphic markers is one of the methods for examining similarity of genotype. There is no simple relationship between similarity and taxonomic level (Bussell et al., 2005). Our results exhibited that percentage of average polymorphism within genotypes for SCOT, ISSR and RAPD primers was rather low and is on the similar level (Table 8 and 9). The percentage of average polymorphism among varieties was higher than that of genotypes and it was 61, 47 and 31 for SCOT, ISSR and RAPD, respectively. Wolfe and Liston (1998) compared 54 RAPD-based studies of genetic relationship and reported a mean of 18.6% polymorphic loci within a population of cultivars (n=3) and 62% between populations or varieties of species (n=19). Another way to show the rate of similarity is Jaccard's coefficient of similarity. Our results demonstrate that high genetic diversity exists between the investigated genotypes and varieties. As the parents of genotypes are tetraploids and they differ very much in their pedigree, this range of diversity is normal. The rate of genetic diversity for genotypes was nearly the same using SCOT, ISSR and RAPD markers. Although the rate of diversity for

the three marker type was approximately equal; we anticipate that the source of detected diversity is different, as each technique targets different regions of the genome. The rate of similarity between varieties is less than that of genotypes. The main reason for this is that the parents and origin of varieties which were used in this study are highly different. Overall Shannon index for ISSRs (4.41) was found to be similar to SCOTs (4.47) and RAPD (4.35). This indicates that the relative genetic diversity of the genotypes is similar when SCOT, ISSR and RAPD markers are used. For varieties, overall Shannon index of SCOTs (6.70) was more than ISSRs (5.87) and RAPDs (5.32). This suggested that the relative genetic diversity of the varieties is more when SCOT markers are used but it is fairly similar when ISSR and RAPD markers are used. The AMOVA analysis indicated that more than 90% of the total genetic diversity by SCOT, ISSR and RAPD markers is distributed within groups and only a little of the diversity is attributed to differences between regions. This can be helpful during strategy development for variety collections and evaluations. This low variability between regions was reported in fig varieties and lines from Europe, Asia and Tunisian collections (Ikegami et al., 2009; (Salhi-Hannachi et al., 2005).

Many earlier reports manifested discrepancy between dendrograms when two different molecular marker techniques were used (Sonia and Gopalakrishna, 2007; Arif et al., 2009). Discordance between dendograms or trees obtained using different marker types could be explained by the genetically inert nature of markers when compared to functionally active, different regions of the genome targeted by different markering techniques, level of polymorphism detected and the number of loci and their coverage of the overall genome (Souframanien and Gopalakrishna, 2004). Our results promote the previous reports by clustering genotypes and varieties in different groups using different marker techniques. However, some common groups were identified between clustering patterns of each marker and similar results were reported in potato (McGregor et al., 2000; Norero et al., 2003), Shishma (Arif et al., 2009). Comparison of clustering patterns also revealed that location specificity of SCOT technique was higher than other markers because it discriminated a lot of varieties according to their relationship and location where they were released. PCoA analysis (3Dimension) from SCOT, ISSR and RAPD data showed similar results using cluster analysis (Fig. 5, 7 and 9).

Bootstrap values in general were low for the main clusters (data not shown). The number of markers and some biological factors which interfere with the DNA data could cause these results. Similar results were obtained for the species *Magnaporthe grisea* as well (Kumar et al., 1999; Sonia and Gopalakrishna., 2007). Moreover, interpretation of bootstrap values has been a controversial topic and its reliable estimation could be obtained with sufficient polymorphic loci (Sanderson and Wojciechowski, 2005; Kalinowski, 2005).

5.4. Development of IT-SCoT marker

Gene-targeted markers are preferred for numerous applications in plant molecular genetics especially for QTL mapping since recombination levels between gene-based markers and gene/QTL are generally lower compared with 'indirect random markers' such as RAPDs, ISSRs, or SSRs (Andersen and Lubberstedt 2003). It-SCoT can be considered to SCoT when primers are used as single, IT when primer pair is used and TRAP when single primers are used with other random primers such as ISSR.

Due to the basis of IT-SCoT primer design, we expect SCoT markers to be distributed within gene regions that contain genes on both plus and minus DNA strands. It is also possible that pseudogenes and (genes within) transposable elements may be used as primer binding sites when single primer is used. An important factor is that the technique capable combine advantages of three marker techniques namely IT, SCoT and TRAP. The present study demonstrated that the total of polymorphic and scorable bands produced by one pair of primer was more than that when IT, SCoT and TRAP primers were used separately. Therefore, the total cost per polymorphic band will be less when we use IT-SCoT primers.

5.5. Genetic linkage map and QTLs

A genetic linkage map represents the relative order of genetic markers along chromosomes. A complete linkage map of potato would comprise 12 sets of four homologous chromosomes (linkage groups, LGs), and the 12 LGs would be numbered from I to XII to have the same nomenclature with those now adopted for the 12 unique

chromosomes of potato (Dong et al., 2000; Celebi-Toprak et al., 2005; Bradshaw et al., 2008). This ideal case was not achieved at the present study, but 13 LG groups were established for WL and 14 LG groups for S440 after omitting of double-simplex markers. Double-simplex markers are very uninformative about recombination and should be omitted from the linkage maps of the parents (Bradshaw et al., 2008). The 13 WL groups (total map length 951 cM) were aligned with those from S440 (total map length 1096 cM). There were insufficientnumber of bridging markers between WL and S440 to accurately align the parental maps. For that reason QTL models were also fitted to the two parents separately. The same result was previously reported by Bradshaw et al. (2008) for tetraploid potato. Furthermore the developed genetic map was not an improvement on the previously published one and consequently we could not compare our map with the previously published one.

The linkage map for chromosomes XII was the most appropriate because it was identified by two well characterized chromosome specific IT and SSR marker. Bradshaw et al. (2008) identified chromosomes IV and V as the most acceptable with two well characterized chromosome specific SSR markers. Based on a rule of thumb at least three putatively homologous markers per LG should be used for chromosome identification (Rouppe van der Voort et al., 1997). Hence, chromosomal identification of chromosomes VII and XI of WL and IX and XII of S440 which fond in present study is tentative, with varying degrees of ambiguity, until they can be associated with well characterized SSR, IT or other chromosome specific markers.

For mapping and QTL analysis in potato, it is important to have adequate simplex markers on each of the 48 chromosomes of both parents. Duplex markers are necessary for identifying homologous chromosomes. As recombination frequencies from simplex/duplex markers in coupling and repulsion are estimated with equal accuracy, in theory, a single duplex marker per LG would be sufficient (Bradshaw et al., 2008). In the this study we used 65 simplex and 24 duplex markers for mapping of WL and 95 simplex and 16 duplex markers for mapping of S440. Hackett et al. (1998) used 14 simplex and 4 duplex markers per set of four homologous chromosomes in their simulation study and concluded that a population size of at least 150, and preferably 250, should be used to identify homologous chromosomes. Hence we just constructed an overall map for each

LG. Our results also showed that there was no even distribution of duplex markers for parents. This result is in agreement with the results of Bradshaw et al (2008) found in potato. They suggested that increasing the number of primer combinations should provide more than enough duplex markers present in one parent and absent in the other. It remains to be studied if in future SNPs will provide the required density of markers more cheaply and easily, but the same problems have to be faced over linkage phase.

The use of genetic linkage maps can help to identify the loci contributing to adaptive changes in populations. Maps with a density of markers around 20–30 cM are sufficient for detecting the presence of QTLs and increasing marker density allow more precise positioning of the QTLs (Darvasi and Soller, 1994). Moreover the introduction of DNA-based molecular markers allow the identification of genetic factors (QTLs) underpinning the variation of quantitative traits (Tanksley, 1993; Quarrie, 1996; Ribaut et al., 1997; Tuberosa et al., 1998; Sari-Gorla et al., 1999). Although QTLs for root characteristics have been extensively analyzed in other plants such as rice (Yadav et al., 1997; Price et al., 2000), limited information is available for potato. This study indicates the possibility of using *in vitro* tests to identify QTLs for traits of the root system in tetraploid potato. A total of 14 QTLs with LOD>2 were identified. Only 6 of them were confirmed as major QTL based on the permutation test. Out of them 3-3 QTLs were identified to affect root length and root number, respectively. Three QTLs for root length explained 51, 52.3 and 64.9% of phenotypic variance, individually. This range of phenotypic variance previously reported by Bradshaw et al. (2008) for maturity (one QTL), after cooking blackening (six QTLs) and tuber shape (four QTLs) of potato. Three major QTLs for root number explained 19.2, 26.8 and 43.1% of variation, independently. This range of phenotypic variance previously accounted for four QTLs of Sprouting and fry colour of potato (Bradshaw et al. 2008). For each major QTL, we identified closely linked markers that mapped at 2 to 4 cM from them. These markers were able to verify 40 to 55% of *in vitro* results. The development of near-isogenic lines at this QTL region would provide a valuable opportunity to validate and further characterize their effects on other quantity traits such as yield under osmotic stress conditions. The phenotypic variance explanation of 8 remaining QTLs which were not identified as major QTL based on permutation test was relatively high and ranged from 18.2 to 54.5%. Bradshaw et al. (2008) confirmed

that some QTLs of large effect may have been missing through inadequate marker coverage on some chromosomes, but it is more likely that many QTLs of minor effect remain undetected. QTLs with minor to intermediate effects, rather than ones of large effect, have been reported for some feature of potato such as yield, dormancy, specific gravity, tuberization, cold sweetening, sugar content (Bonierbale et al. 1993; Freyre et al. 1994; Freyre and Douches, 1994; Van den Berg et al. 1996a, b; Schaefer-Pregl et al. 1998; Menendez et al. 2002).

5.6. cDNA subtraction

The suppression subtractive hybridization method proved once more to be an efficient tool to generate cDNA libraries under specific circumstances. In the present study, it was used to subtract the cDNA pool of White Lady that is resistance against PVY^{NTN}. The result of subtraction revealed that the banding pattern of unsubtracted cDNA ligated with both adaptors was different from the banding pattern of experimental subtracted DNA samples that show subtraction was successfully performed.

The generation of cDNA library presented a high amount of genes that could be related to pathogenesis response.

SUMMARY AND FUTURE DIRECTIONS

We successfully combined different alleles of an extreme resistance gene of PVY (Ry_{sto}, Ry_{adg} and Ry_{hou}) in specific advanced parental lines. These triplex and duplex lines could be effectively used in breeding programs focusing on combination of PVY resistance with quality traits of virus sensitive varieties by increasing the ratio of PVY resistant genotypes in progenies. More over these progenitors have the potential to provide a durable PVY genetic control and reduce the present influence of this virus on the potato crop.

Development of osmotic stress tolerant varieties is a demand for potato improvement. The evaluation of osmotic stress tolerance of potato genotypes in conventional field trials is rather time consuming and labor intensive and the results are often confounded by field and environmental conditions. Our study demonstrated that root number and root length are appropriate traits to study osmotic stress tolerance under *in vitro* conditions and could be used to identify QTLs responsible for this feature.

For the identification of QTL markers which are closely linked in coupling to the genes affecting the phenotype, a linkage maps is essential. In order to develop a linkage map, the use of several different types of molecular markers is advantageous. For this reason, we used different marker types to construct a genetic linkage map in tetraploid potato in this study. As far as we know, this is the first report to apply SCoT markers for the construction of a linkage map in tetraploid potato. The constructed genetic maps consist of 13 linkage groups (LGs) for White Lady and 14 LGs for S440. Three LGs were obviously corresponded to chromosomes VII, XI and XII while two others were tentatively assigned with chromosomes IX using Intron targeting and SSR markers. The constructed genetic linkage maps were used to identify QTLs responsible for osmotic stress tolerance. Finally, we could identify 6 major QTLs which were closely mapped in coupling to molecular markers in different linkage groups and these markers could be used to select osmotic stress tolerant genotypes. Nevertheless, further experiments are required to confirm the utility of these markers to discriminate resistant/susceptible genotypes with known root mass production under field conditions. Furthermore,

construction of a genetic linkage map needs some basic but necessary information about efficiency of marker techniques to produce polymorphism and reliable bands which are useable to fingerprint the potato genome as well. Therefore, we checked efficiency of different marker types. The results revealed that SCOT, ISSR and RAPD markers are capable to generate high number of polymorphic markers which can be used in diagnostic fingerprinting studies of tetraploid potato. Based on the average percentage polymorphism, PIC, Rp, diversity index, marker index and overall Shannon index, the efficiency of SCOT for fingerprinting of varieties was more than other markers. In these terms ISSRs are more informative than RAPD markers. The efficiency of SCOT, ISSR and RAPD markers for fingerprinting of genotypes is relatively the same. In general, these three marker types could be used in conjunction with each other for diagnostic fingerprinting of tetraploid potato.

Based on the obtained results, we believe that the development of gene-targeted markers which are located near the candidate genes will be useful for molecular studies in the tetraploid potato. Hence we could develop a new marker technique named IT-SCoT where we could combine advantages of three marker techniques namely IT, SCoT and TRAP.

LIST OF NEW FINDINGS

1) Identification of one heterotriplex parental line having the Ry resistance gene from potato species of *S. stoloniferum, S. tuberosum*, ssp. *andigena* and *S. hougasii.*
2) Comparison of clustering patterns revealed that location specificity (the origin of the variety) of SCOT technique was higher than other markers because it discriminated varieties according to their relationship and location where they were released.

3) The results suggest that efficiency of SCOT, ISSR and RAPD markers was relatively the same in fingerprinting of F1 population of potato but SCOT analysis is more effective in fingerprinting of potato varieties.

4) Development of a new marker technique termed IT-SCoT being capable to combine advantages of three marker techniques namely IT, SCoT and TRAP.

5) Thirteen and 14 linkage groups (LGs) were established for WL and S440, respectively after excluding double-simplex markers. Three linkage groups have been corresponded to chromosomes VII, XI and XII while two others were tentatively assigned with chromosome IX. The linkage map for chromosome XII was the most appropriate because it was identified by two well characterized chromosome specific IT and SSR markers.

6) During the analysis of *in vitro* osmotic tolerance in F1 tetraploid genotypes, 14 QTLs with LOD>2 were identified. Out of them, 6 QTLs (3 for root length and 3 for root number) were confirmed as major QTL-s based on the permutation test. For root length one major QTL was identified on chromosome XII explaining 52.3% of phenotypic variance. For root number one QTL with 19.2% of phenotypic variance was identified tentatively on chromosome IX and one was putatively identified on chromosome XII (LOD of 2.4) explaining 26.8 % of phenotypic variance.

7) For each major QTL, we identified closely linked markers mapped at 2 to 4 cM from them. These markers were able to verify 40 to 55% of *in vitro* results.

8) The linkage between QTLs of root number and root length indicates that the two traits are associated.

ACKNOWLEDGEMENTS

PRAISE AND THANKS THE ALMIGHTY GOD, HIS WORSHIP CAUSING BLESSED AND THANKSGIVING OF HIM INCREASING THE BLESSING

"The work goes on, the cause endures, the hope still lives and the dreams shall never die" with the words of Edward Kennedy in my mind I started my project and now it is time for me to finish it and thank everyone who helped, encouraged and supported me during my PhD course without which the completion of this thesis would have not been possible.

