The Nelson Mandela AFrican Institution of Science and Technology

NM-AIST Repository **https://dspace.mm-aist.ac.tz**

Life sciences and Bio-engineering Research Articles [LISBE]

2015

Molecular Genetic Diversity of Grapevines to Produce Powdery mildew Resistant Genotypes

Mwamahonje, Andekelile

American Journal of Research Communication

http://dspace.nm-aist.ac.tz/handle/123456789/153

Provided with love from The Nelson Mandela African Institution of Science and Technology

Molecular Genetic Diversity of Grapevines to Produce Powdery mildew Resistant Genotypes

Andekelile Mwamahonje[1], Deusdedit Kilambo[2] and Tileye Feyissa[1]

[1]Department of Biodiversity and Ecosystem Management and Sustainable Agriculture, School of Life Science and Bio-engineering, The Nelson Mandela African Institution of Science and Technology, P.O. Box 447, Arusha, Tanzania
[2]Tanzania Coffee Research Institute, P.O. Box 3004, Moshi, Kilimanjaro
Correspondence Author: mwamahonjea@nm-aist.ac.tz

Abstract

Grapevine powdery mildew is a fungal disease caused by *Erysiphe necator* (formerly *Uncinula necator*) which is an obligate parasite. It is considered to be one of the most important fungal diseases in viticulture worldwide causing 20% loss of grapevine production. Conventional breeding has been used to obtain powdery mildew resistant varieties however; it takes long time to obtain new variety. The use of molecular markers has been proposed to be the best method for identifying genes for resistance to powdery mildew which provides basic information in breeding programs. Furthermore, molecular markers have been useful in identifying genetic diversity among grapevine varieties. Inter Simple Sequence Repeat (ISSR) and Simple Sequence Repeat (SSR) is among the most useful markers for genetic diversity studies. This will be among of the useful markers as the guidelines for breeding programs of grapevine in tropical and sub-tropical countries to improve agricultural production in addressing food security.

Key words: Genetic diversity, Grapevine, ISSR, Molecular marker, Powdery mildew

{**Citation:** Andekelile Mwamahonje, Deusdedit Kilambo, Tileye Feyissa. Molecular genetic diversity of grapevines to produce powdery mildew resistant genotypes. American Journal of Research Communication, 2015, 3(8): 10-33} www.usa-journals.com, ISSN: 2325-4076.

1.0 Introduction

Grapevine is a horticultural crop produced worldwide for wine making and used as table grape for spices, juices and eaten raw. It contains high level of glucose, protein, vitamins, amino acids, lecithin and minerals as well as flavonoids which are good antioxidant, eliminate free radicals, and prevent aging (Choudhary et al. 2014). Grapevine powdery mildew is a fungal disease caused by *Erysiphe necator* (formerly *Uncinula necator*) which is an obligate parasite. Most grapevine varieties are attacked by several diseases that hamper its production (Halleen and Holz 2001; Rumbolz and Gubler 2005). Powdery mildew is considered one of the most important fungal diseases in viticulture worldwide (Rumbolz and Gubler 2005) causing about 20% loss in grapevine production and wine quality due to off flavors (Hossein et al. 2012). This ultimately, contributes to drastic loss of yield in many grapevines producing countries (Khiavi et al. 2012). Therefore, scientists have been developing powdery mildew resistant varieties (Seyedimoradi et al. 2012). Additionally, the lack of scientific background on the resistant varieties to powdery mildew in grapevine has led to drastic reduction of grapevine genetic diversity due to loss of natural habitat with poor cultivar identity within germplasm (Tangolar et al. 2009). In this case grapevine germplasm collections for crop breeding lack useful information on genetic diversity, population structure and proper phenotypic assessments (Emmanuel et al. 2013). The identification of grapevine varieties has been reported to be difficult due to vegetative propagation of cultivars which depend on ampelography (Pinto et al. 2003). Research efforts are needed for identifying the resistant varieties against the disease and enhancing the use of existing collections for improvement of crop yield while other efforts should be made to find the proper germplasm (Emmanuel et al. 2013). Scientists have been using conventional breeding techniques which entail crossing grapevine varieties, those having susceptibility to powdery mildew back-crossed to obtain new varieties with resistant genes (Seyedimoradi et al. 2012). DNA markers are useful in identification of powdery mildew resistant genes at molecular level. The identified

genes are incorporated into susceptible farmers' preferred varieties to enhance crop production (Emmanuel et al. 2013).

2.0 Grapevine powdery mildew disease

The disease affects green tissues of grapevines. Leaves show irregular chlorosis of gray-white with white powder on the leaf conveying at upper and lower surface (Wan et al. 2007). Furthermore, it retards the growth of berries and fruit cracking hence, poor grape production (Wan et al. 2007). In the case of severe infection, leaves dry out and drop prematurely, the growth is stunted and affected shoots appear dark brown to black with association of lesions. In addition, the affected fruits appear white and powdery, dark or dusty resulting in shriveling of the fruit (Wilcox 2003).The development of powdery mildew disease is favored by presence of host plant, pathogens and favorable condition. Favorable conditions for powdery mildew infection include temperature between 18-30°C. Dry conditions with high relative humidity favor the disease epidemics (Khiavi et al. 2012). The pathogenic activities of *Erysiphe necator* is favored by relative humidity above 50%. Additionally, it has been reported in Iran that sporulation is favored by the temperature and relative humidity ranging between 20-25°C and 50-100% respectively (Fathi and Karbalaei 2012).The temperature that favors pathogen development differ in some countries which could be due to variation in relative humidity and amount of rainfall per year. Due to high yield loss caused by powdery mildew disease, researchers have proposed different ways of controlling this disease. The study by Halleen and Holz (2001) has reported fungicide sulfur as one of the efficient methods to control powdery mildew in grape. This study contradicts with findings of previous study by Wicks et al. (1997) who reported that sulfur is affected by the weather condition thus, affect application of the fungicides and 58% loss of sulfur after three days of application have been reported. On top of that, spray timing and coverage still lead to control failure. The pathogen, *Erysiphe necator*, has

