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DEVELOPMENT OF SCARS (SEQUENCE CHARACTERIZED AMPLIFIED REGIONS) TO ANCHOR GENETIC LINKAGE MAPS IN VITIS

ASMA (Vitis vinifera L)'DA SCAR MARKÖRLERİ ARACILIĞIYLA GEN HARİTALARININ BİRBİRİNE BAĞLANMASI

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ABSTRACT: This research was conducted to develope SCARs (Sequence Characterized Amplified Regions) to anchor genomic linkage map in Vitis for important traits. RAPD marker from the map of an interspecific hybrid population of Cayuga White x Aurore were used in this study. In this study, 4 RAPD markers from linkage group III (p36f, OP10d, BC 389b and P232c) and 2 RAPD markers from linkage group IX (BC 374a and HB 374d) of Cayuga White x Aurore genomic maps were used. These markers were converted to the SCAR markers and tested in three other populations. These are 88.0514 Horizon x Illinoi 547-1, 89.064 Swenson Red x Flame Seedless, and Joannes Seyve 23-416 x Illinoi 547-1.

From these SCAR markers developed from RAPD markers, two of them worked in the population tested.However the others did not work except its own populations.

ÖZET: Bu araştırma asma'da (Vitis vinifera L.) önemli özelliklere karşı çıkarılan genom haritalarının SCAR markörleriyle birbirine bağlanması amacıyla gerçekleştirilmiştir. Bu amaçla interspesifik hibrit olan Cayuga White ve Aurore üzüm çeşitleri arasında oluşturulmuş olan genome haritasındaki RAPD markörleri kullanılmıştır. Bu genom haritasındaki III. kromozom üzerinde bulunan 4 RAPD markörü (p36f, OP10d, BC 389b and P232c) ve IX. Kromozom üzerinde bulunan 2 RAPD markörü (374a and HB 374d) kullanılmıştır. Bu markörler SCAR markörlerine dönüştürülmüş ve farklı üç populasyonda test edilmiştir. Bunlar 88.0514 Horizon x Illinoi 547-1, 89.064 Swenson Red x Flame Seedless, and Joannes Seyve 23-416 x Illinoi 547-1.

Araştırma sonucunda, RAPD markörlerinden geliştirilmiş olan SCAR markörlerden iki tanesi çalışmış, fakat diğerleri ise kendi populasyonu dışındaki populasyonlarda çalışmadığı belirlenmiştir.

INTRODUCTION

The genetic improvoment of a species through artificial selection depends on genetic effects that can be distinguished from environmental effects. Phenotypic selection based on traits that are conditioned by additive allelic effects can produce dramatic, economically important changes in breeding populations. Genetic markers-heritable entities that are associated with economically important traits – can be used by plant breeders as selection tools (BECKMANN and SOLLER, 1983; DARVASI and SOLLER, 1994). Marker-assisted selection (MAS) provides a potential for increasing selection efficiency by allowing for earlier selection and reducing plant population size used during selection. Nevertheless, the phenotypic variation that marker loci define is often nonadditive, and is a function of genetic linkage , pleiotropy, and environment (LARK et al. 1995). Thus, the efficiency of application of marker loci as predictors of phenotypic variation depends on many factors, and predictions of response to selection (R) or genetic gain (Δ G) are often difficult.

Often the biotechnological information presented in research reports is not tied directly to classical genetic methotodologies and the sophisticated technology presented results in a bewildering array of new terms. For scientists who have a peripheral interest in genome mapping, but would like to understand the potential role of marker-assisted selection in plant improvoment, the wealth of information currently being produced in this area can lead to considerable confusion.

Grape (Vitis sp.) is the most important and widely grown decidious fruit and major crop on every continent due to its multiple uses in the food and beverage industries (ANONYMOUS ,1994 ; TINLOT and ROUSSEAU ,1993).