I wish to express my profound sense of reverence and gratitude to Dr. Zsolt Polgár and Dr. János Taller, firstly for accepting me to be my supervisors and for their constructive guidance, inspiration, persuasion, befitting advisement, unending benevolence, debonair discussion, excellent counsel and solicitous behaviour during the course of investigation which proved a boon for me in making this work a success. Special thanks also go to their kind families who have made my family's stay at Keszthely pleasant and peaceful.

I am grateful to the Seed and Plant Improvement Research Institute, Karaj, Iran, for accepting to carry out my doctoral studies abroad; the University of Pannonia, Georgikon Faculty, Keszthely for providing me an opportunity as well as all the required facilities for conducting my research during my tenure as a PhD student.

I owe my sincere thanks to Dr. Richard Gáborjanyi for his guidance and motivation throughout my project.

Many thanks go to my friends and colleagues, Dr. Péter Poczai for his invaluable tips and advice which made a huge impact on the way I carried out my experiments; Dr. István Cernák for discussions and help to organize my project.

I have to say a special thanks to my lab buddies; Ms. Kinga Matyas, Dr. Kincso Decsi, Mr. Rahim Ahmadvand, Mr. Vignesh Murthy and Mr. Shifarew Abate Gizaw whose presence made the lab a convivial place to work in and also for their timely help, valuable suggestions, corrections in the experiments and molding me in a friendly way.

I extend my heartiest thanks to Mr. Wolf István, Mr. Vaszily Zsolt and Mrs. Ildiko for their help and constant support during the greenhouse experiments.

I take this opportunity to also thank all the excellent staff of the Potato Research Center and the Biotechnology Lab for their assistance during my work and helping me to complete my thesis in time.

My deepest gratitude goes to my family for their unflagging love and support throughout my life; this dissertation is simply impossible without them. Their unflinching support and belief in me has helped me to walk towards my dream. I am sure without my mother's prayers and my father's wishes life would not have been easier. I thank the Almighty for giving me all the people I have mentioned above and for giving me the courage to face the world.

REFERENCES

Adams, M.D., Kelley, J.M., Gocayne, J.D., Dubnick, M., Polymeropoulos, M.H., Xiao, H., Merril, C.R., Wu, A., Olde, B., Moreno, R.E., Kerlavage, A.R., Combie, W.R. and Venter, J.C. (1991) Complementary DNA sequencing: expressed sequence tags and human genome project. *Science* 252, 1651–1653.

Agarwal, M., Neeta, S., and Harish, P. (2008) Advances in molecular marker techniques and their applications in plant sciences. Plant Cell Rep 27:617–631.

Akagi, H., Yokozeki, Y., Imagaki, A., Nkamura, A. and Fujimura, T. (1996) A codominant DNA marker closely linked to the rice nuclear restorer gene, RF-1, identified with inter-SSR fingerprinting. Genome 39: 1205–1209.

Albert, V.A (2005) Parsimony and phylogenetics in the genomic age. In: Albert VA. (Ed.), Parsimony, Phylogeny and Genomics. Oxford University Press, Oxford, pp1-11.

Al-Janabi, S., Honeycutt, R., McClelland, M., and Sobral, B. (1993) A genetic linkage map of *Saccharum spontaneum* L. 'SES 208'. Genetics 134:1249–1260.

Al-Janabi, S., Honeycutt, R., and Sobral, B. (1994) Chromosome assortment in *Saccharum*. Theor Appl Genet 89:959–963.

Andersen, J.R., and Lubberstedt, T. (2003) Functional markers in plants. *Trends in Plant Science* 8, 554–560.

Arif, M., Zaidi, N.W., and Singh, Y.P. (2009).A comparative analysis of ISSR and RAPD markers for study of genetic diversity in Shisham (*Dalbergia sissoo*). Plant Mol Biol Rep 27:488–495.

Asama, K., Ito, H., Murakami, N., and Itoh, T. (1982) New potato variety "Konafubuki". Bull Hokkaido Pref Agr Exp Stn 48: 75–84.

Avise, J.C. (2004) Molecular Markers, Natural History and Evolution, 2nd edn. Sinauer Associates, Inc., Sunderland, Massachusetts.

Bachem, C., Van Der Hoeven, R., Lucker, J.,, Oomen, R., Casarini, E., Jacobsen, E., and Visser, R. (2000) Functional genomic analysis of *potato* tuber life cycle. *Potato Res*. 43, 297-312.

Bachem, C.W.B., van der Hoeven, R.S., de Bruijn, S.M., Vreugdenhil, D., Zabeau, M., and Visser, G.R.F. (1996) Visualization of differential gene expression using a novel method of RNA fingerprinting based on AFLP: analysis of gene expression during potato tuber development. *The Plant Journal* 9, 745–753.

Bálint, A. F., Szira, F., Börner, A., and Galiba, G. (2008) Segregation- and association based mapping of loci influencing osmotic tolerance in barley. Acta Biologica Szegediensis, 52 (1):101-102.

Bamberg, J., and del Rio, A. (2005) Conservation of potato genetic resources. In: Razdan MK, Mattoo AK (eds.) Genetic improvement of Solanaceous crops, volume I: potato. Science Publishers Inc, Enfield, NH, pp. 1–38.

Bardakci, F. (2001) Random amplified polymorphic DNA (RAPD) markers. Turk J Biol 25:185–196.

Barker, H. (1997) Extreme resistance to potato virus V *in* clones of Solanum tuberosum that are also resistant to potato viruses Y and A: evidence for a locus conferring broad-spectrum resistance. *Theor. Appl. Genet*. 95, 1258-1262.

Barker, H., and Solomon, R.M. (1990) Susceptibility to virus infection of transgenic plants expressing structural and non-*structural* genes of *tobacco rattle virus*. *Theor. Appl. Genet*. 80, 188-192.

Barker, H., and Harrison, B.D. (1984) Expression of genes for resistance to *potato virus Y* in potato plant and protoplasts. *Annals of Applied Biology*, 105, 539-545.

Bassam, B.J., Caetano-Anolles, G., and Gresshoff, P.M. (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels, Anal. Biochem. 196 80–83.

Beczner, L., Horvath, J., Romhanyi, I., and Forster, H. (1984) Studies on the etiology of tuber necrotic ringspot disease in potato. Potato Res 27:339–352.

Bernatzky, R., and Tanksley, S. (1986) Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics 112:887–898.

Bhalerao, R., Keskitalo, J., Sterky, F., Erlandsson, R., Bjorkbacka, H., Birve, S.J., Karlsson, J., Gardestrom, P., Gustafsson, P., Lundeberg, J., and Jansson, S. (2003) Gene expression in autumn leaves. Plant Physiol 131:430–442.

Boguski, M.S., and Schuler, G.D. (1995) Establishing a human transcript map. Nat Genet

10:369–371.

Bonierbale, M.W., Plaisted, R.L., and Tanksley, S.D. (1993) A test of the maximum heterozygosity hypothesis using molecular markers in tetraploid potatoes. Theor Appl Genet 86:481–491.

Bonierbale, M.W., and Deahl, K.L. (1998) QTL mapping of foliar glycoalkaloid aglycones *Solanum tuberosum* x *S. berthaultii* potato progenies: quatitative variation and plant secondary metabolism. Theor. Appl. Genet. 97, 563–574.

Bonierbale, M.W., Plaisted, R.L., and Tanksley, S.D. (1988) RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. Genetics 120:1095–1103.

Bonierbale, M.W., Simon, R., Zhang, D.P., Ghislain, M., Mba, C., and Li, X.Q. (2003) Genomics and Molecular Breeding for Root and Tuber Crop Improvement. In: Plant Molecular Breeding (H.J. Newbury, ed.), p. 216–253. Blackwell, Oxford.

Bornet, B., and Branchard, M. (2001) Nonanchored Inter Simple Sequence Repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting. Plant Mol Biol Rep 19: 209–215.

Bornet, B., Goraguer, F., Joly, G., and Branchard, M. (2002) Genetic diversity in European and Argentinian cultivated potatoes (*Solanum tuberosum* subsp. *tuberosum*) detected by inter-simple sequence repeats (ISSRs). Genome 45(3): 481–484.

Bosze, Z., Kazinczi, G., and Horvath, J. (1996) Reaction *of* unknown Solanum stoloniferum. Schlechtd. et Bche and Solanum demissum Lindl. accessions to the tuber necrosis strain of potato Y Potyvirus (PVY^{NTN})..*Acta Phytopathol. Entomol. Hung*. 31, 169-174.

Bowman, K.O., Hutcheson, K., Odum, E.P., and Shenton, L.R. (1969) Comments on the distribution of indices of diversity, Proc. Intl. Symp. Stat. Ecol 3:315-359.

Bradeen, J. M., lorizzo, M., Gao,L., Aversano, R., Quirin, E.A., and Carputo, D. (2008) comparative structural genomics of the potato tertiary genepool: improving access to disease resistance genes. PAG Solanacea workshop: ppg.cfans.umn.edu.

Bradshaw, J.E., Hackett, C.A., Pande, B., Waugh, R., and Bryan, G.J. (2008) QTL mapping of yield, agronomic and quality traits in tetraploid potato (Solanum tuberosum

subsp. tuberosum), Theor Appl Genet, 116:193–211.

Bradshaw, J.E., and Mackay, G.R. (eds) (1994) Potato Genetics. CAB International, Wallingford, 467–497.

Bradshaw, J.E. (2007) Breeding potato as a major staple crop. In: Kang MS, Priyadarshan PM (eds.) Breeding major food staples. Blackwell, Oxford, pp. 277–332.

Bradshaw, J.E., Dale, M.F.B., Swan, G.E.L. and et al. (1998) Early-generation selection between and within pair crosses in a potato (*Solanum tuberosum* subsp. *tuberosum*) breeding programme. Theor Appl Genet 97: 1331–1339.

Bradshaw, J.E., and Mackay, G.R. (1994) Breeding strategies for clonally propagated potatoes. In: Bradshaw JE,Mackay GR (eds.) Potato genetics. CAB International,Wallingford, pp. 467–497.

Bradshaw, J.E. (2000) Conventional breeding in potatoes: Global achievements, In Potato, Global Research and Development, Paul Khurana, S. M., Shekhawat, G. S., Singh, B. P., and Pandey, S. K., Eds., Shimla, India: Indian Potato Association, pp. 41–51.

Brigneti, G., Garcia-Mas, J., and Baulcombe, D.C. (1997) Molecular mapping of the *potato virus Y* resistance gene Ry_{sto} in potato. Theor. Appl. Genet. 94:198–203.

Brown, C.R., and Thomas, P.E. (1994) Resistance to potato leafroll virus derived from *Solanum chacoense*: Characterization and inheritance. Euphytica, 74: 51–57.

Brown, C.R. (1993) Outcrossing rate in cultivated autotetraploid potato. Am. Potato J. 70:725–734.

Brown, J. and Dale, M.F.B. (1998) Identifying superior parents in a potato breeding program using cross prediction techniques, Euphytica, 104, 143–149.

Browning, I., Charlet, K., Chrzanowska, M., Dˇediˇc, P., Kerlan, C., Kryszczuk, A., Schubert, J., Varveri, C., Werkman, A., and Wolf, I. (2004) What is PVY^{NTN} ? The Reaction of Potato Cultivars to Inoculation with a Range of PVY Isolates, p. 151. 12th EAPR Virology Section Meeting, Rennes, France.

Brugmans, B., del Carmen, A.F., Bachem, C.W.B., van Os, H., van Eck, H.J., and Visser, R.G.F. (2002) A novel method for the construction of genome wide transcriptome

maps.*Plant J*. 31, 211-222.

Brugmans, B., Ronald, G.B., Hutten, A., Nico, O., Rookmaker – Richard, G.F., Visser Herman, J., and van, E. (2006) Exploitation of a marker dense linkage map of potato for positional cloning of a wart disease resistance gene. Theor Appl Genet, 112: 269–277.

Bussell, J.D., Waycott, M., and Chappill, J.A. (2005) Arbitrarily amplified DNA markers as characters for phylogenetic inference. Persp Plant Ecol Evol Syst 7:3-26.

Caligari, P.D.S. (1992) Breeding new varieties. In: Harris P M (eds.) The potato crop, Scientific Basis for Improvement. Chapman and Hall, London pp. 334–372.

Camadro, E.L., Carputo, D., and Peloquin, S.J. (2004) Substitutes for genome differentiation in tuberbearing *Solanum*: interspecific pollen-pistal incompatibility, nuclear-cytoplasmic male sterility, and endosperm. Theor Appl Genet 109: 1369–1376.

Carputo, D., and Barone, A. (2005) Ploidy level manipulations in potato through sexual hybridisation. *Annals of Applied Biology*, 146: 71–79.

Celebi-Toprak, F., Watanabe, J.A., and Watanabe, K.N. (2005) Molecular markers in identification of genotypic variation. In: Razdan MK, Mattoo AK (eds) Genetic improvement of Solanaceous crops: potato, vol 1. Science Publishers, Enfield, pp 115–141.

Celebi-Toprak, F., Slack, S.A., and Jahn, M.M. (2002) A new gene, *Nytbr*, for hypersensitivity to *Potato Virus Y* from *Solanum tuberosum* maps to chromosome IV. Theor. Appl. Genet. 104:669–674.

Cernak, I., Decsi, K., Sandor, N., Istvan, W., Polgar, Z., Gergely, G., Yataka, H., and Taller, J. (2008) Development of a locus-specific marker and localization of the Ry_{sto} gene based on linkage to a catalase gene on chromosome XII in the tetraploid potato genome. Breeding Science, 58: 309-314.

Charcosset, A., and Gallais, A. (2003) In: D. de Vienne (Ed.), *Molecular Markers in Plant Genetics and Biotechnology*. Science Publishers, Enfield, New Hampshire, U.S.A.

Charters, Y.M., Robertson, A., Wilkinson, M.J., and Ramsay, G. (1996) PCR analysis of oilseed rape cultivars (*Brassica napus* L. ssp. *olifera*) using 5'-anchored simple sequence repeat (SSR) primers. Theor Appl Genet 92: 442–447.

Chavez, R., Brown, C.R., and Iwanaga, M. (1988) Application of interspecific

sesquiploidy to introgression of PLRV resistance from non-tuber-bearing *Solamum tuberosum* to cultivated potato germplasm. *Theor. Appl. Genet*. 76, 497-500.

Chen, Q., Kawchuk, L.M., Lynch, D.R., Goettel, M.S., and Fujimoto, D.K. (2003) Identification of late blight, Colorado potato beetle and blackleg resistance in three Mexican and two South American wild 2x (IEBN) Solanum species. Am. Potato J. 80, 9-19.

Chrzanowska, M. (1991) New isolates of the necrotic strain of *potato virus* Y (PVNN) found recently in Poland. Potato Res 34:179–182.

Chrzanowska, M. (1994) Diverentiation of *potato virus Y* (PVY) isolates. Phytopathol Pol 8:15–20.

Churchill, G.A., and Doerge, R.W. (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971.

Collard, B.C.Y., and Mackill, D.J. (2009) Start Codon Targeted (SCoT) polymorphism: A simple novel DNA marker technique for generating gene- targeted markers in plants. Plant Mol Biol 27:86-93.

Colon, L.T., Eijlander, R., Budding, D.J., Van Ijzendoom, M.T., Pieters, M.M.J., and Hoogendoom, J. (1993) Resistance to potato late blight (*Phytophthora infestans* (Mont.) de Bary) in *Solanum nigrum, S. villosum* and their sexual hybrids with *S. tuberosum* and *S. demissum*. *Euphytica* 66, 55-64.