ability to develop resistance to fungicides for a short time. Temperatures below 15°C and above 35°C have been reported to affect the performance of sulfur fungicide (Leavitt and Martin-Duval 1997; Leavitt and Martin-Duval 1998). Furthermore, other researchers have suggested using modern technological ways of controlling the disease rather than the sulfur fungicide which need to be applied several times, which is difficult for farmers in developing countries to afford the cost (Wilcox 2003). Although, Wilcox (2005) has recommended copper to be useful in controlling disease, the results by Khiavi et al. (2012) demonstrated that application of chemicals is not environmentally friendly. Therefore, new approaches of controlling disease are needed. Some studies have proposed the use of cultivar selection reduce powdery mildew infection, by planting grapevines in high and open areas so as to provide adequate space for air flowing. However, this cannot completely overcome the problem because of availability of other factors that may cause disease infection (Wilcox 2003). The study by Wilcox (2005) has suggested the use of cultural control based on reducing humidity and improving air circulation. In this regard, plants should be planted in direct exposure to the sunlight which helps to provide good air circulation and inhibit powdery mildew development. In addition, grapevine should be pruned to allow air movement and preventing excess shading. This was also suggested by Wilcox (2003). Due to difficulty of controlling the disease completely, researchers have proposed the use of resistant varieties as the good alternative (Khiavi et al. 2012). Pavlousek (2007) has reported that Augustovskii, Yalovenskii Ustoichivyi, Poloskei Muskotaly and Pleven Ustoichyvii varieties of grapevine to be resistant against powdery mildew and proposed to be used in national and international breeding programs. Wan et al. (2007) recommended powdery mildew resistant grapevine varieties are encouraged to be adopted by breeders to be used in developing new resistant varieties. The resistance of grapevine varieties should be broad to more than one disease so as to reduce cost of control using chemicals (Calonnec et al. 2013). Seedlings can be screened for disease resistance. However, the challenge of this process is usually time-consuming, and does not correlate with reactions of mature vines. Evaluation of young seedlings

for powdery mildew resistance under greenhouse conditions is not clearly correlated with field ratings obtained from adult plants (Aldwinckle et al. 1975; Pool et al., 1981). Molecular markers linked to resistance genes can be employed as an alternative selection technique. For instance, RAPD and AFLP markers have been reported to be useful in identification of disease resistance of powdery mildew on grapevine (Dalbo et al. 2001). Selection of seedlings based on the presence of markers, or marker-assisted selection (MAS), is fast and not affected by environmental factors. Markers linked closely to genes of interest may be obtained by bulked segregant analyses (Michelmore et al. 1991). In order to address the problem of diseases in crop, genes of each variety need to be identified in response to disease susceptibility. The genes of varieties which respond positively for disease resistance are preferred to be selected as the basis for breeding programs. In order to identify these genes, the use of molecular markers for grapevine varieties identification has been shown to supplement ampelography (Dhanorkar et al. 2005). For instance, simple sequence repeats (SSR) markers have been used for compilation and exchange of information of grapevine genetic resources due to their polymorphism, reproducibility and co-dominant (Fernandez et al. 2007). They have been extensively used in grape crop for varieties identification in collections, pedigree analysis, or genetic mapping (Laucou et al. 2011; Dokupilova et al. 2013) and are very useful for distinguishing grape genotypes and determining genetic relationships among *Vitis* cultivars. Based on genetic relationships of grapevine accessions assessed by microsatellite markers, Guo et al. (2010) has reported that, the genetic relationship among accessions of regional interest and some genetic diversity of grape is still unexploited.

3.0 Genetic diversity of grapevine varieties

Genetic diversity refers to the number of genetic traits in the genetic makeup of a species. Most of cultivated grape varieties are introduced by the major producing countries and thus variations

are expected among these varieties (Jogaiah et al. 2013).The variability is based on shape and color, berries size, and quality traits based on berry composition, content of sugars, acidity and organic acids as well as disease resistance and stress tolerance (Coombe 1992; Choudhary et al. 2014). Regarding the ampelography (Galet 1979) and other morphometric methods (Rubio and Yuste 2004; Theocharis et al. 2010) to be successfully used for differentiation of grapevine varieties, they are particularly complex for the identification and study of genetic relations among varieties. This is due to inadequate sensitivity for discrimination at the clonal level (Mullins et al. 2000; Theocharis et al. 2010). It has been reported that some genetically related cultivars are morphologically very similar and difficult to distinguish visually (Aradhya et al. 2003). Therefore, the molecular markers and DNA barcodes have been proposed as solutions for appropriate cultivars identification (Akhare et al. 2008). Microsatellite markers (Sefc et al. 2000; Aradhya et al. 2003; This et al. 2004) are favored among Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP) or Random Amplified Polymorphic DNA (RAPD) markers because of their combination of polymorphism and reproducibility as well as co-dominant nature of SSR marker (Sefc et al. 2001). On the other hand, identification of polymorphism among plants, determination of relationships at species and cultivar level is done by multilocus molecular typing approaches such as RAPD (Qu et al. 1996), Inter simple sequence repeat (ISSR) (Dhanorkar et al. 2005) and A FLP (Theocharis et al. 2010). The markers which are most plentiful with high level of polymorphism, reproducibility (Dhanorkar et al. 2005) and low cost are the best marker for genetic diversity studies (Herrera et al. 2002).