The availability of suitable scientific tools have encouraged scientific research on many aspects of grape breeding including genome analysis. Because of the long generation cycle, breeding and field evaluation of new grape cultivars require extensive labor over a period of several years (REISCH et al., 1994). In addition to that, inbreeding depression make it difficult to perform genetic linkage analysis with the use of inbred parental lines. A faster and more reliable system could be used to aid in the breeding and subsequent selection of grape cultivars. One strategy is based on molecular linkage maps (O'BRIEN, 1993). These maps indicate the order and relative genetic distance among markers and can be used to locate genes for important traits such as *Botrytis* rot resistance, Powder mildew resistance etc.

If we look through marker types; they can be classified as follows.

Morphological Markers: Morphological traits controlled by a single locus that can be used as genetic markers if their expression is reproducible over a range of environments. Although codominant morphological markers have been useful as predictors of genetic response to selection, they can be influenced by environmental and genetic factors (e.g. epistatis). For instance, the expression of the determinate (*de*) character in cucumber (*cucumis sativus* L.) may vary, depending on growing environment and modifying genes (STAUB and CRUBAUGH, 1995). Thus, a description of such a trait has significance only when accompanied by properly documented pedigree information and environmental conditions. The fact that such factors may modify a gene's expression of phenotype may limit its usefullness as a genetic marker. A further drawback of morphological markers is that they may present an altered phenotype that interferes with grower needs.

Isoenzymes: Isoenzymes are differently charged protein molecules that can be seperated using electrophoretic prosedures (MARKERT and MOLLER, 1959). Since enzymes catalyse specific biochemical reactions, it is possible to visualize the location of a particular enzyme on a gel by supplying the appropriate substrate and cofactors, and involving the product of enzymatic reaction in a color producing reaction. The colored product becomes deposited on the gel, forming a visible band where a particular enzyme has been electrophoretically localized. Band visualized from specific enzymes represent protein products, have a genetic basis and can provide genetic information as codominant markers. However, the paucity of isozyme loci and the fact that they are subject to post-translational modifications often restricts their utility(STAUB et al., 1982).

Restricted Fragment Length Polymorphisms (RFLPs): RFLPs are detected by the use of restriction enzymes that cut genomic DNA molecules at specific nucleotide sequences (restriction sites), thereby yielding variable size DNA fragments. Identification of genomic DNA fragments is made by southern blotting, a procedure whereby DNA fragments, seperated by electrophoresis, are transferred to nitrocellulose or naylon filter (SOUTHERN, 1975). Filter immobilized DNA is allowed to hybridize to radioactivelly labeled prob DNA. Porbes are usually small, cloned DNA segments. The filter is placed againist photographic films, where radioactive disintegrations from the probe result in visible bands. Such bands are visualizations of RFLPs, which are codominant markers.

Molecular markers such as isozyme and RFLP (Restricted Fragment Length Polymorphism) markers have been used in many plant species (RICK, 1983, BOTSTEIN et al. 1980, BECKMAN and SOLLER, 1986 and WEEDEN 1989). However, these molecular markers have certain limitations. Isoenzymes are restricted to the gene encoding soluble proteins and RFLP markers are mostly restricted to the coding region of the genome.

In order to overcome these limitations of these techniques, PCR (Polymerase Chain Reaction) based analysis such as RAPD (Random Amplified Polymorphic Dna), Arbitrarily primed PCR (AP-PCR) have been developed and reported (WILLIAMS et al., 1993).

Random Amplified Polymorphic DNA (RAPDs): RAPD markers are generated by PCR amplification of random genomic DNA segments with single primers (usually 10 nucleotides long) arbitrary sequence

(WILLIAMS et al., 1990). The primer are used as substrates for DNA polymerase to copy the genomic sequences 3' to the primers. Iteration of this process yields a discrete set of amplified DNA products that represent target sequences flanked by opposite- oriented primer annealing sites. Amplification products can be seperated by electrophoresis on agarose or polyacrylamide gels and visualized by staining with ethidium bromide or silver. RAPDs are usually dominant markers with polymorphisims between individuals defined as the presence or absence of a particular RAPD band.