Colon, L.T., Turkensteen, L.J., Prummel, W., Budding, D.J., and Hoogendoorn, J. (1995) Durable resistance *to* late blight (Phytophthora infestans) in old potato cultivars. *Eur. J. Plant Pathol*. 101, 387-397.

Correll, D.S. (1962) The potato and its wild relatives. Texas Research Foundation, Renner, TX, 606 pp.

Crookshanks, M., Emmersen, J., Welinder, K.G., and Nielsen, K.L. (2001) The potato tuber transcriptome: Analysis of 6077 expressed sequence tags. *FEBS Lett*. 506, 123-126.

Da Silva, J., Sorrells, M.E., Burnquist, W.L., and Tanksley, S.D. (1993) RFLP linkage map and genome analysis of *Saccharum spontaneum* L. Genome 36:782–791.

Damude, H.G., and Kinney, A.J. (2008) Enhancing plant seed oils for human nutrition.

Plant Physiology 147, 962–968.

Darvasi, A., and Soller, M. (1994) Optimum spacing of markers for determining linkage between marker loci and quantitative trait loci. Theor Appl Genet, 89: 351–357.

Davis, T.M., Yu, H., Haigis, K.M., and McGowan, P.J. (1995) Template mixing: a method of enhancing detection and interpretation of codominant RAPD markers. *Theor Appl Genet*. 91:582–8.

De Bokx, J.A., and van der Want, J.P.H. (eds) (1987) Viruses of Potatoes and Seed-Potato Production. Pudoc, Wageningen, The Netherlands.

De Jong, H., and Rowe, P.R. (1971) Inbreeding in cultivated diploid potato. Potato Res 14: 74–83.

De Maine, M.J., Carroll, C.P., Stewart, H.E., Solomon, R.M., and Wastie, R.L. (1993) Disease resistance in *Solanum phureja* and diploid and tetraploid *S. tuberosum* × *S. phureja* hybrids. *Potato Res*. 36, 21-26.

Dean, M., White, M.B., Amos, J., Gerrard, B., Stewart, C., Khaw, K.T., and Leppert, M. (1990) Multiple mutations in highly conserved residues are found in mildly affected cystic filrosis patients. Cell 61:863–870.

Del Rio, A.H., Bamberg, J.B., and Huaman, Z. (1997 a) Assessing changes in the genetic diversity of potato genebanks. 1. Effects of seed increase. Theor Appl Genet 95:191-198.

Del Rio, A. H., J. B. Bamberg, Z. Huaman, A. Salas and S. E. Vega (1997 b) Assessing changes in the genetic diversity of potato genebanks 2. In situ vs ex situ. Theor Appl Genet 95: 199-204.

Demeke, T., Kawchuk, L.M., and Lynch, D.R. (1993) Identification of potato cultivars and clonal variants by random amplified polymorphic ANA analysis, American Potato Journal 70: 461-470.

Dhanaraj, A.L., Slovin, J.P., and Rowland, L.J. (2004) Analysis of gene expression associated with cold acclimation in blueberry floral buds using expressed sequence tags. Plant Sci 166:863– 872.

Diatchenko, L., Lau, Y.F.C., Campbell, A.P., Chenchik, A., Moqadam, F., Huang, B., Lukyanov, S., Lukyanov, K., Gurskaya, N., Sverdlov, E.D., and Siebert, P.D. (1996) Suppression subtractive hybridization: a method for generating differentially regulated or

tissue-specific cDNA probes and libraries.

Dobranszki, J., Tabori, K.M., and Hudak, A.T. (2003) Growth and developmental responses of potato to osmotic stress under in vitro conditions, Acta Biologica Hungarica 54 (3–4), pp. 365–372.

Dodds, K.S. (1962) Classification of cultivated potatoes. In: Correll DS (ed.) The potato and its wild relatives. Texas Research Foundation, Renner, TX, pp. 517–539.

Dodds, K.S. (1965) The history and relationships of cultivated potatoes. In: Hutchinson JB (eds) Essays in crop plant evolution. Cambridge University Press, Cambridge, England, pp. 123–141.

Dong, F., Song, S., Naess, S.K., Helgeson, J.P., Gebhardt, C., and Jiang, J. (2000) Development and applications of a set of chromosome-specific cytogenetic DNA markers in potato. Theor Appl Genet 101:1001–1007.

Douches, D.S., and Jastrzebski, K. (1993) Potato. In: Kalloo G, Bergh BO (eds.) Genetic improvement of vegetable crops. Pergamon Press, Oxford, pp. 605–644.

Dubos, C., and Plomion, C. (2003) Identification of water-deficit responsive genes in maritime pine (Pinus pinaster Ait.) roots. Plant Mol Biol 51:249–262.

Dudley, J.W. (1993) Molecular markers in plant improvement: manipulation of genes affecting quantitative traits. *Crop Science* 33, 660–668.

Dwivedi, S.L., Crouch, J.H., Mackill, D.J., Xu, Y., Blair, M.W., Ragot, M., Upadhyaya, H.D., and Ortiz, R. (2007) The molecularization of public sector crop breeding: progress, problems and prospects. *Advances in Agronomy* 95, 163–318.

Ehsanpour, A.A., and Razavizadeh, R. (2005) Effect of UV-C on drought tolerance of alfalfa (Medicago sativa) callus. American J. Biochemistry and Biotechnol. 1(2): 107-110.

EPSO (2005) European plant science: a field of opportunities. Journal of Experimental Botany, 417: 1699–1709.

Erusha, K.S., Shearman, R.C., Roirdan, T.P., and Wit, L.A. (2002) Kentucky bluegrass cultivar root and top growth responses when grown in hydroponics. Crop Sci. 42:848–852.

Eujayl, I., Sorrells, M.E., Baum, M., Wolters, P., and Powell, W. (2002) Isolation of EST-derived microsatellite markers for genotyping the A and B genomes of wheat. Theor Appl Genet 104:399– 407.

Evans, K., Trudgill, D.L., Raman, K.V., and Radcliffe, E.B. (1992) Pest Aspects of Potato Production. In: The Potato Crop (P. Harris, ed.), p. 438–506.

Faivre-Rampant, O., Cardle, L., Marshall, D., Viola, R., and Taylor, M.A. (2004) Changes *in* gene expression during meristem activation processes in Solanum tuberosum with a focus on the regulation of an auxin response factor gene. *J. Exp. Bot*. 55, 613-622.

Falconer, D.S., and Mackay, T.F.C. (1996) Introduction to Quantitative Genetics, 4th edition. Longman, Harlow, England.

Falque, M., and Santoni, S. (2007) Molecular markers and high-throughput genotyping analysis. In: Morot- Gaudry, J.F., Lea, P. and Briat, J.F. (eds) *Functional Plant Genomics*. Science Publishers, Enfield, New Hampshire, pp. 503–527.

Fauquet, C.M., Mayo, M.A., Maniloff, J., Desselberger, U., and Ball, L.A. (2005) Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses. Elsevier, San Diego, CA, U.S.A.

Flis, B., Hennig, J., Strzelczyk-_Zyta, D., Geberhart, C., and Marczewski, W. (2005) The *Ry-fsto* gene from *Solanum stoloniferum* for extreme resistant to *Potato Virus Y* maps to potato chromosome XII and is diagnosed by PCR marker GP122718 in PVY resistant potato cultivars. Mol Breed 15:95–101.

Fowler, S., and Thomashow, M.F. (2002) *Arabidopsis* transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. Plant Cell 14:1675–1690.

Freyre, R., and Douches, D.S. (1994) Development of a model for marker assisted selection of specific gravity in diploid potato across environments. Crop Sci 34:1361–1368.

Freyre, R., Warnke, S., Sosinski, B., and Douches D.S. (1994) Quantitative trait locus analysis of tuber dormancy in diploid potato (Solanum spp.). Theor Appl Genet 89:474–480.

Frusciante, L., Barone, A., Carputo, D., Ercolano, M.R., Rocca, della F., and Esposito, S.

(2000) Evolution and use of plant biodiversity for food and pharmaceuticals. Fitoterapia, 71: S66–S72.

Fukuoka, S., Inoue, T., Miyao, A., and Monna, L. (1994) Mapping of sequence-tagged sites in rice by single conformation polymorphism. DNA Res 1:271–277.

Galván, M., Aulicino, M., García Medina, S., and Balatti, P.A. (2001) Genetic diversity among northwestern argentinian cultivars of common bean (*Phaseolus vulgaris* L.) as revealed by RAPD markers. Genet Res and Crop Evol 48: 251–260.

Gebhardt, C., and Valkonen, J.P.T. (2001) Organization of genes controlling disease resistance in the potato genome. Annual Review of Phytopathology, 39: 79–102.

Georgieva, M., Djilianov, D., Konstantinova, T., and Parvanova, D. (2004) Screening of Bulgarian raspberry cultivars and elites for osmotic tolerance in vitro. Biotechnol. & Biotechnol. Eq. 95-98.

Ghislain, M., Zhang, D., Fajardo, D., Huamann, Z., and Hijmans, R.H. (1999) Marker-assisted sampling of the cultivated Andean potato Solanum phureja collections using RAPD markers. Genet Resour. Crop Evol., 46, 547–555.

Glais, L., Kerlan, C., and Robaglia, C. (2002) In: J.A. Khan and J. Dijkstra (eds), Plant Viruses as Molecular Pathogens, p. 225. Haworth Press, Binghampton, NY, U.S.A.

Glave, L. M. (2001) The conquest of highlands, In The Potato Treasure of the Andes from Agriculture to Culture, Graves, C., Ed., Lima: Peru: International Potato Center, 41, 42–51.

Glendinning, D.R. (1976) Neo-Tuberosum: New potato breeding material. 4. The breeding system of Neo-Tuberosum, and the structure and composition of the Neo-Tuberosum gene pool. *Potato Res,* 19:27–36.

Gopal, J., and Iwama, K. (2007) In vitro screening of potato against water-stress mediated through sorbitol and polyethylene glycol. *Plant Cell Rep*, 26:693–700.

Gopal, J. (1998) Identification of superior parents and crosses in potato breeding programmes, Theor. Appl. Genet., 96, 287–293.

Gopal, J., and Oyama, K. (2005) Genetic base of Indian potato selections as revealed by pedigree analysis. Euphytica 142:23–31.

Gorji, M.,A., Wolf, I., Decsi, K., Taller, J., Ahmadvand, R., and Polgár, Z. (2011) Development of hetero multiplex potato genotypes carrying PVY resistance genes, IX, Magyar Genetikai Kongresszus es XVI, Sejt-es Fejlődésbiológiai Napok, pp 176-177.

Gort, G., van Hintum, T., van Eeuwijk, F. (2009) Homoplasy corrected estimation of genetic similarity from AFLP bands, and the effect of the number of bands on the precision of estimation. *Theor Appl Genet* 119(3):397-416.

Grivet, L., D'Hont, A., Roques, D., Feldmann, P., Lanaud, C., and Glaszmann, J. (1995) RFLP mapping in cultivated sugarcane (*Saccharum* spp.): Genome organisation in a highly polyploidy and aneuploid interspecific hybrid. *Genetics* 142:987–1000.

Gupta, P.K., Chyi, Y.S., Romero-Severson, J., and Owen, J.L. (1994) Amplification of DNA markers from evolutionary diverse genomes using single primers of simple-sequence repeats. *Theor Appl Genet* 89:998-1006.

Gupta, P.K., and Rustgi, S. (2004) Molecular markers from the transcribe/expressed region of the genome in higher plants. *Funct Integr genomics* 4:139-62.

Gupta, P.K., Varshney, R.K., Sharma, P.C., and Ramesh, B. (1999) Molecular markers and their application in wheat breeding. *Plant breed* 118:369-90.

Hackett, C.A., Bradshaw, J.E., Meyer, R.C., McNicol, J.W., Milbourne, D., and Waugh, R. (1998) Linkage analysis in tetraploid species: a simulation study. *Genet Res* 71(2):143–154.

Hackett, C.A., Bradshaw, J.E., and McNicol, J.W. (2001) Interval mapping of QTLs in autotetraploid species. *Genetics* 159:1819–1832.

Hackett, C.A., and Luo, Z.W. (2003) TetraploidMap: construction of a linkage map in autotetraploid species. *J Hered* 94:358–359.

Hackett, C.A., Pande, B., and Bryan, G.J. (2003) Constructing linkage maps in autotetraploid species using simulated annealing. *Theor Appl Genet* 106:1107–1115.

Haley, C.S., and Knott, S.A. (1992) A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity* 69, 315–324.

Hallden, C., Hansen, M., Nilsson, N.O., Hjerdin, A., and Sall, T. (1996) Competition as a source of errors in RAPD analysis. *Theor Appl Genet* 93:1185-1192.

Hamalainen, J.H., Watanabe, K.N.,, Valkonen, J.P.T., Arihara, A., Plaisted, R.L., Pehu, E., Miller, L., and Slack, S.A. (1997) Mapping and marker-assisted selection for a gene for extreme resistance to potato virus Y. *Theor. Appl. Genet*. 94, 192-197.

Hamalainen, J.H., Kekarainen, T., Gebhardt, C., Watanabe, K.N., and Valkonen, J.P.T. (2000) Recessive *and* dominant genes interfere with the vascular transport of Potato virus A in diploid potatoes. *Mol. Plant-Microbe Interact*. 13, 402-412.

Hamalainen, J.H., Sorri, V.A., Watanabe, K.N., Gebhardt, C., and Valkonen, J.P.T. (1998) Molecular examination of a chromosome region that controls resistance to potato Y and A potyviruses in potato. *Theor. Appl. Genet*. 96:1036–1043.

Hawkes, J.G. (1990) The potato: Evolution, biodiversity, and genetic resources. Belhaven Press, London. 259 pp.

Hawkes, J.G. (1994) Origins of cultivated potatoes and species relationships. In: Bradshaw JE, Mackay GR (eds.) Potato genetics. CAB International, Wallingford, UK, pp. 3–42.

Hawkes, J.G., and Francisco-Ortega, J. (1992) The potato in Spain during the late 16th century. *Econ Bot* 46: 86–97.

Hawkes, J.G., and Francisco-Ortega, J. (1993) The early history of the potato in Europe. *Euphytica* 70: 1–7.

Hayashi, K. (1992) PCR-SSCP—rapid and easy detection of DNAsequence changes. *Hum Cell* 5:180–184.

Hayashi, K. (1993) How sensitive is PCR-SSCP? *Hum Mutat* 2:338–346.

Hermanova, V., Barta, J., and Curn, V. (2007) Wild Potato Species : Charachterization an Biological Potential for Potato Breeding.

Hermsen, J.G.Th. (1994) Introgression of genes from wild species, including molecular and cellular approaches. In: Bradshaw JE, Mackay GR (eds.) Potato genetics. CAB International, Wallingford, UK, pp. 515–538.

Hide, G.A. and Lapwood, D.H. (1992) Disease Aspects of Potato Production. In: The Potato Crop (P. Harris, ed.), p. 403–437. Chapman & Hall, London.

Hijmans, J.R., Jacobs, M., Bamberg, J.B., and Spooner, D.M. (2003) Frost tolerance in

wild potato species: Assessing the predictivity of taxonomic, geographic and ecologic factors. *Euphytica*, 130: 47–59.