3.1 Types of molecular markers used for grapevines genetic diversity analyses

Molecular marker methods used for grapevines genetic diversity analyses including RFLP (Bowers and Meredith 1996), AFLP (Sensi et al. 1996), RAPD (Sefc et al. 2001), SSRs, ISSR as well as Single Nucleotide Polymorphism (SNP) have been reported to be useful in cultivars

identification because they are not affected by the environmental factors and their interpretation is more realistic (Almadanim et al. 2007). However, some markers are more powerful than others. Grapevine varieties need high efficiency markers for varieties identification. For instance, according to Jing et al. (2013) construction of primers for ISSR and SSR require high temperature compared to RFLP, AFLP and RAPD markers thus, making microsatellites useful for identification of genetic diversity in grapevine varieties (Sabir et al. 2008).

3.1.1 Restriction Fragment Length Polymorphism (RFLP)

RFLP is a marker which is highly suited to inter-laboratory experiments (Jones et al. 1997). RFLP analysis was successfully used to detect cultivar fingerprints to differentiate varieties of grapevine and rootstock. RFLP marker offers the advantages of robustness in various environments and higher efficiency of noticing polymorphism, identification of varieties by distribution of different enzymes (Jones et al. 1997). However, complex banding patterns may result in difficulties in the assessment of results and high quality DNA may be required, more time is consumed and the cost of prior formulating of probes and procedure analysis is high.

3.1.2 Amplified Fragment Length Polymorphism (AFLP)

Amplified fragment length polymorphism (AFLP) is a useful DNA marker and PCR-based technique which is produced by selective amplification of restricted DNA fragments (Upadhyay et al. 2007, Lamia et al. 2010). In this marker, adapters ligate to the ends of the restricted fragments and either a pre-selection step performed using magnetic beads followed by a round of selective PCR, or two selective rounds of PCR amplification are applied. The amplified products are separated on a sequencing gel by electrophoresis and after that visualized by radioactive or fluorescent labeling AFLP (Vos et al. 1995). The character of this kind of marker is the highest number of bands, between 50-100 that can analyze several samples at once (Zulini et al. 2005).This helps for identifying large portion of genome thus, helps to identify spot mutation which may distinguish clonal variations in a given grape cultivar (Zulini et al. 2005). AFLP

marker has been reported to identify and compare grapevine varieties of the unknown ancestors with those having known ancestors suggesting the presence of genetic similarities and distances of those intraspecific hybrids in comparison with original varieties (Theocharis et al. 2010). AFLP is useful when identifying closely related cultivars (Argade et al. 2009). However, the use of AFLP marker has limited level of polymorphism in some cultivated species and requires both high quality and medium quality DNA where heterozygotes are scored as homozygotes which may not be appropriate markers for grape varieties identification.

3.1.3 Random Amplified Polymorphic DNA (RAPD)

The RAPD marker is a type of molecular marker which uses single arbitrary primer in a PCR reaction and result into amplification of several discrete DNA products. These products are derived from a genome region which contains two short segments in inverted orientation that are templates for design of primer sequences and sufficiently close together for the amplification. The use of short primers is important to enhance high possibility that, although the sequences are random, they are able to find homologous sequences suitable for annealing (Mondin et al. 2009). Advantages of RAPD marker is the simplicity of the technique as this marker requires no primary knowledge of the genome analysis (Weising et al. 2005). The study by Stavrakaki and Biniari (2009) has shown that there is genetic variability among studied Greek grapevine varieties thus recommending RAPD-PCR as a reliable and useful method for the identification and genetic analysis of grape cultivars as similar study reported by Herrera et al. (2002). RAPDs are useful tools for the molecular characterization of grapevine cultivars. However, RAPD markers have low reproducible, need high quality DNA as well as time consuming during analysis (Sefc et al., 2000).

3.1.4 Simple sequence repeats (SSRs)

SSRs sometimes called microsatellites have become widely used genetic markers for the characterization of grapevingermplasm. SSRs have been proved valuable markers for genetic analysis of grapevines (Sefc et al. 2001). Microsatellites are short (1-4 bp) and tandem repeated

DNA sequences. The merits of microsatellite markers include their co-dominant inheritance, high polymorphisms and high reproducibility. This marker system requires prior information of primer binding, which raise cost for markers improvement (Scott et al. 2000). Due to this drawback, for species that lack prior sequence information, ISSR markers are suggested to be the appropriate markers since no prior information of primers is needed and the price is low. However, as prior sequence information for grapevine is available, SSR markers are useful for identifying grapevine varieties, reconstruction of pedigree and genes relatedness, as well as population genetic studies, genome mapping and enhancing marker assisted selection (Sefc et al. 2001). According to Guo et al. (2014), eighty-six alleles were detected in 9 simple sequence repeat (SSR) loci with an average of 9.6 alleles per locus as in Table 1. Genetic similarity ranged from 0.38 to 0.83 with an average value of 0.58. These results show high genetic diversity among accessions analyzed. Based on cluster analysis and principal coordinate analysis, the results indicated different origin of accessions according to eco-geographical grouping. Wine grapes are distantly related to table grapes either from China or other country. The study showed some genetic diversity of grapes is still unexploited. Therefore, further research need to be done (Guo et al. 2014). Dallakyan et al. (2013) has also reported usefulness of SSR marker sets for grape rootstocks identification and might be further optimized and studied in more details. There is high genetic diversity among accessions analyzed based on cluster analysis and principal coordinate analysis, where the results indicated that all accessions could be different in origin according to eco-geographical grouping .The distant relationship with table grapes (Guo et al. 2014) could be due to different geographical location, soil conditions and genetic makeup of the varieties which change with change in time. Previously, Zoghlami et al. (2009) developed ten SSR loci of grapevine varieties for characterization and assessment of genetic diversity, cultivar closeness, and parentage in Tunisia. The results showed high heterozygosity of 0.9 at 4 loci (VVMD28, VVMD5, VVIP31 and VVS2) compared to the expected heterozygosity which ranged between 0.621 and 0.855, this could be due to high efficiency of SSR markers to have