In random Amplified Polymorphic DNA analysis unknown fragments of target DNA are amplified exponentially by using short sequence oligonucleotide primers (usually 10-mer) and thermostable Taq DNA polymerase (RAFALSKI et al., 1991; WILLIAMS et al., 1993).

These RAPD markers show great potential as genetic markers for construction of genome mapping (WILLIAMS et al., 1990; MARTIN et al., 1991; PARAN et al. 1991; MICHELMORE et al. , 1991).

However, RAPD amplified products often contain repetetive DNA sequences. Therefore they can not be used as an hybridization probes. RAPD markers are also dominant/null as compared to codominant isoenzyme and RFLP markers. In addition, RAPD markers are sensitive to amplification conditions and there is a lack of reproducibility between laboratories.

Some of disadvantages of RAPD markers could be overcome by converting these markers to co-dominant SCAR markers (PARAN and MICHELMORE, 1993). A SCAR is a genomic DNA fragments at a single genetically defined locus which is identified by PCR amplification using a pair of specific oligonucleotide primers.

Sequence Characterized Amplified Regions (SCARs): Utility of a desired RAPD marker can be inreased by sequncing its termini and designing longer primers (e.g., 24 nucleotide) for specific amplification of markers (PARAN and MICHELMORE, 1993). DNA sequence differences are manifest by the presence or absence of a single unique band. SCARs are more reproducible than RAPDs and can be developed into plus/minus arrays where electrophoresis is not needed.

SCARs have advantageous over RAPD markers, as they detect only a single locus. They are less sensitive to amplification conditions and they potentially can be converted into co-dominant markers.

HAYMES et al., (2000) stated that , two dominant SCAR markers (linked at 3.0 cM, cuopling phase) were constructed for the strawberry (*Fragaria x ananassa* Duch.) gene *Rpf1*. This gene confers resistance to red stele root rot; caused by the soil-born fungus *Phytophthora fragariae* Hickman var. *fragariae*. The SCAR markers were developed originally from the sequence of RAPD OPO-16$C_{(438)}$ that is linked in repulsion phase to the *Rpf1* allele. The SCAR markers, as well as some additional RAPD markers known to be linked to *Rpf1*, were shown to be higly conserved in linkage to the gene based on examination of 133 European and North American *Fragaria* L.sp. cultivars and breeding selections. It is also stated that these flanking RAPD and SCAR-PCR markers can be used in breeding programs for the selection of red stele *(Rpf1)* resistance.

KASAI et al., (2000) developed SCARs based on nucleotide differences within resistance gene-like fragments isolated from a potato plant carrying the Ry_{adg} gene, which confers extreme resistance to potato Y potyvirus (PVY). It originates from *Solanum tuberosum subsp. andigena*, and a susceptible potato plant. SCARs were tested using 103 potato breeding lines and cultivars with diverse genetic backrounds derived from Europe, North america and Japan. Two markers showed high accuracy for detection of the Ry_{adg} gene. The SCAR marker RYSC3 was generated only in genotypes carrying Ry_{adg}. The SCAR marker RYSC4 was detected in all genotypes carrying other Ry_{adg} but also in four PVY-susceptible genotypes. They concluded that these SCAR markers should be powerful tools in marker assisted selection for Ry_{adg} in potato breeding programs, and should also be useful for cloning of the Ry_{adg} gene.

FRANÇOISE et al. (2001) are also developed two SCAR markers from a RAPD marker linked to a major locus involved in seedlessness, *sdl* in Vitis. They stated that SSC8 was found to be a usefull marker at least in the seedless x seedless progenies and to show a good linkage disequilibrium with seedlessness in our set of varieties.

This research was conducted to develop SCARs by converting some of the RAPD markers- linked to some important traits- so as to test them in other populations to anchor genetic linkage maps in Vitis.