Hijmans, R.J. (2001) Global distribution of the potato crop. *Am J Potato Res* 78: 403–412.

Hijmans, R.J., Gavrilenko, T., and Stephenson, S. et al. (2007) Geographical and environmental range expansion through polyploidy in wild potatoes (*Solanum* section *Petota*). Global Ecol Biogeogr 16: 485–495.

Hoopes, R.W., and Plaisted, R.L. (1987) Potato, In Principles of Cultivar Development, Fehr, W., Ed., New York: Macmillan, pp. 385–436.

Horvath, J., and Wolf, I. (1991) Reaction of *Solanum stoloniferum* accessions to potato *virus Y* and henbane mosaic virus. Indian J. Virol. 7, 176-178.

Hosaka, K., and Hanneman, R.E.Jr. (1998) Genetics of self-compatibility in a self-incompatible wild diploid potato species *Solanum chacoense*. 2. Localization of an S locus inhibitor (*Sli*) gene on the potato genome using DNA markers. *Euphytica* 103: 265–271.

Hosaka, K., Hosaka, Y., Mori, M., Maida, T., and Matsunaga, H. (2001) Detection of a simplex RAPD marker linked to resistance to potato virus Y in a tetraploid potato. *Am J Potato Res* 78: 191–196.

Hougas, R.W., Peloquin, S.J., and Ross, R.W. (1958) Haploids of the common potato. *J Hered* 49: 103–107.

Howden, R., and Cobbett, C.S. (1992) Cadmium-sensitive mutants of *Arabidopsis thaliana*. *Plant Physiol* 99:100–107.

Hsissou, D., and Bouharmont, J. (1994) In vitro selection and characterization of drought-tolerant plants of durum wheat (*Triticum durum Desf*) Agronomy, 2: 65-70.

Hudson, T.J., Stein, L.D., Gerety, S.S., Ma, J., Castle, A.B., Silva, J., Slonim, D.K., Baptista, R., Kruglyak, L., and Xu, S.H. et al. (1995) An STS-based map of the human genome. *Science* 270:1945–1954.

Huson, D.H., and Brayant, D. (2006) Application of phylogenetic networks in evolutionary studies, *Molecular Biology and Evolution*, 23 (2):254-267.

Hyne, V., and Kearsey, M.J. (1995) QTL analysis – further uses of marker regression. *Theoretical and Applied Genetics* 91, 471–476.

Ikegami, H., Nogata, H., Hirashima, K., Awamura, M., and Nakahara, T. (2009) Analysis of genetic diversity among European and Asian fig varieties (Ficus carica L.) using ISSR, RAPD, and SSR markers, *Genet Resour Crop Evol* 56:201–209.

Ingram, K.T., Bueno, F.D., Namuco, O.S., Yambao, E.B., and Beyrouty, C.A. (1994) Rice root traits for drought resistance and their genetic variation. In: KirkGJD (ed) Rice roots: nutrient and water use. IRRI, Manila, Philippines, pp 67–77.

Isidore, E. et al. (2003) Toward a marker-dense meiotic map of the potato genome: Lessons from linkage group I, *Genetics*, 165, 2107–2116.

Iwama, K., and Yamaguchi, J. (2006) Abiotic stresses. In: Gopal J, Khurana SM Paul (eds) Handbook of potato production, improvement and postharvest management. Food Product Press, New York, pp 231– 278.

Jacobs, J., Van Eck, H., Arens, P., Verkerk Bakker, B., Te Lintel Hekkert, B., Bastiaanssen, H., El-Kharbotly, A., Pereira, A., Jacobsen, E., and Stiekema, W. (1995) A genetic map of potato (*Solanum tuberosum*) integrating molecular markers, including transposons and classical markers. *Theor Appl Genet* 91:289–300.

Jansen, R.C. (1996) A general Monte Carlo method for mapping multiple quantitative trait loci. *Genetics* 142, 305–311.

Jansen, R.C., and Stam, P. (1994) High resolution of quantitative traits into multiple loci via interval mapping. *Genetics* 136, 1447–1455.

Jansky, S. (2006) Overcoming hybridization barriers in potato. *Plant Breed* 125: 1–12.

Jansky, S. (2009) Breeding, genetics and cultivar development. In: Singh J, Kaur L (eds.) Advances in potato chemistry and technology. Academic Press, Burlington, VT, pp. 27–62.

Jansky, S.H., and Rouse, D.I. (2003) Multiple disease resistance in interspecific hybrids of potato. *Plant Dis*. 87, 266-272.

Jeffries, C.J. (1998) Potato. FAO/IPGRI Technical Guidelines for the Safe Movement of Germplasm no. 19. Plant Production and Protection Division of the Food and Agriculture Organization of the United Nations (FAO) and the International Plant Genetic Resources

Institute (IPGRI), Rome, Italy.

Johnston, S.A., and Hanneman, R.E. (1980) Support of the endosperm balance number hypothesis utilizing some tuber-bearing *Solanum* sp. *American Potato Journal*, 57: 7–14.

Johnston, S.A., der Nijs, T.P.M., Peloquin, S.J. et al. (1980) The significance of genic balance to endosperm development in interspecific crosses. *Theor Appl Genet* 57: 5–9.

Jondle, R.J. (1992) Legal aspects of varietal protection using molecular markers, pp. 50–52. In Applications of RAPD technology to plant breeding. Joint Plant Breeding symposia series.

Jones, R.A.C. (1990) Strain group specific and virus specific hypersensitive reactions to infection with potyviruses in potato cultivars. Ann. Appl. Biol. 117, 93-105.

Jones, J.D.G., and Dangl, J.L. (2006) The plant immune system. Nature 444, 323-329.

Kalinowski, S. T (2005) Do polymorphic loci require large sample sizes to estimate genetic distances? Heredity 94, 33–36.

Kantety, R.V., Zeng, X.P., Bennetzen, J.L., and Zehr,B.E. (1995) Assessment of genetic diversity in dent and popcorn (*Zea mays* L.) inbred lines using inter-simple sequence repeat (ISSR) amplification. *Mol Breed* 1: 365–373.

Kao, C.H., and Zeng, Z.B. (1997) General formulas for obtaining the MLEs and the asymptotic variance– covariance matrix in mapping quantitative trait loci when using the EM algorithm. *Biometrics* 53, 653–665.

Kao, C.H., Zeng, Z.B., and Teasdale, R.D. (1999) Multiple interval mapping for quantitative trait loci. *Genetics*152, 1203–1216.

Karim, M.S., Percival, G.C., and Dixon, G.R. (1997) Comparative composition of aerial and subterranean potato tubers (*Solanum tuberosum* L). *Journal of the Science of Food and Agriculture*, *75*, 251–257.

Kearsey, M.J., and Hyne, V. (1994) QTL analysis: a simple 'marker regression' approach. *Theoretical and Applied Genetics* 89, 698–702.

Keightley, P.D., and Bulfield, G. (1993) Detection of quantitative trait loci from frequency changes of marker alleles under selection. *Genetical Research* 62, 195–203.

Knapp, S.J. (1991) Using molecular markers to map multiple quantitative trait loci:

models for backcross, recombinant inbred and doubled haploid progeny. *Theoretical and Applied Genetics* 81, 333–338.

Knight, T.A. (1807) On raising of new and early varieties of the potato (*Solanum tuberosum*). *Trans Hort Soc* Lond 1: 57–59.

Knight, J. (2003) A dying breed, Nature, 421, 568–570.

Knoll, J., and Ejeta, G. (2008) Marker-assisted selection for early-season cold tolerance in sorghum: QTL validation across populations and environments. *Theoretical and Applied Genetics* 116, 541–553.

Kojima, T., Nagaoka, T., Noda, K., and Ogihara, Y. (1998) Genetic linkage map of ISSR and RAPD markers in einkorn wheat in relation to that of RFLP markers. *Theor Appl Genet* 96: 37–45.

Kozub, J.G. et al. (2000) A relational database system for potato breeding programs, *Am. J. Potato Res*, 77, 95–110.

Kumar, J., Nelson, R.J., and Zeigler, R.S. (1999) Population structure and dynamics of M. grisea in the Indian Himalayas. *Genetics* 152, 971–984.

Lahlou, O., and Ledent, J.F. (2005) Root mass and depth, stolons and roots formed on stolons in four cultivars of potato under water stress, Eur. *J. Agron*. 22: 159-173.

Lander, E.S., and Botstein, D. (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 121,185–199.

Le Romancer, M., Kerlan, C., and Nedellec, M. (1994) Biological characterization *of* various geographical isolates of Potato virus Y inducing superficial necrose on potato tubers. *Plant Pathol*. 43, 138-144.

Leport, L., Petrivalsky, M., and Chappart, M. (1993) Effectors for the osmoinduced proline response in higher plants. *Plant Physiol Biochem* 31: 911-922.

Li, L., Strahwald, J., Hofferbert, H.R., Lu¨beck, J., Tacke, E., Junghans, H., Wunder, J., and Gebhardt, C. (2005) DNA variation at the invertase locus invGE/GF is associated with tuber quality traits in populations of potato breeding clones. *Genetics* 170:813–882.

Lilley, J.M., Ludlow, M.M., McCouch, S.R., and O'Toole, J.C. (1996) Locating QTL for osmotic adjustment and dehydration tolerance in rice, Journal of Experimental Botany,

302: 1427-1436.

Lippman, Z.B., and Zamir, D. (2007) Heterosis: revisiting the magic. *Trends in Genetics* 23, 60–66.

Liu, B.H. (1998) Statistical Genomics: Linkage, Mapping and QTL Analysis. CRC Press, Boca Baton, Florida, 611 pp.

Lörz, H., and Wenzel, G. (eds) (2009) Molecular Marker Systems in Plant Breeding and Crop Improvement. Biotechnology in Agriculture and Forestry, Vol. 55. Springer-Verlag, Berlin.

Luo, Z.W., Hackett, C.A., Bradshaw, J.E., McNicol, J.W., and Milbourne, D. (2000) Predicting parental genotypes and gene segregation for tetrasomic inheritance. *Theor Appl Genet* 100(7):1067–1073.

Luo, Z.W., Hackett, C.A., Bradshaw, J.E., McNicol, J.W., and Milbourne, D.M. (2001) Construction of a genetic linkage map in tetraploid species using molecular markers. *Genetics* 157:1369–1385.

Lynch, M., and Walsh, B. (1998) Genetics and Analysis of Quantitative Traits. Sinauer Associates, Sunderland, Massachusetts, 980 pp.

Maas, E.V. (1985) Crop tolerance to saline sprinkling water. *Plant Soil* 89: 273–284.

Mackay, G.R. (2005) Propagation by traditional breeding methods. In: Razdan MK, Mattoo AK (eds.) Genetic improvement of Solanaceous crops, volume I: potato. Science Publishers Inc, Enfield, NH, pp. 65–81.

Makino, R., Yazyu, H., Kishimoto, Y., Sekiya, T., and Hayashi, K. (1992) F-SSCP (fluorescence-based polymerase chain reaction-singlestrand conformation polymorphism (PCR-SSCP) analysis. PCR Methods Appl 2:10–13.

Maliepaard, C., Jansen, J., and Van Ooijen, J. (1997) Linkage analysis in a full sib family of an outbreeding plant species: overview and consequences for applications. *Genet Res* 70:237–250.

Manjit, S., Kang, and Priyadarshan, P.M. (2007) Breeding major food staples.

Mark, P.S., Li-Bing, Z., Colleen, T.W., and Kai, M. (2007) A penalty of using anonymous dominant markers (AFLPs, ISSRs, and RAPDs) for phylogenetic inference.

Molecular phylogenetics and Evolution 42:528-542.

Martin, G.B., Williams, J.G.K., and Tanksley, S.D. (1991) Rapid identification of markers linked to a Pseudomonas resistance gene in tomato by using random primers and near-isogenic lines. Proc Natl Acad Sci 88:2336–2340.

Martinez, O., and Curnow, R.N. (1992) Estimating the locations and the sizes of the effects of quantitative trait loci using flanking markers. *Theoretical and Applied Genetics* 85, 480–488.

Maruyama, H., Koyama, R., Oi, T., Yagi, M., Takeda, M., Kanechi, M., Inagaki, N., and Uno, Y. (2008) In vitro evaluation of osmotic stress tolerance using a novel root recovery assay. *Plant Cell Tiss Organ Cult*, 95:101–106.

McGregor, C.E., Lambert, C.A., Greyling, M.M., Louw, J.H,. and Warnich, L. (2000) A comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, AFLP and SSR) in tetraploid potato (*Solanum tuberosum* L.) germplasm, *Euphytica* 113: 135–144.

Mendoza, H.A., Mihovilovich, E.J., and Saguma, F. (1996) Identification of triplex (YYYy) potato virus Y(PVY) immune progenitors derived from *Solanum tuberosum* ssp. *andigena*, *Am. Potato Res*. 73: 13-19.

Mendoza, H.A., and Jayasinghe, U. (1993) Progress in breeding for combined resistance to potato viruses Y, X and leafroll. 12th Triennial Conference of EAPR, Paris, July 18-23, 1993.p.457-458.

Mendoza, H.A., Mihovilovich, E. J., and Saguma, F. (1996) Identification of triplex (YYYy) Potato Virus Y (PVY) immune progenitors derived from Solanum tuberosum ssp. Andigena. Am. J. Pot. Res. 73. No. 1. pp13-19.

Menendez, C.M., Ritter, E., Scha¨fer-Pregl, R., Walkemeier, B., Kalde, A., Salamini, F., and Gebhardt, C. (2002) Cold sweetening in diploid potato: mapping quantitative trait loci and candidate genes. *Genetics* 162:1423–1434.

Meyer, R., Milbourne, D., Hackett, C., Bradshaw, J., McNichol, J., and Waugh, R. (1998) Linkage analysis in tetraploid potato and association of markers with quantitative resistance to late blight (*Phytophthora infestans*). *Mol Gen Genet* 259:150–160.

Meyer, W., Michell, T.G., Freedman, E.Z., and Vilgalys, R. (1993) Hybridization probes for conventional DNA fingerprinting used as single primers in polymerase chain reaction

to distinguish strain of *Cryptococcus neoformans*. *J Clin Biol* 31: 2274-2280.

Meyers, B.C., Kozik, A., Griego, A., Kuang, H., and Michelmore, R.W. (2003) Genome-wide analysis of NBS-LRR-encoding genes in *Arabidopsis*. *Plant Cell* 15, 809-834.

Milbourne, D., Meyer, R., Collins, A., Ramsay, L., Gebhardt, C., and Waugh, R, (1998) Isolation, characterization and mapping of simple sequence repeat loci in potato. *Mol. Gen. Genet*. 259: 233-245.

Milla, M.A.R., Butler, E., Huete, A.R., Wilson, C.F., Anderson, O., and Gustafson, J.P. (2002) Expressed sequence tag-based gene expression analysis under aluminum stress in rye. *Plant Physiol* 130:1706– 1716.

Mohamed, M.A.H., Harris, P.J.C., and Henderson, J. (2000) *In vitro* selection and characterization of a drought clone of *Tagetes minuta*. *Plant Sci*. 159: 213-222.