high ability of identifying grapevine cultivars. Similar study by Zoghlami et al. (2009) has shown high genetic variability of grapevine cultivars using SSRs markers for detection of polymorphism. This finding has indicated SSR marker as one of the most appropriate markers to determine parentage of grapevine in Tunisia (Zoghlami et al. 2009). Further studies have shown ISSR markers to be the best for identifying closeness and distant among grapevine varieties in comparison with SSR markers. In the previous study, Jing et al. (2013) has reported 97.02% of the genetic variability in 80 *Vitis* cultivars indicating that *Vitis* species, *V. ficifolia* and *V. yeshanensis* as different species (Jing et al. 2013). The genetic diversity of wild *Vitis species* in China provides the basic foundation for grape breeding. Similar study was reported by Venkat et al. (2014) where a total of 42 genotypes of grapevine analyses by seven SSR markers with twelve SSR primers characterized in other studies (Bowers et al. 1996 and 1999b). There was genetic variability detected within Indian grapevine germplasm among varieties studied indicating high reliability, efficient, and effectiveness of markers recommended to be used for diversity analysis and subsequently in crop improvement programs. However, these data indicated the presence of a lower genetic variability in the Indian grapevine germplasm, comparable to the variability found in the Algeria and Mediterranean basin and similar to Spanish grape germplasm (Martin et al. 2003).

3.1.5 Inter Simple Sequence Repeat (ISSR) marker

ISSR marker has been reported to be easy, rapid and consistent (Moreno et al. 1998). It has been proved to be simple since no need of prior sequence information. It does not only play an important function of analyses of intra-varietal variations in grapevines but also identifies cultivars (Hanorkar et al. 2005). In grapes, ISSR markers are used for analysis of various number of plant species (Kandasamy et al. 2013). It is useful for varietal fingerprinting or genetic diversity analysis, characterization of genetic relatedness among populations, detection of clonal variation, cultivar identification, phylogenetic analysis, detection of genomic instability, and

assessment of hybridization (Bornet and Branchard 2004). Previously, Herrera et al. (2002) used ISSR markers to compare four planted grape varieties in Chile. Argade et al. (2009) have reported ISSR analysis to be applied on grapevine varieties. According to this study, few primers of ISSR marker are useful for characterization of grape varieties. Sabir et al. (2008) has reported data analyzed from 16 table grape cultivars using 14 ISSR markers, a total of 110 bands produced by 14 ISSR primers and 88 of a total 100 bands were polymorphic. The total number of bands per primer ranged from 4 to 11 with the mean value of 7.86. Moreno et al. (1998), Herrera et al. (2002) and Wu et al. (2006) have also reported 2 to 13 bands per ISSR primer in the previous studies. The highest polymorphism rates were reported from UBC 824, UBC 840 and UBC 857 primers with 100 % polymorphism as shown in Table 3. Further, Wu et al. (2006) generated a total of 105 bands with 91 % polymorphism rate in the same study where 15 cultivars were analyzed using fifteen ISSR primers. In addition, previous researchers have reported a polymorphism ranging between 60 and 100%. Dhanorkar et al. (2005) obtained a high polymorphism rate of 96 % in a genetic assessment study which included *V. vinifera, V. labrusca* and *V. rotundifolia* cultivars with 13 selected ISSR primers for 43 cultivars. Therefore, these results recommend ISSR primers to be powerful and reliable tool for identification of grape varieties (Sabir et al. 2008). Regarding various molecular markers to be useful in grapevine varieties identification, ISSR and SSR markers have reported to have high efficiency due to high polymorphic, repetition motifs as well as reasonable cost compared to AFLP, RFLP and RAPD. ISSR markers are suggested to be the best markers in evaluation of genetic variability and polymorphism of grape varieties (Choudhary et al. 2014). In spite of ISSR markers being useful for identification of plant varieties, some of ISSR primers show less response. Therefore, screening for high polymorphism, reproducibility and resolving power is important in order to identify the best primers. Furthermore, even if the screening for appropriate primers is done, still the selected primers differ in efficiency resulting in some errors. Seyedimoradi et al. (2012) have reported wide variability in allele produced per each primer in

grapevine cultivars in the previous study. Relationships among grapevine varieties are not very clear and more studies on morphological and agronomic traits and analysis of the clones with more number of reliable DNA markers like SSR, SNP and ISSR may be helpful in confirming the results (Choudhary et al. 2014). Knowledge of the degree of genetic relationship among these varieties is important for germplasm collection, *in situ* conservation and *Vitis* breeding programs. The results of the present study will be useful in DNA fingerprinting and in determining the genetic diversity among the grapes (Choudhary et al. 2014).

Table1. Characterization of microsatellite markers used in grapevines diversity

Locus	Annealing temperature **(°C)**	No. of allele (n)	H_e	H_o	PIC
VVS2	51	8	0.82	0.76	0.79
VVMD5	52	10	0.78	0.73	0.72
VVMD7	51	12	0.86	0.81	0.82
VVMD27	52	8	0.75	0.62	0.68
VrZAG62	52	9	0.82	0.72	0.75
VrZAG79	52	10	0.83	0.79	0.76
VrZAG112	52	10	0.76	0.69	0.71
VrZAG47	52	9	0.8	0.75	0.76

According to research article published by Herrera et al. (2002) red wine for instance, Carmenere, Cabernet Franc, Merlot, Cabernet Sauvignon, and white wine such as Sauvignon Blanc, Sauvignonasse and Chardonnay were compared using primers listed in Table 2. Both red wine and white wine grape cultivars were effectively identified by 11 ISSR primers. In this study

all primers resulted in high polymorphic bands. However, there was low similarity index since only 64% fingerprint reported to be generated by Jaccard's method taking into consideration that cultivars were vegetative propagated which discounted mutation as the source of differences during data analysis. In addition, other study by reported high polymorphism in grapevine varieties based on ISSR UBC 880 and UPR4R primers as shown in Fig. 1. (Seyedimoradi et al. 2012).