MATERIAL AND METHODS

Plant Material:

RAPD marker from the map of an interspecific hybrid population of Cayuga White x Aurore were used in this study. This cross was developed by B. I. Reisch at the New York State Agricultural Experiment Station, Geneva, N.Y. (NYSAES) in 1981. Genetic Linkage maps of Cayuga White and Aurore using a double pseudotestcross strategy were previously developed in 1994 (LODHI , 1994).

SCAR markers were tested in three other populations. These are 88.0514 Horizon x Illinoi 547-1, 89.064 Swenson Red x Flame Seedless, and Joannes Seyve 23-416 x Illinoi 547-1.

DNA Extraction and RAPD Reaction

DNA for RAPD analysis was extracted from young unexpanded leaves using a modified CTAB procedure (LODHI et al. 1994). However, the DNA of Flame Seedless cv. was extracted from rachis tissue by using a modified extraction method as described by YEE et al. (1994-unpublished research).

The final DNA pellets were dissolved in sterile water and DNA concentartions were measured in a TKO 100 mini flourometer. DNA was diluted to a working concentration of 100-200 ng/ul for RAPD reactions.

Amplification reaction was performed in 25 ml volumes containing 10 mM Tris-HCl (pH 9.0), 3.0 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, 0.12 mM each of the four dNTPs (Boehringer Mannheim), 0.2 µM oligonucleotide primer, 100-200 ng grape template DNA and 0.5 unit of Taq DNA polymerase (Promega, WI, USA).

The reaction mixtures were overlaid with a drop of sterile mineral oil (Sigma, St. Louis, MD, USA) and the DNA was amplified on a PTC-100 Thermocycler (MJ Corp., MA, USA) for 35 cycles of 30 seconds at 94 °C, 60 seconds at 35 °C, and 105 seconds at 72 °C, followed by a final extention of 8 minutes at 72 °C. The amplification products were seperated by electrophoresis on 2% agarose gel (1% agarose/ 1%Nusieve GTG agarose, FMC Corp., ME, USA) in 1X TBE. The DNA fragments were visualized under UV light after staining with 0.01 mg/ml ethidium bromide. The gel were photographed using Type 55 Polaroid film.

Oligonucleotide Primers

RAPD primers were obtained from Operon Technologies (OP, Alemeda, CA, USA), from the University of British Columbia (UBC, Canada) and from New York State Center for Advanced Technology in Biotechnology, Cornell University, Ithaca , N. Y.

The sequences of primers found to identify polymophism are (5' to 3') as follows;

P36f*	5'-ATA CCA AAC GAC GAG-3'
OP10d	5'- GTG ACG TAG G-3'
BC374a	5'- GGT CAA CCC T-3'
BC 389b	5'- CGC CCG CAG T-3'
P232c	5'- CCG CTT GTT G-3'
HBC 374d	5'- GGT CAA CCC T-3'

*: P36f: p36 is the primer, "f" is the RAPD fragment.

Cloning of the RAPD Products and Generation of SCAR Markers

In this study, 4 RAPD markers from linkage group III (p36f, OP10d, BC 389b and P232c) and 2 RAPD markers from linkage group IX (BC 374a and HB 374d) of Cayuga White x Aurore genomic maps were used. The amplified products of RAPDs were excised from agarose gels and the DNA was reamplified using the same primer that identified the RAPD polymorphism.

These six RAPD markers were cloned into pCR II (Invitrogen Corp., San Diego) and introduced into compotent E. Coli INVaF'. The procedure was as described by the manufacturer except for certain modifications. For the cloning of six RAPD markers, ligation reaction contained 1.5 ul undiluted PCR reaction product.

Recombinants were identified as white or pale blue colonies on medium containing X-gal. Plasmid DNA was isolated from several independent transformants by a modified mini alcaline lysis/Peg precipitation procedure as recommended by ABI.