Mohler, V., and Schwartz, G. (2005) Genotyping tools in plant breeding: from restriction fragment length polymorphisms to single nucleotide polymorphisms. In: Lorz, H. and Wenzel, G. (eds) Molecular Marker Systems in Plant Breeding and Crop Improvement. Biotechnology in Agriculture and Forestry, Vol. 55. Springer-Verlag, Berlin, pp. 23–38.

Mohler, V., and Singrun, C. (2004) General considerations: marker-assisted selection. In: Lorz, H. and Wenzl, G. (eds) Biotechnology in Agricultural and Forestry, Vol. 55. Molecular Marker Systems in Plant Breeding and Crop Improvement. Springer-Verlag, Berlin, pp. 305–317.

Moreau, L., Charcosset, A., Hospital, F., and Gallais, A. (1998) Marker-assisted selection efficiency in populations of finite size. *Genetics* 148, 1353–1365.

Mullis, K.B., and Faloona, F. (1987) Specific synthesis of DNA in vitro via polymerase chain reaction. *Methods Enzymol* 155:350–355.

Munoz, F.J., Plaisted, R.L., and Thurston, H.D. (1975) Resistance to potato virus Y in Solanum tuberosum ssp. andigena. *Am Potato J* 52: 107–115.

Murphy, A.M., De Jong, H., and Proudfoot, K.G. (1999) A multiple disease resistant potato clone developed with classical breeding methodology. *Can. J. Plant Pathol*. 21(3):207–212.

Nagaoka, T., and Ogihara, Y. (1997) Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD

markers. *Theor Appl Genet* 94: 597-602.

Nicholas, F.W. (2006) Discovery, validation and delivery of DNA markers. *Australian Journal of Experimental Agriculture* 46, 155–158.

Norero, N., Malleville J., Huarte M., Feingold S (2003) Cost efficient potato *(Solanum tuberosum* L.) cultivar identification by microsatellite amplification, *Potato Research* 45: 131-138.

Nürnberger, T., Brunner, F., Kemmerling, B., and Piater, L. (2004) Innate immunity in plants and animals: striking similarities and obvious differences. *Immunol Rev* 198, 249-266.

Oomen, R., Bergervoet, M., Bachem, C.W.B., Visser, R.G.F., and Vincken, J.P. (2003) Exploring the use of cDNA-AFLP with leaf protoplasts as a tool to study primary cell wall biosynthesis in potato. *Plant Physiol. Biochem*.41, 965-971.

Orita, M., Iwahana, H., Kanazawa, H., Hayashi, K., and Sekiya, T. (1989 b) Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphism. Proc Natl Acad Sci USA 86:2766–2770.

Orita, M., Suzuki, Y., Sekiya, T., and Hayashi, K. (1989 a) Rapid and sensitive detection of point mutations and DNA polymorphism using the polymerase chain reaction. *Genomics* 5:874–879.

Ortiz, R. (1998) Potato breeding via ploidy manipulations. In: Janick J (ed.) Plant Breed Revs Volume 16. Wiley, New York, NY, pp. 15–86.

Ortiz, R. (2001) The state of the use of potato genetic diversity. In: Cooper HD, Spillane C, Hodgkin T (eds.) Broadening the genetic base of crop production. CAB International, Wallingford, England, pp. 181–200.

Pandey, S.K., and Kaushik, S.K. (2003) Origin, evolution, history and spread of potato. In: Khurana SMP, Minhas JS, Pandey SK (eds.) The potato – production and utilization in sub-tropics. Mehta Publishers, New Delhi, pp. 15–24.

Pavek, J.J., and Corsini, D.L. (2001) Utilization of potato genetic resources in variety development. *Amer J Potato Res* 78:433-441.

Peleman, J.D., and van der Voort, J.R. (2003) Breeding by design. *Trends in Plant*

Science 8, 330–334.

Peloquin, S.J., and Hougas, R.W. (1959) Decapitation and genetic markers as related to haploidy in *Solanum tuberosum*. Eur Potato J 2: 176–183.

Peloquin, S. J. (2000) New approaches to breeding for the potato in the year 2000, In Research for the Potato in the Year 2000, Hooker, W. J., Ed., Lima, Peru: International Potato Center, pp. 32–34, 1983.

Pérez, W., Salas, A., Raymundo, R., Huamán, Z., Nelson, R., and Bonierbale, M. (2000) Evaluation of wild potato species for resistance to late blight. CIP Program Report 1999–2000. Research on Potato, Lima, 49–62.

Peuke, A.D., Scharml, C., Hartung, W., and Rennenberg, H. (2002) Identification of drought-sensitive beech ecotypes by physiological parameters. *New phytologist*, 154: 373-387.

Phillips, R.L. (2006) Genetic tools from nature and the nature of genetic tools. *Crop Science* 46, 2245–2252.

Picoult-Newberg, L., Ideker, T.E., Pohl, M.G., Taylor, S.L., Donaldson, M.A., Nickerson, D.A., and Boyce-Jacino, M. (1999) Mining SNPs from EST databases. *Genome Res* 9:167–174.

Plaisted, R.L., and Hoopes, R.W. (1989) The past record and future prospects for the use of exotic potato germplasm. *American Potato Journal*, 66, 603-627.

Plaisted, R.L. (1980) Potato. In: Fehr WR, Hadley HH (eds.) Hybridization of crop plants. The American Society of Agronomy, Inc, Madison, WI, pp. 483–494.

Poehlman, J.M., and Sleper, D.A. (1995) Breeding Field Crops, Fourth Edition, Iowa State University Press, Ames.

Polgár, Z., Wolf, I., and Horváth, S. (2002) Development of parental lines having resistance genes against PVY NTN in hetero multiplex state. 15th Triennial Conference of the European Association for Potato Research, July 14-19, Hamburg, Germany, Abstracts of Papers, p. 98.

Powell, W., Phillips, M.S., McNicol, J.W., and Waugh, R. (1991) The use of DNA markers to estimate the extent and nature of genetic variability in *Solanum tuberosum*

cultivars. *Ann. Appl. Biol*. 118:423–432.

Prevost, A., and Wilkinson, M. J. (1999) A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars , *Theor Appl Genet* 98: 107-112 .

Price, A.H., Steele, K.A., Moore, B.J., Barraclough, P.B., and Clark, L.J. (2000) A combined RFLP and AFLP linkage map of upland rice (Oryza sativa L.) used to identify QTLs for root-penetration ability. *Theor Appl Genet*. 100: 49–56.

Provan, J., Powell, W., and Waugh, R. (1996) Microsatellite analysis of relationships within cultivated potato (*Solanum tuberosum*). *Theor*. *Appl. Genet*. 92, 1078-1084.

Quarrie, S.A. (1996) New molecular tools to improve the efficiency of breeding for increased drought resistance. *Plant Growth Regul*. 20: 167–178.

Rajamaki, M.L., Maki-Valkama, T., Makinen, K., and Valkonen, J.P.T. (2004) In: N.J. Talbot (ed.), *Plant-Pathogen Interactions*, p. 68. Blackwell Publishing, Sheffield, U.K.

Ribaut, J.M., Jiang, C., Gonzáles de León, D., Edmeades, G.O., and Hoisington, D.A. (1997) Identification of quantitative trait loci under drought conditions in tropical maize. II. Yield components and marker-assisted selection strategies. *Theor Appl Genet*. 94: 887–896.

Ritter, E., Gebhardt, C., and Salamini, F. (1990) Estimation of recombination frequencies and construction of RFLP linkage maps in plants from crosses between heterozygous parents. *Genetics* 125:645–654.

Ritter, E., and Salamini, F. (1996) The calculation of recombination frequencies in crosses of allogamous plant species with applications to linkage mapping. *Genet Res* 67:55–67.

Rockman, M.V., and Wray, G.A. (2002) Abundant raw material for *cis*-regulatory evolution in humans. *Molecular Biology and Evolution* 19, 1991–2004.

Ronning, C.M., Stegalkina, S.S., Ascenzi, R.A., Bougri, O., Hart, A.L., Utterbach, T.R., Vanaken, S.E., Riedmuller, S.B., White, J.A., Cho, J., Pertea, G.M., Lee, Y., Karamycheva, S., Sultana, R., Tsai, J., Quackenbush, J., Griffiths, H.M., Restrepo S., Smart, C.D., Fry, W.E., van der Hoeven, R., Tanksley, S.D., Zhang, P., Jin, H., Yamamoto, M.L., Baker, B.J. and Buell, C.R. (2003) Comparative analyses of potato expressed sequence tag libraries. *Plant Physiol*. 131, 419-429.

Ross, H. (1958) Inheritance of extreme resistance to virus Y in Solanum stoloniferum and its hybrids with Solanum tuberosum. In: Proc Third Conf Potato Virus Diseases, pp. 204–211.

Ross, H. (1986) Potato breeding-Problems and perspectives. Advances in plant breeding. Supplement 13 to Journal of Plant Breeding. Verlag Paul Parey. Berlin and Hamburg, 132 pp.

Rossouw, F.T., and Waghmarae, J. (1995) The effect of drought on growth and yield of two South African potato cultivars. *S Afr J* Sci 91:149–150.

Rouppe van der Voort, J., Van Eck, H.J., Draaistra, J., Van Zandvoort, P.M., Jacobsen, E., and Bakker, J. (1998) An online catalogue of AFLP markers covering the potato genome. *Mol Breed* 4(1):73–77.

Rouppe van der Voort, J., van Zandvoort, P., van Eck, H.J., Folkertsma, R.T., Hutten, R. C. B., Draaistra, J., Gommers, F.J., Jacobsen, E., Helder, J., and Bakker, J. (1997) Use of allele specificity of comigrating AFLP markers to align genetic maps from different potato genotypes. *Mol Gen Genet* 255:438–447.

Rousselle-Bourgeois, F., Rousselle, P., Ame´lioration ge´ne´tique, In La pomme de terre, Rousselle, P., Robert, Y., and Crosnier, J. C. (eds) (1996) Paris: Institut National de la Recherche Agronomique (INRA), chap. 4.

Ruiz de Galarreta, J.I., Carrasco, A., Salazar, A., Barrena, I., Iturritxa, E., Marquinez, R., Legorburn, F.J., and Ritter, E. (1998) Wild Solanum species as resistance sources against different pathogens of potato. *Potato Res* 41, 57-68.

Sakthivelu, G., Akitha Devi, M.K., Giridhar, P., Rajasekaran, T., Ravishanker, G.A., Nedev, T., and Kosturkova, G. (2008) Drought-induced alterations in growth, osmotic potential and *in vitro* regeneration of soybean cultivars. *Gen. Appl*. Plant Physiol., Special issue, 34(1-2): 103-112.

Salhi-Hannachi, A., Chatti, K., Mars, M., Marrakchi, M., and Trifi, M. (2005) Comparative analysis of genetic diversity in two Tunisian collections of fig cultivars based on random amplified polymorphic DNA and inter simple sequence repeats fingerprints. Genet Resour Crop Evol 52:563–573.

Sanderson, M.J., and Wojciechowski. (2005) Improved bootstrap confidence limits in large-scale phylogenies, with an example from Neo-Astragalus (*Leguminosae*). Syst. Bio

l49: 671– 685.

Sari-Gorla, M., Krajewski, P., Di Fonzo, N., Villa, M., and Frova, C. (1999) Genetic analysis of drought tolerance in maize by molecular markers. II. Plant height and flowering. *Theor Appl Genet*. 99: 289–295.

Satagopan, J.M., Yandell, B.S., Newton, M.A., and Osborn, T.G. (1996) A Bayesian approach to detect quantitative trait loci using Markov chain Monte Carlo. *Genetics* 144, 805–816.

Sato, M., Nishikawa, K., Komura, K., and Hosaka, K. (2006) *Potato virus Y* resistance gene, Ry_{chc}, mapped to the distal end of potato chromosome 9. *Euphytica* . 149: 367–372.

Sax, K. (1923) The association of size differences with seed coat pattern and pigmentation in *Phaseolus vulgaris*. *Genetics* 8, 552–560.

Scha¨fer-Pregl, R., Ritter, E., Concilio, L., Hesselbach, J., Lovatti, L., Walkemeier, B., Thelen, H., Salamini, F., and Gebhardt, C. (1998) Analysis of quantitative trait loci (QTLs) and quantitative trait alleles (QTAs) for potato tuber yield and starch content. *Theor Appl Genet* 97:834–846.

Schafleitner, R., Rosales, R.O.G., Gaudin, A., Aliaga, C.A.A., Martinez, G.N., Marca, L.R.T., Bolivar, L.A., Delgado, F.M., Simon, R., and Bonierbale, M. (2007) Capturing candidate drought tolerance traits in two native Andean potato clones by transcription profiling of field grown plants under water stress. *Plant Physiology and Biochemistry* 45: 673-690.

Schittenhelm, S., and Hoekstra, R. (1995) Recommended isolation distances for the field multiplication of diploid tuber-bearing *Solanum* species. *Plant Breed* 114: 369–371.

Schluter, P.M., and Harris, S.A. (2006) Analysis of multilucous fingerprinting data sets containing missing data. *Mol. Notes*: 6:569-572.

Semagen, K., Bjornstad, A., and Ndjiondjop, M.N. (2006) An overview of molecular marker methods for plants. *Afr J Biotechnol* 5:2540-68.

Sen, S., and Churchill, G.A. (2001) A statistical framework for quantitative trait mapping. *Genetics* 159, 371–387.

Shukla, D.D., Ward, C.W., and Brunt, A.A. (1994) Genome structure, variation and

function. In The Potyviridae, pp. 74±110. Wallingford, UK: CAB International.

Simmonds, N.W. (1995) Potatoes. In: Smartt J, Simmonds NW (eds.) Evolution of crop plants, 2nd edn. Longman Scientific & Technical, Harlow, pp. 466–471.

Simmons, M.P., Zhang, L.B., Webb, C.T., and Müller, K. (2007) A penalty of using anonymous dominant markers (AFLPs, ISSRs and RAPDs) for phylogenetic inference. *Mol Phylogenet Evol* 42:528-542.

Singh, M., Singh, R.P., and Somerville, T.H. (1994) Evaluation of tuber-bearing Solanum species for symptomology, as diagnostic hosts, and sources of immunity to potato virus Y necrotic strain (PVYN).American Potato Journal 71(9):567-579.

Singh, R.P., Nie, X., and Tai, G.C.C. (2000) A novel hypersensitive resistance response against potato virus A in cultivar 'Shepody'. *Theor. Appl. Genet*. 100: 401–408.

Small, I., Peeters, N., Legeai, F., and Lurin, C. (2004) Predotar: A tool for rapidly screening proteomes for N-terminal targeting sequences. *Proteomics* 4, 1581–1590.

Smith, J.S.C., and Williams, J.G.K. (1994) Arbitrary primer mediated fingerprinting in plants: case studies in plant breeding, taxonomy and phylogeny. In: Schierwater, B., Streit B., Wagner G.P., DeSalle R. (Eds.) Molecular Ecology and Evolution: Approaches and Applications. Birkhauser Verlag, Basel pp. 5-15.

Smith, S. (1998) Cultivar identification and varietal protection. In: G. Caetano-Annolés & P.M. Gresshoff (Eds.), DNA Markers: Protocols, Applications and Overviews, pp. 383–400. Wiley-VCH, New York.

Soller, M., and Beckmann, J.S. (1990) Marker-based mapping of quantitative trait loci using replicated progenies. *Theoretical and Applied Genetics* 80, 205–208.