Table 2. Inter Simple Sequence Repeat (ISSR) primers used for identification of red and white grape cultivars Where B: (C, G, T); D: (A, G, T but not C); R: (A, G); Y: (C, T); V: (A, C, G)

Primer	Primer Sequence (5′- - - - -3′)
811	GAGAGAGAGAGAGAGAC
818	CACACACACACACACAG
820	GTGTGTGTGTGTGTGTC
825	ACACACACACACACACT
826	ACACACACACACACACC
836	AGAGAGAGAGAGAGAGYA
840	GAGAGAGAGAGAGAGAYT
841	GAGAGAGAGAGAGAGAYC
848	CACACACACACACACARG
888	BDBCACACACACACACA
890	VHVGTGTGTGTGTGTGT

3.1.6 Single nucleotide polymorphism (SNP)

Single Nucleotide Polymorphism is a DNA sequence variation that occurs within a population whereby a single nucleotide in the genome differs in the paired chromosomes. For instance, two

sequenced DNA fragments from different individuals, AAGCCTA to AAGCTTA, contain a difference in a single nucleotide (Nachman and Michael, 2001). The SNP can be obtained by advanced sequencing so as to make data available for molecular marker development (Weising *et al*. 2005). Each locus of SNP contains two, three or four alleles whereas SNPs are bi-allelic markers, representing two alleles which can differ in a given nucleotide position in a diploid genome. They are named according to their location of the genome, effect on coding as well as regulatory sequences (Weising *et al*. 2005). The SNPs can be recognized by an experiment whereby the target genes or genome regions are screened for SNPs. The techniques used for screening SNPs include: microchip hybridization, direct sequencing or electrophoresis of PCR fragments containing gene sequences on DNA single strand conformation polymorphism (SSCP) or denaturing gradient (DGGE) gels (Ergul *et al*. 2002). The technique of SNP genotyping categorized into: direct sequencing, Cleaved Amplified Polymorphic Sequences (CAPS), allele-specific PCR, allele specific primer extension, allele specific oligonucleotide hybridization (Weising *et al*. 2005). The identification and discovery of SNP for the development of molecular marker systems of whole genome sequences of grapevines have been recently increased dramatically (Pinto *et al*. 2008). Among the analyzed genotypes, 247 SNPs which present useful markers for genetic analysis have been discovered in grapevine. Further, regarding high relationship of the grapevine accessions, 0.34 of the heterozygosis has been documented (Emanuelli et al. 2013)a .The study for genetic diversity of grapevines is still important for appropriate identification of grapevine cultivars (Emanuelli et al. 2013). According to the study by Maul et al. (2012) the total set of 384 SNPs have reported to be imperative by previous SNP-discovery and validation project. The similar study by reference has reported 48 SNPs to be useful for identification of grapevine varieties. In addition, one SNP per every 64bp has been reported for grapevine which is higher than the one reported by Velasco et al. (2007) having an average of one SNP per 100bp for sequenced genome of clone pinot Noir. This reports proved high polymorphism in grapevine which is explained by low mutation rate with double mutation

on single locus (Nielsen, 2000; Riahia et al. 2012). All of the detected SNPs in grapevines are bi-allelic which is similar with previous report by Vashney et al. (2007).