Inserts were tested by Quick prep. For plasmid DNA without digestion. The cloned RAPD markers were sequenced using T7 and Sp6 sequencing primers, except for p36f which was sequenced using T7 and M13 sequencing primers at Cornell University, Cornell Biotechnology Facility, Ithaca, N. Y.

For each cloned RAPD amplification product, two oligonucleotides were designed to be used as SCAR primers. Each primer contained the original 10 bases of the RAPD primer plus the net 8 to 11 internal bases from the end. All primers were synthezed by Cornell University, Cornell Biotechnology Analytical /Synthesis Facility (Ithaca, N. Y.) (Table 1).

Amplification of genomic DNA with SCAR primers was performed in a standart PCR reaction with some modifications. Two ml of primer (1+1 from each) was used per reaction DNA was amplified on a PTC-100 Thermocycler for 35 cycles of 30 sec. at 94°C, 180 sec. at 60°C for BC 389b, 51°C for OP10d, 61°C for BC374a and 46°C for HB374d, 60 sec. at 72°C, followed by a final extention of 8 minutes at 72°C. Amplification products were resolved in 2% agarose gel (1% agarose / 1% Nusieve GTG agarose) in 1X TBE.

RESULTS and DISCUSSION

Six RAPD fragments were converted into SCARs; VLG III BC 389b R, F; VLG III.P232c R, F; VLG III OP10d R, F; VLG III P36f R, F; VLG IX BC 374a R, F, and VLG IX HB 374d R, F. A variety of primer length was between 18-22 nucleotides and annealing temperatures varied from 46 °C to 67 °C.

Two pairs of SCAR primers (VLG III BC 389b R, F; and VLG IX BC 374a R, F) resulted in the amplification of single major bands of the same size as the RAPD fragment cloned. Polymorphisim was detected as the presence or absence of amplification of the band and were therefore dominants markers. The initial pair of primers for VLG III P232c R,F; VLG III OP10d R,F and VLG III P36f amplified also the same sized fragment from both (Cayuga White and Aurore) parents. In addition, VLG IX HB 374d R,F marker which is heterozygous in both parent ampilified also the same-sized fragment from both progenies that have RAPD fragment and the progenies have no fragment. However, when the primers that started 3 to 6 bases inside the sequence of the RAPD primer were used VLG III OP10d R,F, two bands were amplified from Cayuga White cv. and only one band was amplified from Aurore. In addition , VLG IX HB 374d SCAR marker amplified three bands in Cayuga White cv. and two bands in Aurore (Table 2).

Table 1. SCAR Primer Pairs Derived From Cloned RAPD Bands

SCAR PRIMER PAIR*		SEQUENCE	N**	BAND SIZE
VLG III BC 389b	R	CGC CCG CAG TCG GTT GTA	18	850 bp
VLG III BC 389 b	F	CGC CCG CAG TTG ATG GTA	18	
VLG III P232 c	R	CCG CTT GTT CCA ACA TAT CAG	21	680 bp
VLG III P232 c	F	CCG CTT GTT CAT CTA CAA GAA C	22	
VLG OP10d	R	GTG ATC GCA GAA CAT CTG	18	500 bp
VLG OP10d	F	GTG ATC GCA GAG GTA TCA	18	
VLG P36 f	R	ATA CCA AAC GAC GAG TCT C	19	400 bp
VLG P36 f	F	ATA CCA AAC GAC GAG GTT A	19	
VLG IX BC 374a	R	GGT CAA CCC TCT AGA ATC	18	1050bp
VLG IX BC 374a	F	GGT CAA CCC TTA TTA TAG	18	
VLG IX HB 374d	R	GGT CAA CCC TTT CTA CCC	18	690 bp
VLG IX HB 374d	F	GGT CAA CCC TAG TGA TCG	18	

* Bold sequences are derived from orijinal RAPD primers. Letters and Numbers preceding the VLG III (Vitis Linkage Group III), VLG IX (Vitis Linkage Group IX), R(reverse), F (forward).