Solomon-Blackburn, R.M., and Barker, H. (1993) Resistance to potato leafroll luteovirus ean be greatly improved by combining two independent types of heritable resistance. *Ann. Appl. Biol*. 122, 329-336.

Solomon-Blackburn, R.M., and Barker, H. (2001) Breeding virus resistant potatoes (Solanum tuberosum): a review of traditional and molecular approaches, Heredity, 86, 17–35.

Song, Y.S., Heptin, L., Schweizer, G., Hartl, L., Wenzel, G., and Schwarzfischer, A. (2005) mapping of extreme resistance to PVY (Ry_{sto}) on chromosome XII using abther-

culture derived primary dihaploid potato lines. *Theoretical and applied genetics*, 111, 879-887.

Sonia, C., and Gopalakrishna, T. (2007) Comparative assessment of REMAP and ISSR marker assays for genetic polymorphism studies in *Magnaporthe grisea*. *Current Science* 93:688-692.

Sorensen, D., and Gianola, D. (2002) Likelihood, Bayesian and MCMC Methods in Quantitative Genetics. Springer-Verlag Inc., New York.

Sorri, V.A., Watanabe, K.N., and Valkonen, J.P.T. (1999) Predicted kinase-3a motif of a resistance gene analogue as a unique marker for virus resistance. *Theor Appl Genet* 99:164–170.

Sosinski, B., and Douches, D.S. (1996) Using polymerase chain reaction-based DNA amplification to fingerprint North American potato cultivars, *Hort Science* 31(1): 130-133.

Souframanien, J., and Gopalakrishna, T. (2004) Acomparative analysis of genetic diversity in black gram genotypes using RAPD and ISSR markers. *Theor Appl Genet* 109:1687-1693

Spitters, C.J.T., and Ward, S.A. (1988) Evaluation of breeding strategies for resistance to potato cyst nematodes using a population dynamic model. *Euphytica* 39, 87-98.

Spooner, D.M., and Van den Berg, R.G. (1992) An analysis of recent taxonomic concepts in wild potatoes (*Solanum* sect. *Petota*). *Genetic Resources an Crop Evolution* 39: 23-37.

Spooner, D.M., Hijmans, R.J. (2001) Potato systematics and germplasm collecting 1989–2000. *American Journal of Potato Research*, 78: 237–268.

Spooner, D.M., McLean, K., and Ramsay, G. et al. (2005) A single domestication for potato based on multilocus amplified fragment length polymorphism genotyping. Proc Natl Acad Sci 102: 14694–14699.

Spooner, D.M., and Bamberg, B.J. (1994) Potato genetic resources: sources of resistance and systematics, *Am. Potato J*, 71, 325–338.

Spooner, D.M., van den Berg, R.G., Rodriguez, A., Bamberg, J., Hijmans, R.J., and Cabrer, S.I.L . (2004) Wild Potatoes (Solanum section Petota; Solanaceae) of North and

Central America. Systematic Botany Monographs Volume 68.

Stam, P. (1993) Construction of integrated genetic linkage maps by means of a new computer package: Joinmap. *Plant J* 3:739–744.

Stam, P., and Van Ooijen, J. (1995) JoinMap version 2.0: Software for the calculation of genetic linkage maps. CPRO-DLO, Wageningen, The Netherlands.

Stam, P. (1991) Some aspects of QTL analysis. Proceedings of the Eighth Meeting of the Eucarpia Section Biometrics on Plant Breeding, 1–6 July 1991, Brno, Czechoslovakia, pp. 24–32.

Strange, R.N., and Scott, P.R. (2005). Plant disease: a threat to global food security. *Annu Rev Phytopathol* 43, 83-116.

Strong, E.E., and Lipscomb, D. (1999) Character coding and inapplicable data. Cladistics 15:363:372.

Struik, P.C., and Wiersema, S.G. (1999) Development of cultivars, In Seed Potato Technology, Struik, P. C. and Wiersema, S. G., Eds., Wageningen: Wageningen Pers, chap. 3.

Stuber, C.W., Goodman, M.M., and Moll, R.H. (1982) Improvement of yield and ear number resulting from selection at allozyme loci in a maize population. *Crop Science* 22, 737–740.

Stuber, C.W., Moll, R.H., Goodman, M.M., Schaffer, H.E., and Weir, B.S. (1980) Allozyme frequency changes associated with selection for increased grain yield in maize (*Zea mays*). *Genetics* 95, 225–336.

Sukhotu, T., and Hosaka, K. (2006) Origin and evolution of Andigena potatoes revealed by chloroplast and nuclear DNA markers. *Genome* 49: 636–647.

Sun, G., Wang-Pruski, G., Mayich, M., and Jong, H. (2003) RAPD and pedigree-based genetic diversity estimates in cultivated diploid hybrids. *Theor. Appl. Genet*. 107:110–115.

Szajko, K., Chrzanowska, M., Witek, K., Strzelczyk-ôyta, D., Zagórska, H., Gebhardt, C., Hennig, J., and Marczewski, W. (2008) The novel gene Ny-1 on potato chromosome IX confers hypersensitive resistance to Potato virus Y and is an alternative to Ry genes in

potato breeding for PVY resistance. *Teor. Appl. Gent*. 116: 297-303.

Takacs, A.P., Horvath, J., Kazinczi, G., and Pribek, D. (1999) 51st International Symposium on Crop Protection. Part II, May 4. 1999, *Gent Belgium*.

Tanksley, S.D. (1993) Mapping polygenes. Annu. Rev. Genet. 27: 205–233.

Tarn, T.R., Tai, G.C.C., De Jong, H., Murphy, A.M., and Seabrook, J.E.A. (1992) Breeding potatoes for long-day, temperate climates, In: J. Janick (eds.), *Plant Breeding Reviews*, Volume 9, 217–332. John Wiley & Sons, New York.

Thoday, J.M. (1961) Location of polygenes. *Nature* 191, 368–370.

Trindade, L.M., Horvath, B., Bachem, C., Jacobsen E., and Visser, R.G.F. (2003) Isolation and functional characterization of a stolon specific promoter from potato (*Solanum tuberosum* L.). *Gene* 303, 77-87.

Trindade, L.M., Horvath, B.M., van Berloo, R., and Visser, R.G.F. (2004) Analysis of genes differentially expressed during potato tuber life cycle and isolation of their promoter regions. *Plant Science* 166: 423-433.

Tsumura, Y., Ohba, K., and Strauss, S.H. (1996) Diversity and inheritance of inter-simple sequence repeat polymorphisms in Douglas-fir (*Pseudotsuga menziesii*) and sugi (*Cryptomeria japonica*). *Theor Appl Genet* 92: 40-45.

Tuberosa, R., Sanguineti, M.C., Landi, P., Salvi, S., Casarini, E., and Conti, S. (1998) RFLP mapping of quantitative trait loci controlling abscisic acid concentration in leaves of drought-stressed maize (Zea mays L.). *Theor Appl Genet*. 97: 744–755.

Vada, M.E. (1994) Environmental Stress and its Impact on Potato Yield. In: Potato Genetics (J.E. Bradshaw and G.R. Mackay, eds.), p. 239–261. CAB International, Wallingford, UK.

Valkonen, J.P.T. (1997) Novel resistances to four potyviruses in tuber-bearing potato species, and temperature-sensitive expression of hypersensitive resistance to potato virus Y. *Ann. Appl. Biol.* 130, 91-104.

Valkonen, J.P.T., Contreras, A., Pehu, E., and Salazar, L.F. (1992) Naturally occurring viral infections in Solanum brevidens and S. fernandezianum. *Potato Research* 35, 411-417.

Valkonen, J.P.T., Slack, S.A., and Watanabe, K.N. (1995) Resistance to cucumber mosaic virus in potato. *Ann. Appl. Biol*. 126, 143-151.

Valkonen, J.P.T., Rokka, V.M., and Watanabe, K.N. (1998) Examination of the leaf-drop symptom of virus-infected potato using anther culture-derived haploids. *Phytopathology* 88, 1073-1077.

Valkonen, J.P.T. (2007) Potato Viruses: economical losses and biotechnological potential. In *Potato biology and biotechnology*. pp 619–641, (eds) D Vreugdenhil, J Bradshaw, C Gebhardt, F Govers, M Taylor D, MacKerron, H Ross, Amsterdam, The Netherlands: Elsevier.

Valkonen, J.P.T., Jones, R.A.C., Slack, S.A. et al. (1996) Resistance specificities to viruses in potato: standardization of nomenclature. Plant Breed 115:433–438.

Valkonen, J.P.t., Wiegmann, K., Hamalainen, J.H., Marczevski, W., and Watanabe, K.N. (2007) Evidence for utility of the same PCR-based markers for selection of extreme resistance to potato virus Y controlled by Ry_{sto} of *Solanum stoniferum* derived from different sources.*Annals of Applied Biology* ISSN 0003-4746.

Vallejo, R.L., Collins, W.W., and Young, J.B. (1995) Inheritance of resistance to p*otato virus Y* and p*otato virus X* in hybrid *Solanum phureja* × *S. stenotomum* diploid potatoes *J. Hered*. 86, 89-93.

Van den Berg, J.H., Ewing, E.E., Plaisted, R.L., McMurry, S., and Bonierbale, M.W. (1996a) QTL analysis of potato tuberization. *Theor Appl Genet* 93:307–316.

Van den Berg, J.H., Ewing, E.E., Plaisted, R.L., McMurry, S., and Bonierbale, M.W. (1996b) QTL analysis of potato tuber dormancy. *Theor Appl Genet* 93:317–324.

van der Zaag, D.E., Brinkman, H., Bus, C.B., van Delft, M., Hotsma, P.H., Meijers, C. P., Mulder, A., Turkensteen, L.J., and Wustman, R. (1996) potato diseases: diseases, pests and defects.66-82.

van Eck, H., Rouppe van der Voort, J., Draaistra, J., van Zandvoort, P., van Enckervort, E., Segers, B., Peleman, J., Jacobsen, E., Helder, J., and Bakker, J. (1995) The inheritance and chromosomal localization of AFLP markers in a non-inbred potato offspring. *Mol Breed* 1:397–410.

van Ooijen, J.W. (1992) Accuracy of mapping quantitative trait loci in autogamous

species. *Theoretical and Applied Genetics* 84, 803–811.

Vaneck, H.J., Rouppe Van Der Voort, J., Draaistra, J., Van Zand Voort, P., Van Enckevort, E., Segers, B., Peleman, J., Jacobsen, E., Helder, J., and Bakker, J. (1995) The inheritance and chromosomal localization of AFLP markers in a non-inbred potato offspring. *Mol. Breed*. 1: 397–410.

Voinnet, O. (2005) Induction and suppression of RNA silencing: insights from viral infections. *Nat Rev Genet* 6, 206-220.

Vos, P., Hogers, R., Bleeker, M., Rerjans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res* 23:4407–4414.

Vreugdenhil, D., Bradshw, J., Gebhardt, C., Govers, F., Mackerron, D.K.L., Talor, M.A., and Ross, H.A. (2007) PotatoBiology and Biotechnology Advances and Prespectives.

Walbot, V., and Werren, C. (1988) Regulation of *Mu* element copy number in maiz line with an active or inactive mutator transposonable element system. *Mol Gen Genet* 211:27-34.

Watanabe, K.N. (2002) Challenges in biotechnology for abiotic stress tolerance on roots and tubers. JIRCAS Working Report, 75–83.

Watanabe, s., Kojima, K., Ide, Y., and Sasaki, S (2000) Effects of Saline and osmotic stress on praline and sugar accumulation in *populus euphratica in vitro*. Plant cell, Tissue and Organ Culture, 63: 199-206.

Waterworth, H.E., and Hadidi, A. (1998) In: *Plant Virus Disease Control*, p. 1. APS Press, St. Paul, MN, USA.

Wei, H, Dhanaraj, A.L., Rowland, L.J., Fu, Y., Krebs, S.L., and Arora, R. (2005) Comparative analysis of expressed sequence tags from cold acclimated and non-acclimated leaves of Rhododendron catawbiense Michx. *Planta* 221:406–416.

Whittaker, J.C., Haley, C.S., and Thompson, R. (1997) Optimal weighting of information in marker-assisted selection. *Genetical Research* 69, 137–144.

Williams, J.G.K., Hanafey, M.K., Rafalski, J.A., and Tingey, S.V. (1993) Genetic analysis using random amplified polymorphic DNA markers. *Methods in Enzymology*

218:704-740.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., and Tingey, S.V. (1991) DNA polymorphisms amplified by arbitrary primers are usef*l* as genetic markers. *Nucleic Acids Res* 18:6531–6535.

Witek, K., Strzelczyk-_ Zyta, D., Hennig, J., and Marczewski, W. (2006) A multiplex PCR approach to simultaneously genotype potato towards the resistance alleles Ry-fsto and Ns. *Mol Breeding*, 18: 273-275.

Wolfe, A.D., and Liston, A. (1998) Contribution of PCR based methods to plant systematic and evolutionary biology. In: Soltis, P.S., Soltis, D.E., Doyle, J.J. (Eds), Molecular systematic of plants II. Chapman and Hall, New York, pp. 43-86.

Wollenweber, B., Porter, J.R., and Lubberstedt, T. (2005) Need for multidisciplinary research towards a second green revolution. Commentary. *Current Opinion in Plant Biology* 8, 337–341.

Wright, A.J., and Mowers, R.P. (1994) Multiple regression for molecular-marker, quantitative trait data from large F2 populations. *Theoretical and Applied Genetics* 89, 305–312.

Wu, K., Burnquist, W., Sorrells, M., Tew, T., Moore, P., and Tanksley, S. (1992) The detection and estimation of linkage in polyploids using single-dose restriction fragments. *Theor Appl Genet* 83:294–300.

Wu, S.J., Ding, L., and Zhu, J.K. (1996) SOS1, a genetic locus essential for salt tolerance and potassium acquisition. *Plant Cell*, 8:617–627.

Wu, R., Ma, C., and Casella, G. (2007) Statistical Genetics of Quantitative Traits: Linkage, Maps and QTL (Statistics for Biology and Health). Springer, Berlin.

Wu, W.R., and Li, W.M. (1994) A new approach for mapping quantitative trait loci using complete genetic marker linkage maps. *Theoretical and Applied Genetics* 89, 535–539.

Wu, W.R., and Li, W.M. (1996) Model fitting and model testing in the method of joint mapping of quantitative trait loci. *Theoretical and Applied Genetics* 92, 477–482.

Xu, Y. (1997) Quantitative trait loci: separating, pyramiding and cloning. *Plant Breeding Reviews* 15, 85–139.

Xu, Y. (2002) Global view of QTL: rice as a model. In: Kang, M.S. (eds.) Quantitative Genetics, Genomics and Plant Breeding. CAB International, Wallingford, UK, pp. 109–134.

Xu, Y. (2003) Developing marker-assisted selection strategies for breeding hybrid rice. *Plant Breeding Reviews* 23, 73–174.

Xu, Y. (2010) Molecular plant breeding, chap 2-9.

Xu, Y., and Crouch, J.H. (2008) Marker-assisted selection in plant breeding: from publications to practice. *Crop Science* 48, 391–407.

Xu, Y., and Zhu, L. (1994) Molecular Quantitative Genetics (in Chinese). China Agriculture Press, Beijing, China, 291 pp.

Xu, Y., McCouch, S.R., and Zhang, Q. (2005) How can we use genomics to improve cereals with rice as a reference genome? *Plant Molecular Biology* 59, 7–26.