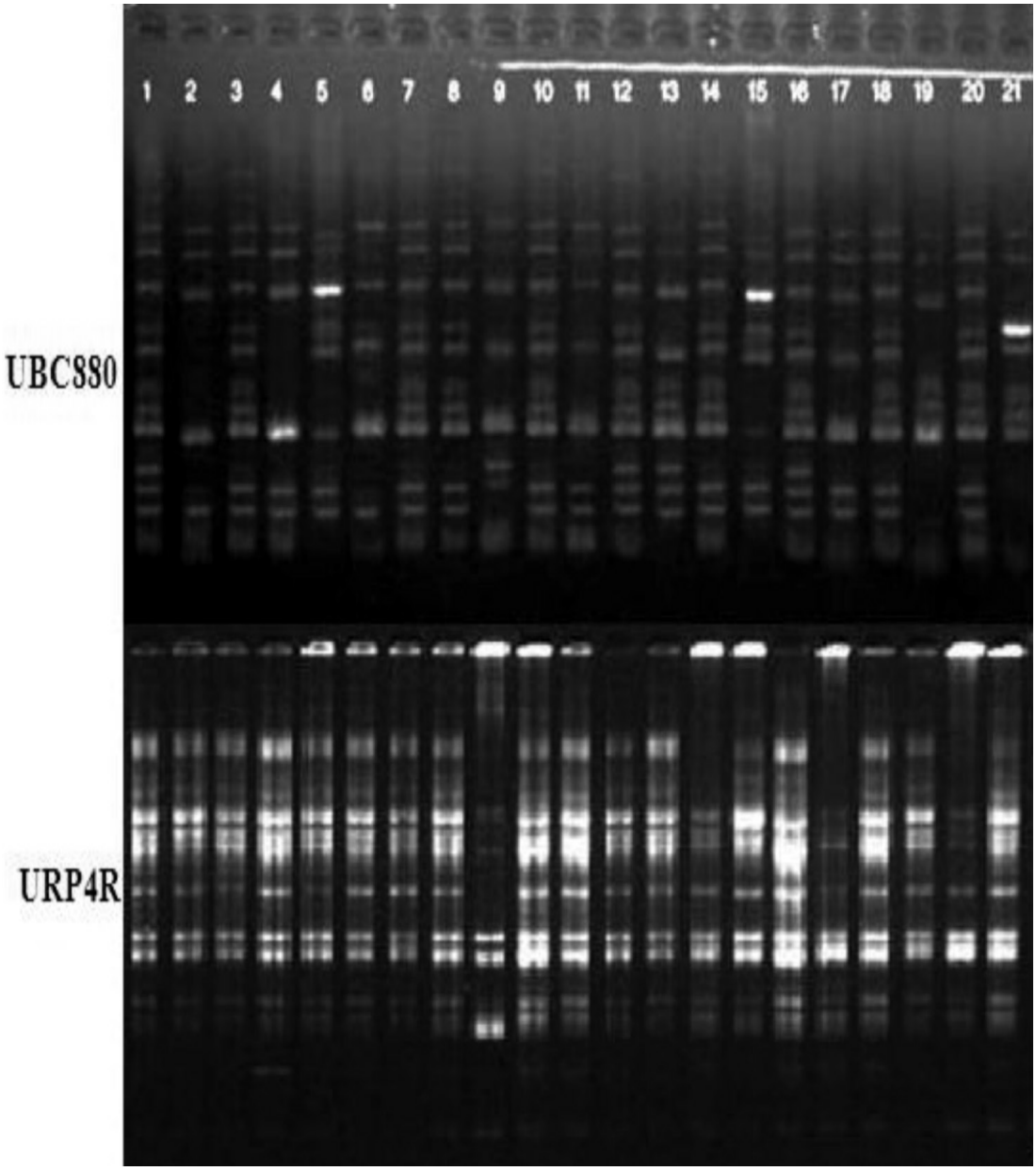

Fig. 1. Amplification profile resulted from UBC880 and URP4R primers as detected in grapevine accessions.

Table 3. Number of Total Bands (NTB), Number of Polymorphic Bands (NPB), Polymorphism Rate (PR), Polymorphism Information Content (PIC) and Resolving Power (RP) of ISSR Primers

No	Primer	NTB	NPB	PR	PIC	RP
1	UBC 808	11	9	81.8	0.675	0.861
2	UBC 809	8	7	87.5	0.686	0.979
3	UBC 810	9	8	88.9	0.549	1.172
4	UBC 815	6	3	50	0.837	0.792
5	UBC 823	8	7	87.5	0.688	0.946
6	UBC 824	5	5	100	0.723	0.925
7	UBC 826	6	5	83.3	0.709	1.063
8	UBC 830	4	3	75	0.664	1.083
9	UBC 840	8	8	100	0.68	0.968
10	UBC 846	10	7	70	0.596	1.143
11	UBC 848	11	9	81.8	0.635	1.125
12	UBC 857	7	7	100	0.749	0.833
13	UBC 887	11	6	54.5	0.536	1.125
14	UBC 888	6	4	66.7	0.486	1.208
Total		110	88	–	–	14.223
Mean		7.86	6.29	80.5	0.658	–

4.0 Genetic relationships in grapevine varieties

Genetic variability can be normal or induced and is potential for crop breeding (This et al. 2006a).The study of genetic relationships and correct identification of varieties is imperative for plant breeding as well as germplasm conservation (Thomas et al. 1993). Molecular markers have been proposed to be used for analysis of genetic relationships (Tamhankar et al. 2001). Among over 900 grape cultivars, roughly 75% of them showed relationships to other cultivars (Myles et al. 2011). Other studies showed that there is far similarity of the cultivars which have difference in gene origin. Some of the cultivars used in this study showed distant relationship (Sabir et al. 2008). In order to clearly identify the genetic diversity among cultivars, it is recommended to use high number of primers which enhance high reproducibility, polymorphic and informative for proper information (Sabir et al. 2008).

5.0 Conclusion

In order to overcome powdery mildew disease susceptibility in grapevines, the interaction of plant pathology and plant molecular breeding technology is imperative. For instance, the use of molecular markers may be helpful in identification of grapevine varieties and the gene sequence which are resistant to powdery mildew disease. This may be done by setting field trials planted with grapevine varieties and should be inoculated with *Erysiphe necator* to screen for resistance varieties. Ultimately, those varieties with positive results should be recommended for further studies at molecular level to identify useful gene sequence for cultivar improvement. Furthermore, more research in needed to inovate new markers which can easily identify *Erysiphe necator* in grapes so as to reduce powdery mildew infection hence, improving crop production.

Acknowledgements

The Author thanks the Co-authors for their contribution of technical and high efficient ideas of designing this review. He acknowledges Nelson Mandela African Institution of Science and Technology for financial support. He also grateful to Mlalila N. Mnyambwa N. and Reymond J. for their constructive comments during the writing of this review.

References

Akhare AA, Sakhare SB, Kulwal PL, Dhumale DB and Kharkar A. RAPD profile studies in Sorghum for identification of hybrids and their parents. *Int J Integrative Biol*, 2008, 3(1): 18-24.

Aldwinckle HS, Watson JP, and Gustafson HL. Relationship between greenhouse and field resistance of grape seedlings to powdery mildew. *Plant Dis Rptr*, 1975, 59:185–188.

Almadanim MC, Baleiras-Couto MM, Pereira HS, Carneiro L and Fevereiro C P. Genetic diversity of the grapevine (*Vitis vinifera* L.) cultivars most utilized for wine production in Portugal. *Vitis*, 2007, 46 (3): 116–119.