** Number of nucleotides in each primers.

Table 2. SCAR Primer Pairs Derived From Six RAPD Loci

SCAR PRIMERS		SEQUENCE	N	BS	AT	POLYMORPHISM
VLG III BC 389b	**R**	**CGC CCG CAG TCG GTT GTA**	18	850 bp	60°C	**Polymorphism**
VLG III BC 389 b	**F**	**CGC CCG CAG TTG ATG GTA**	18			
VLG III P232 c	R	CCG CTT GTT CCA ACA TAT CAG	21	680 bp	60-68°C	No Polymorphism
VLG III P232 c	F	CCG CTT GTT CAT CTA CAA GAA C	22			
VLG III P232 c	R_1	CCG CTT GTT CCA ACA TA	17	680 bp	60-65°C	No Polymorphism
VLG III P232 c	F_1	CCG CTT GTT CAT CTA CAA	18			
VLG III P232 c	R_2	CCG CTT GTT CCA ACA	15	680 bp	60-65°C	No Polymorphism
VLG III P232 c	F_2	CCG CTT GTT CAT CTA	15			
VLG OP10d	R	GTG ATC GCA GAA CAT CTG	18	500 bp	60-67°C	No Polymorphism
VLG OP10d	F	GTG ATC GCA GAG GTA TCA	18			
VLG OP10d	**R_1**	**GTG ATC GCA GAA CAT**	15	500 bp	51°C	**Polymorphism**
VLG OP10d	**F_1**	**GTG ATC GCA GAG GTA**	15			
VLG P36 f	R	ATA CCA AAC GAC GAG TCT C	19	400 bp	60-65°C	No Polymorphism
VLG P36 f	F	ATA CCA AAC GAC GAG GTT A	19			
VLG IX BC 374a	**R**	**GGT CAA CCC TCT AGA ATC**	18	1050bp	61°C	**Polymorphism**
VLG IX BC 374a	**F**	**GGT CAA CCC TTA TTA TAG**	18			
VLG IX BC 374d	R	GGT CAA CCC TTT CTA CCC	18	1050bp	59-67°C	No Polymorphism
VLG IX BC 374d	F	GGT CAA CCC TAG TGA TCG	18			
VLG IX BC 374d	R_1	GGT CAA CCC TTT CTA	15	690bp	50-59°C	No Polymorphism
VLG IX BC 374d	F_1	GGT CAA CCC TAG TGA T	16			
VLG IX HB 374d	R_2	GGT CAA CCC TTT	12	690 bp	40-50°C	No Polymorphism
VLG IX HB 374d	F_2	GGT CAA CCC TAG	12			
VLG IX BC 374d	**F_1**	**GGT CAA CCC TAG TGA T**	16	690bp	46°C	**Polymorphism**
VLG IX HB 374d	**F_2**	**GGT CAA CCC TAG**	12			

The letters preceding N: Number of nucleotides in each primers, AT: Annealing Temperature, and BS: Band Size.

PCR amplifications involving sequence-specific primer pairs VLG III.P232c R, F; VLG III.P232c R_1, F_1; VLG III.P232c R_2, F_2; VLG III P36f R, F; VLG IX HB 374d R, F.; VLG IX HB 374d R_1, F_1., and VLG IX HB 374d R_2, F_2 resulted in a loss of polymorphism. Digestion of these amplification products with restriction enzymes (Hae III, Hinf I, Rsa I, Tag I, Sau3A I, Hha I, Dde I, Msp I and Alu I) did not reveal a polymorphism between these cultivars. On the other hand, different annealing temperatures- decreased and increased- were also tested to obtain polymorphism. But this application also did not result in restoration of polymorphism.

Consequently, out of six RAPD fragments cloned and sequenced , four of them converted to the SCAR markers that segregated as co-dominant markers and the other two have yet to be converted to a SCAR due to the lack of detectable polymorphism within the amplified fragment from diverse genotypes.