Xu, Y., Shen., Chen Z., and Zhu, L. (1995) A statistical technique and generalized computer software for interval mapping of quantitative trait loci and its application. *Acta Agronomica Sinica* 21, 1–8 (in Chinese with English abstract).

Xu, Y., Skinner, D.J., Wu, H., Palacios-Rojas, N., Araus, J.L., Yan, J., Gao, S., Warburton, M.L., and Crouch, J.H. (2009) Advances in maize genomics and their value for enhancing genetic gains from breeding. *International Journal of Plant Genomics* Volume 2009, Article ID 957602, 30 pages. Available at: http://www.hindawi.com/journals/ijpg/2009/957602.html (accessed 21 December 2009).

Yadav, R., Courtois, B., Huang, N., and McLaren, G (1997) Mapping genes controlling root morphology and root distribution on a double-haploid population of rice. *Theor Appl Genet*. 94: 619–632.

Yu, K., and Pauls, K. (1993) Segregation of random amplified polymorphic DNA markers and strategies for molecular mapping in tetraploid alfalfa. *Genome* 36:844–851.

Zeng, Z.B. (1993) Theoretical basis of separation of multiple linked gene effects on mapping quantitative trait loci. *Proceedings of the National Academy of Sciences of the United States of America* 90, 10972–10976.

Zeng, Z.B. (1994) Precision mapping of quantitative trait loci. *Genetics* 136, 1457–1468.

Zeng, Z.B. (1998) Mapping quantitative trait loci: interval mapping, composite interval mapping and multiple interval mapping. *Summer Institute for Statistical Genetics*, Module 7. Department of Statistics, North Carolina State University, Raleigh, North Carolina.

Zietkiewicz, E., Rafalski, A., and Labuda, D. (1994) Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 20: 176-183.

Zipfel, C., and Felix, G. (2005) Plants and animals: a different taste for microbes? *Curr Opin Plant Biol* 8, 353-360.

PUBLICATION LIST

Referred articles related to thesis:

Gorji, M.A., Poczai, P., Polgar, Z., and Taller, J. (2011): Efficiency of Arbitrarily Amplified Dominant Markers (SCOT, ISSR and RAPD) for Diagnostic Fingerprinting in Tetraploid Potato. American Potato Research Journal, pp 1-12. Doi: 10.1007/s12230-011-9187-2.

Gorji, M.A., and Polgar Z. (2010) Application of genetic engineering in potato breeding, *Acta Agronomica Hungarica*, 58(4), pp. 427–441.

Poczai, P., Cernák, I,, **Gorji**, **M.A**., Nagy, S., Taller, J., and Polgár, Z. (2010) Development of intron targeting (IT) markers for potato and cross-species amplification in *Solanum nigrum* (Solanaceae). *American Journal of Botany*, 97: e142-e145.

Conference abstracts related to the thesis:

Gorji, M.A., Wolf, I., Decsi, K., Taller, J., Ahmadvand, R., and Polgár, Z. (2011) Development of hetero multiplex potato genotypes carrying PVY resistance genes, IX, Magyar Genetikai Kongresszus es XVI, Sejt-és Fejlődésbiológiai Napok, pp 176-177.

Decsi K., **Gorji, M.A.,** Taller, J., Cernak, I., Wolf, I., and Polgár, Z. (2011) Burgonya fitoftóra rezsztencia gén molekuláris markerezése és térképezése, IX, Magyar Genetikai Kongresszus es XVI, Sejt-es Fejlodesbiologial Napok, pp 206-207.

Other publications:

Poczai, P., Mátyás, K., Szabó, I., Varga, I., Hyvönen, J., Cernák, I., **Gorji**, M.A., Decsi, K., and Taller, J. (2011) Genetic Variability of Thermal Nymphaea (Nymphaeaceae) Populations Based on ISSR Markers: Implications on Relationships, Hybridization, and Conservation, Plant Mol Biol Rep DOI 10.1007/s11105-011-0302-9.

Gorji, M.A., and Shavakhi, F. (2006) Evaluation of Physical-Chemical Properties of New Potato Varieties and Introducing Proper Varieties for Process Technology, Journal of Agricultural Engineering Research.

Gorji, M.A., Sheidaei, M., Tehrani, A., Mirzaei, H**.** (2005) Evaluation of genetic variance in annual alfalfa with karyotype study, Seed and Plant Journal of Agricultural Research.

Other conference abstracts

Mátyás, K., Murthy, V., **Gorji**, M.A., and Taller, J. **(2011)** Az ürömlevelű parlagfű (*Ambrosia artemisiifolia* L.) populáció genetikai vizsgálata Európában-molekuláris markerek alakalmazásával**.**

Gorji, M.A., Sheidaei, M., Tehrani, A., Mirzaei, H. (2002) Evaluation of genetic diversities on protein coding alleles in annual alfalfa with Electrophoresis method, 7th Crop Sciences congress, Karaj, Iran.

Gorji, M.A., and Zarbakhsh, A.J. (2006) The Determinate of correlation and heritability of quantities and quality traits of potato, The 9th Genetics Congress, Tehran, Iran.

Gorji, M.A., Samadi, F.S., Forouzesh A., and Bakhshi, B. (2008) Effect of Planting Date on Specific Gravity, Tuber Volume, Big and Medium Tuber Percentage of Agria Potato Variety, 5th International Crop Science Congress & Exhibition, CS1-S1, P192.

Gorji, M.A., Samadi, F.S., and Forouzesh, A.(2008) Plant Height, Main Stem Number, Tuber Number, Total Yield, and Marketable Yield of 25 Potato Varieties Grown under Karaj (Iran) Condition, 5th International Crop Science Congress & Exhibition CS1-S1, P184.

Gorji, M.A., Forouzesh, A., and Samadi, F.S. (2008) The Evaluation of Early Planting on Tuber Yield, Shoot Dry Matter, Tuber Dry Matter, and Tuber Dry Matter per Unit Area of Some Potato Cultivars, 5th International Crop Science Congress & Exhibition, CS1-S1, P199.

Gorji, M.A., Samadi, F.S., and Forouzesh, A., and Parvizi K. (2008) Plant Height, Main Stem Number, Dry Matter Content, Maturity, Dormancy, and Yield of 18 Potato Varieties Grown under Hamadan (Iran) Condition, 5th International Crop Science Congress & Exhibition, CS1-S1, P187.

Gorji, M.A., Samadi, F.S., Forouzesh, A., and Bakhshi, B. (2008) Effect of Planting Date on Tuber Yield, Shoot Dry Matter, Tuber Dry Matter, and Tuber Dry Matter per

Unit Area of Agria Potato Variety, 5th International Crop Science Congress & Exhibition, CS1-S1, P193.

Gorji, M.A., Forouzesh, A., and Samadi, F.S. (2008) Evaluation of Specific Gravity, Tuber Volume, Big and Medium Tuber Percentage of Some Potato Cultivars, 5th International Crop Science Congress & Exhibition, CS1-S1, P202.

Gorji, M.A., Samadi, F.S., and Forouzesh, A., and HasanZadeh, S. (2008) Plant Height, Main Stem Number, Tuber Number, Total Yield, and Marketable Yield of 25 Potato Varieties Grown under Mashhad (Iran) Condition, 5th International Crop Science Congress & Exhibition, CS1-S1, P185.

Gorji, M.A., Forouzesh, A., Samadi, F.S., and Hasanpanah, D. (2008) Plant Height, Main Stem Number, Tuber Number, Total Yield, and Marketable Yield of 25 Potato Varieties Grown Under Ardebil (Iran) Condition, 5th International Crop Science Congress & Exhibition, CS1-S1, P186.

Gorji, M.A., Forouzesh, A., Samadi, F.S., and Mohammadi, A. (2008) Plant Height, Main Stem Number, Tuber Number, and Yield of 21 Potato Varieties Grown under Shahroud (Iran) Condition, 5th International Crop Science Congress & Exhibition, CS1-S1, P188.

Gorji, M.A., Samadi, F.S., Forouzesh, A., and Bakhshi, B. (2008) Effect of Planting Date on Specific Gravity, Tuber Volume, Big and Medium Tuber Percentage of Marfona Potato Variety, 5th International Crop Science Congress & Exhibition, CS1-S1, P194.

Gorji, M.A., Samadi, F.S., Forouzesh, A., and Bakhshi, B. (2008) Effect of Planting Date on Tuber Yield, Shoot Dry Matter, Tuber Dry Matter, and Tuber Dry Matter per Unit Area of Marfona Potato Variety, 5th International Crop Science Congress & Exhibition, CS1-S1, P195.

Gorji, M.A., Samadi, F.S., and Forouzesh, A. (2008) Effect of Planting Date On Specific Gravity, Tuber Volume, Big And Medium Tuber Percentage Of Picasso Potato Variety, 5th International Crop Science Congress & Exhibition, CS1-S1, P196.

Gorji, M.A., Samadi, F.S., and Forouzesh, A. (2008) Effect of Planting Date on Tuber Yield, Shoot Dry Matter, Tuber Dry Matter, and Tuber Dry Matter per Unit Area of Picasso Potato Variety, 5th International Crop Science Congress & Exhibition, CS1-S1, P197.

Gorji, M.A., Forouzesh, A., and Samadi, F.S. (2008) The Evaluation of Early Planting on Specific Gravity, Tuber Volume, Big and Medium Tuber Percentage of Some Potato Cultivars, 5th International Crop Science Congress & Exhibition, CS1-S1, P198.

Gorji, M.A., Forouzesh, A., and Samadi, F.S. (2008) The Evaluation of Late Planting on Specific Gravity, Tuber Volume, Big and Medium Tuber Percentage of Some Potato Cultivars, 5th International Crop Science Congress & Exhibition, CS1-S1, P200.

Gorji, M.A., Forouzesh, A., and Samadi, F.S. (2008) The Evaluation of Late Planting on Tuber Yield, Shoot Dry Matter, Tuber Dry Matter, and Tuber Dry Matter per Unit Area of Some Potato Cultivars, 5th International Crop Science Congress & Exhibition, CS1-S1, P201.

Gorji, M.A., Forouzesh, A., and Samadi, F.S. (2008) Evaluation of Tuber Yield, Shoot Dry Matter, Tuber Dry Matter, and Tuber Dry Matter per Unit Area of Some Potato Cultivars, 5th International Crop Science Congress & Exhibition, CS1-S1, P203.

Samadi, F.S., Allahdadi, I., Sepahvand, N.A., Akbari, G.A., and **Gorji, M.A.** (2008) Plant Height, Main Stem Number, and Main Stem Width of Some Potato Varieties Grown under Karaj (Iran) Condition, 5th International Crop Science Congress & Exhibition, CS1-S1, P39.

Hasanpanah, D., **Gorji, M.A.,** Dehdar, B., and Hoseinzade, H. (2005) Evaluation of Qualitative Characteristics, Yield and yield Components of Potato Cultivars for Processing in Ardabil. Plant Genomics and Biotechnology: Challenges and Opportunities. Indira Gandhi Agricultural University, Raipur.

Ghafari, H., **Gorji, M.A.,** et al. (2005) Study on yield trial and adaptation of white bean lines and varieties in Lordegan, Zanjan and Eghlid, The1st Iranian pulse Symposium, Research center for plant Sciences, Ferdowsi University of Mashhad. Mashhad, Iran.

Ghafari, H., **Gorji, M.A.** (2005) Study on yield and component yield of mungbean lines and varieties, The 1st Iranian pulse Symposium, Research center for plant Sciences, Ferdowsi university of Mashhad, Mashhad, Iran.

Hasanpanah, D., **Gorji, M.A.,** Dehdar, B., and Hoseinzade, H. (2005) Investigation of qualitative and quantitative characters of advanced potato cultivars in Ardabil . The 4th Iranian Horticultural Sciences Congress, Mashhad, Iran.

APPENENDIX

Table 10. Primer features

GenBank Accession No. of the target	Gene information	Primer label	Forward Sequence	Reverse Sequence	Tm (^{o}C)
FG548468.1	**Down regulated on PVY infection**	**Int-Ntn2**	**GAATTCACCACTGCTTGATG**	**ACTTGGATCACTGTTCAGC**	**52**
FG548461	"	**Int-Ntn3**	**GCATTCAGGAGAAGACAGAG**	**ATCTACCGATGTTGCTGAAG**	**52.5**
FG548447	"	**Int-ML**	**TTGCTGCAGTCGAAGAAC**	**CTGCAACAATCCTTGCTATG**	**52.5**
FG548436.1	"	**Int-Ntn5**	**GTCCAAGATTTTCACATAGC**	**GATCTTGATGGCGAATACAG**	**50**
FG550790.1	"	**Int-LB3**	**GCGCCATCTTCCTACTTC**	**TCCTCTGCCAATTTCTCC**	**52.5**
FG548434.1		**Int-PC1**	**GCTTGTCCTCCATCATCTTC**	**TCAGATAAGACCACCACCTC**	**53**
	"	**Int- Ntn7**	**TGTTCTTGTCTGAACCTTCC**	**TGAACAAGAAGCAACACCTC**	**52.5**
FG548569.1	**Up regulated on PVY infection**	**Int- Ntn8**	**GGCTTTTCTTGGGTGTTC**	**CTCACAGCAAAGTCCAATCT**	**53**
		Int-ST1	**TGGACCTGTAGGCAAAGA**	**CAGGAATGAACGGAAAGGAG**	**52.5**
		Int- ST2	**CCACTGGAACTGGAATTG**	**AAAGTAGTGAGCTGCTGGTG**	**52.5**
		Int-ST3	**GGCTTTTCTTGGGTGTTC**	**CTCTGCTCACAGCAAAGTC**	**51**
FG548565.1	"	**Int-Ntn9**	**GGACCTCCTTCAGATTGTTG**	**GGACCTGGATGATTATGG**	**52.5**
		Int-MF	**CACACAGTCATGGGTAGAAG**	**GGCCCTTCATTTTTCTGC**	**51**

		Int-ST4	**ATCCTTGTAGATCCCTCCTC**	**CCATCAAGGAATTCACAAAC**	**53.5**
FG548564.1		**“**	**Int-Ntn10**	**GTGGTGATCCGGAAGAAG**	**GTTTCCTGGTGTTCAAGGTC**	**52**
			Int-Ntn11	**CTACTGCTGGCATAAACAGG**	**CTGAGCAAGTTGAGACTGAG**	**52**
			Int-Ntn12	**TGCAAGCTCGACAAGATAAG**	**CCTCTAAGTTTTGCACCAAG**	**52**
			Int-MF2	**CCACACTGCAAAGGGATA**	**CTTGTGCAAGGGATGTTG**	**52.5**
			Int-MF3	**CCTCTAAGTTTTGCACCAAG**	**AGCTCGACAAGATAAGAAGC**	**53**
FG548561.1	**Down regulated on PVY infection**		**Int-LB5**	**TAGTGGTAGTACAGCCGAGA**	**GTGTAGCCCATCTTTTCG**	**52**
FG548559		**“**	**Int-Ntn14**	**GTCTCAGGCTGACAAGTCTC**	**CGTCACATTACACATCTTGC**	**52.5**
FG548550.1		**“**	**Int-Ntn16**	**GTGGATGTTCGCAAGAAG**	**GAATCTATGATGCCGTGCTC**	**51.5**
FG548550.1		**“**	**Int-Ntn17**	**ACGGCGGTTATAAGAAGC**	**ATGTTCAACCAGGTGTGC**	**53.5**
			Int-Ntn18	**CTCCTGCTCCATCACATC**	**CCAATCTGGCATCAAGAG**	**53.5**
			Int-Ntn19	**AGGAGTGGTGTTGGCTTC**	**TGACCGAATAGGCACTTG**	**52.5**
		“	**Int-CS2**	**ACAGCGATGAACGAAGAG**	**AGGAGTGGTGTTGGCTTC**	**53**
			Int-CS3	**CCAATCTGGCATCAAGAG**	**CTCCTGCTCCATCACATC**	**52**
			Int-CS4	**ATGTTCAACCAGGTGTGC**	**ACGGCGGTTATAAGAAGC**	**53**
			Int-CS5	**CGTGCTCATGATGGCTAAC**	**GAGTACGAACGCAAAGAG**	**51**
FG548549.1	**Up regulated on PVY infection**		**Int-Ntn20**	**GACGACAAGGATCAAACCTC**	**CAGCTCCCAGTTAATGCTC**	**51.5**
			Int-LB6	**CCAGTTAATGCTCCCAAG**	**GCCTTCCATTCAAGACAC**	**51.5**