Aradhya MK, Dangl GS, Prins BH, Boursiquot JM, Walker MA, Meredith CP and Simon CJ. Genetic structure and differentiation in cultivated grape, *Vitis vinifera* L. *Genetical Research*, 2003, 81: 179–192.

Argade NC, Tamhankar SA, Karibasappa GS, Patil SG and Rao VS. DNA Profiling and assessment of genetic relationships among important seedless grape (*Vitis vinifera*) varieties in India using ISSR Markers. *J Plant Biochemistry & Biotechnology*, 2009, Vol. 18(1): 45-51.

Bowers JE, Dangl GS, Vignani R and Meredith CP. Isolation and characterization of new polymorphic simple sequence repeat loci in grape (*Vitis vinifera* L.). *Genome*, 1996, 39: 628–633.

Calonnec A, Wiedemann-Merdinoglu S, Delie`re L, Cartolaro P, Schneider C and Delmotte F. The reliability of leaf bioassays for predicting disease resistance on fruit: a case study on grapevine resistance to downy and powdery mildew. *Plant Pathology*, 2013, 62: 533–544.

Coombe BG. Research on development and ripening of the grape berry. *Am J Enol Vitis*, 1992, 43(1): 101-110

Choudhary RS, ZagadeVS, Maboodurrahman GD, Khalakar GD and Singh NK. ISSR based genotypic differentiation of grape (*Vitis vinifera* L.). *The Bioscan*, 2014, 9(2): 823-828.

Dalbo MA, Ye GN, Weeden NF, Wilcox WF and Reisch BI. Marker-assisted Selection for powdery mildew resistance in grapes. *J Amer Soc Hort Scl*, 2001, 126(1): 83–89

Dallakyan MV, Yesoyan SS, Harutyunyan FA, Melyan GH,Yesayan AH and Hovhannisyan NA. Application of microsatellite markers for varietal identification grape rootstocks. Preliminary analysis. *Biolog Journal of Armenia*, 2013, 3 (65).

Dhanorkar VM, Tamhankar SA, Patil SG and Rao VS. ISSR-PCR for assessment of genetic relationships among grape varieties cultivated in India. *Vitis*, 2005, 44 (3): 127–131.

Dokupilova I, Sturdik E and Mihalik D. Characterization of vine varieties by SSR markers. *Acta Chimica Slovaca*, 2013, Vol. 6, No. 2, pp. 227—234.

Emmanuaelli F, Lorenzi S, Grzeskowiak L, CatalanoV, Stefanini M, Troggio M, Myles S, Zapater JM, Eva ZF, Moreira FM and Grando MS. Genetic diversity and population structure assessed by SSR and SNP markers in a large germplasm collection of grape. *BMC Plant Biology*, 2013, 13:39.

Fang Z. Methods in Plant Pathology. *Agricultural Press, Beijing PR, China*, 1979, p. 345.

Fathi H and Karbalaei KH. Study of biology and epidemiology of *Erysiphe necator* caused powdery mildew disease. *Technical Journal of Engineering and Applied Sciencesi*, 2012, 2 (3): 56-61.

Fernandez GM, Mena A, Izquierdo P and Martinez J. Genetic characterization of grapevine (*Vitis vinifera* L.) cultivars from Castilla La Mancha (Spain) using microsatellite markers. *Vitis*, 2007, 46 (3): 126–130.

Galet P. A Practical Ampelography: Grapevine Identification. *Cornell University Press, Ithaca*, NY, 1979, p.272.

Guo DL, Zhang JY, Liu CH, Zhang GH and Li M. Genetic Relationships of Chinese Grape Accessions to European and American Cultivars Assessed by Microsatellite Markers. *Biotechnology & Biotechnological Equipment*, 2010, 24(4): 2054-2059.

Halleen F and Holz G. An Overview of the Biology, Epidemiology and Control of *Eryphise necator* (Powdery Mildew) on Grapevine, with Reference to South Africa. *S Afr J Enol Vitic*, 2001, Vol. 22, No.2

Hanorkar VMD, Amhankar SAT, Atil SGP and Ao VSR. ISSR-PCR for assessment of genetic relationships among grape varieties cultivated in India. *Vitis*, 2005 44 (3): 127–131.

Inglis DA, Hagedorn DJ and Rand RE. Use of dry inoculum to evaluate beans for resistance to anthracnose and angular leaf spot. *Plant disease*, 1988, 72:771-774.

Jing ZB, Wang XP and Cheng JM. Analysis of genetic diversity among Chinese wild *Vitis* species revealed with SSR and SRAP markers. *Genetic and Molecular Research*, 2013, 12 (2): 1962-1973.

Jogaiah S, Oulkar DP, Vijapure AN, Maske SR, Sharma AK and Somkuwar RG. Influence of canopy management practices on fruit composition of wine grape cultivars grown in semiarid tropical region of India. *Afr J Agric Res*, 2013, 8(26): 3462-3472.

Jones N, Ougham H and Thomas H. Markers and mapping.We are all geneticists now New *Phytol*, 1997, 137: 165-177

Kandasamy T, Kumari K, Kaprakkaden A, Lohot VD and Ghosh J. Molecular diversity analysis of flower colour variants of butea monosperma (lam.) Taub using Inter Simple Sequence Repeats. *The Bioscan*, 2013, 8(3): 969-974.

Lamia K, Hedia B, Jean-Marc A and NeilaT. Comparative analysis of genetic diversity in Tunisian apricot germplasm using AFLP and SSR markers. *Scientia Horticulturae*, 2010, 127: 54–63.

Laucou V, LacombeT, Dechesne F, Siret R, Bruno JP and Dessup M. High through-put analysis of grape genetic diversity as a tool for germplasm collection management. *Theoretical and Applied Genetics*,2011, 122: 1233–1245.