Testing SCARs in other populations

SCAR markers from III linkage group were tested in three other populations; Johannes Seyve (JS) 23-416 x Illinois 547-1; 89.064 Swenson Red x Flame Seedless and 88.0514 Horizon x Illinois 547-1. VLG III BC 389b R,F SCAR marker did segregate in JS x Illinois 547-1 population. This SCAR marker is present in JS and absent in Illinois 547-1 cvs. When the second SCAR marker (VLG III OP10d R_1, F_1) from III linkage group was tested in the same population. It is present in both parents and did not segregate. Therefore there were unable to test the linkage If these marker are linked in this population or not due to both unsegregation of VLG III OP10d R_1, F_1 SCAR marker and presence of this marker in both parents.

The second population tested with these two markers was 89.064 Swenson Red x Flame Seedless. Two SCAR markers are present in both parents. Both of them did not segregate in this population. The third population tested with these markers was 88.0514 Horizon x Illinois 547-1. VLG III BC 389b R,F SCAR marker segregated also in this population and present in 88.0514 Horizon and absent in Illinois 547-1. In addition, secondary band was also amplified with original SCAR band. However, VLG III OP10d R_1, F_1 SCAR marker did not segregate.Therefore in this population it was not possible to test the linkage of these two SCAR markers.

SCAR markers from IX linkage group VLG IX BC 374a R,F and VLG IX HBC 374d R_1, F_1 were tested in three different populations for linkage.

In JS 23-416 x Illinois 547-1 population VLG IX BC 374a R,F SCAR marker is absent in JS and present in Illinois and segregated in the progenies. However, second SCAR marker VLG IX BC 374a F_1,F_2 amplified different sized (100 bp lower) band which is present in both parent and segregated in the progenies. In addition, when the segregation patterns in the porgenies is compared, it was clear that they are not linked.

Second population tested with these marker was 89.064 Swenson Red x Flame Seedless. VLG IX BC 374a R,F SCAR marker segregated and present in Swenson Red and absent in Flame Seedless VLG IX HBC 374d F1,F2 SCAR marker also segregated and amplified two other bands.This marker is absent in Swenson Red and present in Flame Seedless. In this case, it was not also possible to test linkage in this populations due to the segregation pattern of these two markers in both parents.

The third population tested with these markers was 88.514 Horizon x Illinois 547-1. VLG IX BC374a SCAR marker segregated and present in Horizon, absent in Illinoi and did not segregate in the progenies.

Along with recent exploding advancement of plant molecular genetics, various kinds of PCR-based molecular markers have been developed for plant breeding, such as RAPD , SCAR, and AFLP. Molecular markers are considered to be an important tool leading to enhanced progress in grape breeding, as well as in breeding of other crop species.

Among the molecular markers developed thus far, SCAR markers seem to be the most valuable. For example, PCR-based markers do not require large amounts of prufied DNA, in contrast to hybridization methods like RFLP. With SCAR markers, scoring the results is more straightforward than other PCR-based markers, such as RAPD and AFLP. In comparison to CAPS markers, the SCAR marker can be revealed by only a single PCR procedure, without subsequent digestion with a restriction enzyme. Another advantage of PCR-baseed markers is that genomic information can be obtained directly by analysing PCR products. Consequently, SCAR markers do not only offer the most practical methods for screening numerous samples in a time- and labor- saving manner, but may also offer starting points for cloning the target genes.

Consequently , in this research 6 RAPD markers, which are closely related with important traits such as powder mildew resistance, shoot tip color, *Botrytis* bunch rot resistance from Cayuga White and Aurore genome map, were converted to the SCAR marker and tested in two other different populations to anchor genomic linkage map in Vitis. However, two of them were worked but the others were not. Therefore, in order to construct genomic linkage map among the populations , two more SCAR markers should be developed and tested in the populations.

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