		Int-LB7	**TTGATCCTTGTCGTCCTG**	**CCTGCCTGTTTGAAGTTG**	**52**
FG548548.1	“	**Int-Ntn21**	**TGGATCGAGGAAGATGAC**	**CAGTCCCCTAAATCACCAC**	**52**
		Int-Sc2	**CCCACATCTCTCTGTCTTTC**	**CCCACATCTCTCTGTCTTTC**	**53.5**
FG548547.1	“	**Int-Ntn22**	**CCCTTCAGTGGAGATTGA**	**AAGATGGAGAGTGAGTGACC**	**52.5**
FG548540.1	“	**Int-SM2**	**CAACAGTAATGCCAGCATCC**	**AAGCCACGGTTTGGAATC**	**51.5**
		Int-SM4	**CCAGCCACCTTCTCTTTC**	**GTACCCCAACGACCATATAC**	**49.5**
		Int-CA5	**GTTGATGTTGAAGAGCACCT**	**CCGTAATTCCAGAGAATGG**	**55.6**
FG548539.1	“	**Int-Ntn23**	**CTTTTAGAGCAGTTTCATGC**	**GCAACCCAAATCACAATC**	**52**
FG548512.1	“	**Int-MF5**	**GGATACTTGCCCTCAAGCTG**	**CAGCATCCTCCTTGTTGAGTC**	**51**
		Int-MF6	**CCCTGGTGTTGTTTCTTGTG**	**GTGATCTGGGAGATGTTG**	**55**
		Int-MF7	**CAACATCTCCCAGATCAC**	**GGGTTCAATTGAGGGTCTAC**	**52.5**
		Int-S2 F	**TGGTCATGTCCACTACACAAAC**	**ATCTCTCCAACAAGCTCCAC**	**55**
		Int-S3	**CGGTGTACCAACTTCACACAG**	**CAACATCTCCCAGATCAC**	**52**
FG548510.1	“	**Int-Ntn29**	**CCATGTCTGCCTTTGTGG**	**GTGCCAGTACTTTCATCTGCTG**	**55**
		Int-Ntn30	**AAGGGCACAGTGGAATTG**	**TTCTGAAGGCTCAGTAGC**	**50.5**
		Int-Ntn32	**TCTGCTATTACTTCTGCTGCAA**	**GCACTCAATGACCAGCATGT**	**53.5**
CV497719.1	**Mixed leaf clone**	**Int-Ntn33**	**GTGAGCAGACATCCAATTTC**	**CCCTATTACTTTCGGCATAG**	**53.5**
FG548490.1	**Down regulated on PVY infection**	**Int-SH2**	**CGAATGAACCAGAGAACC**	**GAGCTCTGAGATCCGAACC**	**51.5**

		Int-SH3	**CGGATCTCAGAGCTCATTTC**	**TTGAGTGGCGATTCTTCG**	**51.5**
FG548480.1	**Down regulated on PVY infection**	**Int-Ntn36**	**TGTTCAGAGAAGACCACAGATG**	**CACTTCTCCTCCAATAGCTGAA**	**53**
		Int-Ntn37	**CTGCAAGAAAAAGGATCTCAAC**	**CTTCCAAATCTTCCCCATTG**	**53.5**
		Int-tn38	**CCAATGGGGAAGATTTGG**		
		Int-SC4	**ACGAGCAATGTTGGACAGG**	**CCAATGGGGAAGATTTGG**	**53.5**
FG548474.1	**“**	**Int-Ntn43**	**CAAGCCAATTGAAACAGCAG**	**AATGCATCTCTCAACCTCAGAC**	**52.5**
		Int-Rs2	**GGCCAGGAACTTTTGGAAG**	**TTCCGTATCTCAAGGCTCTC**	**52.5**
		Int-Rs4	**CAAATCTAGCTGCCATCCTG**	**TCCCATGTCTTGCTGTATCC**	**53.5**
FG550860.1		**Int-Ntn45**	**CGTAGTGGGAGATATCCTTG**	**GCGTTCCTGGAAATCTTCTG**	**53.5**
	“	**Int-Ntn46**	**GCGTTCCTGGAAATCTTCTG**	**CGTAGTGGGAGATATCCTTG**	**53.5**
		Int-Ntn48	**GTGCCCTTCTTCTACCCTTC**	**CGAGCCATTCGACCTTTA**	**52**
		Int-Cys	**CTCAGGCAATTGTCCTACTTG**	**CTTGACCAACTCTGGGAAATG**	**54.5**
FG550855.1	**“**	**Int-ST13**	**ACTGGTATCCGATTGACACG**	**GGAGACATGGCAGTCTTG**	**53**
		Int-ST15	**GTGCAGGATGTTTTCGTG**	**CCTCCGGAAGATGGTTTC**	**53.5**
FG550854.1	**“**	**Int-Ntn49**	**TGCCAATATGCTGTCAGAGG**	**GAAATGTCAGTGGCTTCTGC**	**55**
		Int-Ntn51	**CTGGATGATCCGAATGTGG**	**CGTCTCTCAATGAAGTCTGC**	**54**
		Int-Ntn52	**GCAGACTTCATTGAGAGACG**	**GCCGCGAATGATACTGTTC**	**55**
		Int-Ntn53	**GTGCCAGCAAAGGAGTAATG**	**CCAGTTTGCAGTCTCTTC**	**53.5**

		Int-Ntn54	**ACTGCAAACTGGGGTCTTG**	**TGTCCAGTGGGATTTCCAG**	**52.5**
		Int-Ntn55	**TGGAGACGAAGTTTATCAGC**	**GACAAGCTGAACCTCCTTGC**	**54**
		Int-S5 151	**CACAACACTTGCCTCCTAAC**	**CCGTACTGGCAATGTAGCAC**	**55**
FG550851	**“**	**Int-Ntn56**	**CCATCCAGATCCAACTCCAG**	**CACCATTTTCGTCAACATCAG**	**53.5**
		Int-Ntn57	**ATGGTGTCGATAGCGTTG**	**CAGAGACATTTGGGCTCTTG**	**55.5**
		Int-S6	**GCTTGACAGTGACCATTTCC**	**CCGAAAGTTGAGGTTGATCG**	**55.5**
		Int-S8	**ATCCTCTTGACGCACTTTCC**	**GGCAGATACACTTCCTGCTG**	**55**
		Int-S9	**CAGAGACATTTGGGCTCTTG**	**CCGAAGTGTTATGTAGTCTGGA**	**54**
FG550845.1	**“**	**Int-Ntn59**	**CAAGTATGGCACCCCACAG**	**GTTTAAGTGGTTGGGGAGGAC**	**55**
		Int-Ntn60	**GTCACGGTCTACAGATGACAAC**	**CACTTTACGGTGCCTGTGAG**	**53.5**
		Int-SC7	**CCATCTCTCAGGCACTGTTG**	**CAGGTGTTTAGTCCATCCTTCC**	**54.5**
FG550844.1	**“**	**Int-Ntn61**	**GCCACTTGAAAACCAAGGAG**	**TGGATGATGTCTCGAGGAAG**	**51.5**
		Int-Ntn62	**GCACATGCAGGTTCTTCCTC**	**GCAGTGGTGAAAGTGGATTC**	**54**
		Int-Sc8	**CCTTCTTCTAGCCTCTTTGACA**	**AATTTCCATGGGTTCACTCG**	**55**
		Int-S10	**GTTCACTCGTTCGTCAAGAGC**	**CCCATCCAAGATGAATCCAC**	**56**
		Int-S11	**GTGGATTCATCTTGGATGG**	**TTCCAGTGGTGGATAAAGC**	**54**
		Int-S12	**TCTCGAGGAAGAACCTGCAT**	**ACTGCATTCCTGTGCTGAAG**	**52.5**
FG550842.1	**“**	**Int-S14**	**ATGTAACAGAGCAGGAGGAC**	**CTTGAGACAAGCTCCCTTGC**	**54**

		Int-S15	**ACGCAAGGGAGCTTGTCTC**	**TGGGAGTTGCCTTAGGTTCA**	**54.5**
		Int-S16	**TGCTGAACCTAAGGCAAC**	**GCAGCATCAGATTCTTGG**	**53.5**
		Int-S17	**TATAGCCCCAGCATGTTCAG**	**GCAGTGCTTGGATTTTGGAG**	**56**
		Int-LB11	**GTCATTCCAACCAACCATGC**	**ATGAAGGCAATGGAAAGTG**	**55**
		Int-LB12	**GCATGGTTGGTTGGAATGAC**	**TATAGCCCCAGCATGTTCAG**	**53.5**
		Int-LB13	**TGTTGGAAATGCTGCTGAAC**	**CATGGCAATTTGAGTTGCTG**	**54.5**
		Int-LB14	**TGGGAGTTGCCTTAGGTTCA**	**TGGGAGTTGCCTTAGGTTCA**	**55.5**
FG550836.1	**“**	**Int-Ntn63**	**AGAGAGGAATCCAGCAACTG**	**ATGCATAGGCAGGCAACTG**	**54**
FG550835.1	**“**	**Int-Ntn64**	**CCAGTGTTACTGCTGGATGC**	**GGGAGATCAATTTCCTTTCTTC**	**54**
FG550825.1	**“**	**Int-Ntn65**	**CCGTGGACTCCATTTTCAAG**	**CACACCAGTGGGGTTATGTG**	**55.5**
		Int-Ntn66	**ACCAAAGATCAGTGGGAGCA**	**CCACAAACATGCGAACAGAC**	**52.5**
		Int-LB17	**GAACTTGGGAGTTGGTGCATA**	**ACTAGTAGCTGTTCTGCTTTTC**	**52**
		Int-LB18	**GTTGTTAGAAAAGCAGAACAGC**	**TGTCAGCACCAAGTATCAGC**	**55.5**
		Int-PC3	**AGCAACCACTCCATTGTCG**	**ATGCACCAACTCCCAAGTTC**	**57**
		Int-PC4	**GTTGAACTTGGGAGTTGGTG**	**CATTTACTAGTAGCTGTTCTGC**	**57**
	“	**Int-PC5**	**GTTAGAAAAGCAGAACAGC**	**TGTCAGCACCAAGTATCAGC**	**53.5**
		Int-PC7	**CTCATTGAGGGTTGGAGGTG**	**CACGGGTTGCTGGATCATAG**	**52.5**
FG550820.1	**“**	**Int- tn67**	**TCCCTGCACTCTTGACAACC**	**GGAAGGAGGCGACTAGGAAC**	**56**

		Int-Ntn68	**AGAGCACAAGAGGTGTTTGTGG**	**AAGTGGAGTCTGGAGCTCATTG**	**53.5**
		Int-Ntn70	**TGATATGGCAGATGGAAGGAG**	**CCAAGATTGAGGATGTGACC**	**55**
		Int-S19	**CACCTTCAACTATGTAGGTAGC**	**GGGAGACTCACTTAGGATTTTG**	**55**
		Int-S21	**ATCGTCGAGCTTGTTTGTGG**	**CTCTTCTTGCAGCCTTCACC**	**54**
		Int-S22	**TCCCATTCGTTCTGAAGAGG**	**GGTAGCAATTGTCCAAGGAG**	**53.5**
FG550819	“	**Int-Ntn71**	**CAGACCTGCTGCTATTGACC**	**CTGAAGTCTTCCAGCTGTATCC**	**55**
FG550810.1	“	**Int-Ntn73**	**ACTCGTTCGAAGTGGGAATG**	**CGGACACGTAGACAGCTAAAG**	**53**
		Int-Ntn74	**TGGATTTTGCCGCTCTTC**	**GGTGGTCAAAACAGGACACA**	**54**
FG550805.1	“	**Int-Ntn75**	**CAGCAGCTCGTGCTATTACTTC**	**GTTGGGGTTCATTTCAGC**	**54.5**
		Int-LB21	**GTCACTCCTGACAAAGAAGGAC**	**CCGTGACATAGGCAGGATG**	**54**
		Int-LB23	**GATGGCGATGAGTCGTGTAG**	**GTTGGGGTTCATTTCAGC**	**55**
		Int-LB24	**GTCACTCCTGACAAAGAAGG**	**CCGTGACATAGGCAGGATG**	**54.5**
FG550804.1	“	**Int-Ntn76**	**CCTTCAATTATCCCCACACC**	**GGCTCCTTCTACATGTTTGCTC**	**55**
		Int-SST16	**GAGTTGGTTCCGCCATTG**	**GAAACGGCCACAAATAGAGC**	**48.5**
		Int-SST17	**GGCTCCTTCTACATGTTTGCTC**	**AATTATCCCCACACCAGCTC**	**54.5**
FG550802.1	“	**Int-Ntn77**	**CTTCATCATCCCAGTCCTCTG**	**GGACATTCTCCCACCAAAGA**	**54**
		Int-S25	**TGGTAGCTTGTACTCTGACTGG**	**GCTGTCCATTCACCATCTTC**	**55**
		Int-S26	**GAGGACTGGGATGATGAAGAAG**	**CCACTTCCCCTTGTAGTTGG**	**51.5**

		Int-S27	**AATCAAGAACCCCAACTAC**	**GGGAAAACATATAGATCTGG**	**56.5**
DN938803.1	**After cooking darkening A**	**Int-Ntn78**	**AGCTGAAGATGCCTTCTCG**	**AAGAGCCGATCCTCAAACTG**	**54**
FG550799.1	**Down regulated on PVY infection**	**Int-Ntn80**	**TGAGAAGGGGAAGAAGCAAG**	**GGGAGCCCAAATATGAACAG**	**52**
FG550791.1	**“**	**Int-LB25**	**GTCACTCCTGACAAAGAAGGAC**	**CCGTGACATAGGCAGGATG**	**52.5**
FG550790.1	**“**	**Int-LB26**	**CGATAAGATGCTCTCGAAATG**	**GAACTCCTTCTTCAACTTTGC**	**51**
		Int-S28	**CTTAAAGCTCGCAGGGATGA**	**GGAAAAATCAGAACATCTCC**	**51.5**
		Int-S30	**GTTCATGTGGCTGCTTGTTC**	**TTTCCCTCTCCGATATGCTC**	**51.5**