Leavitt GM and Martin-Duvall T. Rain and its effect on sulfur residues of *Vitis vinifera* variety Thompson Seedless in California. Third Int. *Workshop on grapevine downy and powdery mildew, loxton, Australia. SARDI*, 1998, Research report series no. 22: 54.

Lorenzis G, Imazio S, Brancadoro L, Failla O and Scienza A. Evidence for a Sympatric Origin of Ribolla gialla, Gouais Blanc and Schiava cultivars (*V. vinifera* L.*). South Africa Journal Enol. Vitic*, 2014, Vol 35, No. 1

Martin JP, Borrego J, Cabello F and Ortiz JM. Characterization of the Spanish diversity grapevine cultivars using sequence tagged microsatellite site markers. *Genome*, 2003, 46: 10-18.

Maul E, Sudharma KN, Kecke S, Marx G, Müller C, Audeguin L, Boselli M, Boursiquot JM, Bucchetti B, Cabello F, Carraro F, Crespan M, De Andrés MT, Dias JE, Ekhvaia J, Gaforio L, Gardiman M, Grando MS, Gyropoulos D, Jandurova O, Kiss E, Kontic J, Kozma P, Lacombe T, Laucou V, Legrand D, Maghradze D, Marinoni D, Maletic E, Moreira F, et al.

The European Vitis Database (http://www.eu-vitis.de): a technical innovation through an online uploading and interactive modification system. *Vitis*, 2012, 2:79–85.

Michelmore RW, Paran I and Kesseli RV. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Natl. Acad. Sci. USA*, 1991, 88:9828–9832.

Moreno S, Martín JP and Ortiz JM. Inter-simple sequence repeats PCR for characterization of closely related grapevine germplasm. *Euphytica*, 1998, 101:117-125.

Mullins GM, Bouquet A and Williams EL. Biology of the grapevine. 5th edn, *Cambridge University Press, UK*, 2000, p 252.

Myles S, Boyko AR, Owens CL, Brown PJ. Grassi F, Aradhya MK, and Prins B. Genetic structure and domestication history of the grape. *Proceedings of the National Academy of Sciences, USA*, 2011, 108: 3530– 3535.

Nielsen R. Estimation of population parameters and recombination rates from single nucleotide polymorphisms. *Genetics*, 2000, 154: 931–942.

Pavlousek P. Evaluation of resistance to powdery mildew in grapevine genetic. *Central European Agriculture Journal*, 2007,Volume 8 No. 1:105-114.

Pinto-Carnide O, Michelmore JP, Leal F, Castro I, Guedes-Pinto H and Ortiz JM. Characterization of grapevine (*Vitis vinifera* L.) cultivars from northern Portugal using RAPD and microsatellite markers. *Vitis*, 2003, 42 (1): 23–25

Pool RM, Creasy LL and Frackelton AS. Resveratrol and the viniferins, their application to screening for disease resistance in grape breeding programs. *Vitis*, 1981, 20:136–145.

Riahia L, Zoghlami N, Dereeper A, Laucou V, Mliki A and This P. Single nucleotide polymorphism and haplotype diversity of the gene NAC4 in grapevine. *Industrial Crops and Products*, 2012, 43: 718– 724.

Rubio JA and Yuste J. Ampelographic differentiation of Tempranillo clones from different area of origin, according to their synonyms. In: Sequeira JC, de Sequeira OA (eds) Proceedings of the International Symposium on Grapevine Growing, *Commerce and Research, Lisbon*, 2004, pp 73–80.

Rumbolz J and Gubler WD. Susceptibility of grapevine buds to infection by powdery mildew *Erysiphe necator*. *Plant pathology*, 2005, 54:535-548.

Sabır A, Kafkas S, Tangolar S and Buyukalaca S. Genetic Relationship of Grape Cultivars by ISSR (Inter-Simple Sequence Repeats) Markers. *Europ.J.Hort.Sci*, 2008, 73 (2): 84–88.

Scott KD, Eggler P, Seaton G, Rossetto M, Ablett EM, Lee LS and Henry RJ. Analysis of SSRs derived from grape ESTs. *Theor Appl Genet*, 2000, 100:723–726.

Sefc KM, Lefort F,Grando MS, Scott KD, Steinkellner H and Thomas MR. Microsatellite markers for grapevine: a state of the art Molecular Biology and Biotechnology of Grapevine, Edited by KA Roubelakis-Angelakis Kluwer Academic Publishers, *The Netherlands*, (2001).

Sensi E, Vignani R, Rohde W and Biricolti S. Characterization of genetic biodiversity with *Vitis vinifera* L. Sangiovese and Colorino genotypes by AFLP and ISTR DNA marker technology. *Vitis*, 1997, 35:183–188

Seyedimoradi H, Talebi R, Hassan D and Karami F. Comparative genetic diversity analysis in Iranian local grapevine cultivars using ISSR and directly amplified minisatellite DNA (DAMD) molecular markers. *Environmental and experimental biology*, 2012, 10: 125-132.

Stavrakaki M and Biniari K. Genetic Study of Grapevine Varieties (*Vitis vinifera* L.) Using Molecular Markers RAPD and SSR. 32nd World Congress of vine and wine June 28th– July 3rd – July at Laboratory of Viticulture Agricultural University of Athens in Zagreb, Croatia, 2009.

Tamhankar CM, Rodriguez I, Cabezas JA, Chavez J, Martínez-Zapater JM and Cabello F. Morphological and molecular characterization of grapevine accessions Known as Albillo. *Am J Enol Vitic*, 2001, 52:2.

Tangolar SG, Soydam S, Bakir M, Karaagac E, Tangolar S and Ergul A. Genetic Analysis of Grapevine Cultivars from the Eastern Mediterranean Region of Turkey Based on SSR Markers. *Tarim Bilimreli Dergisi*, 2009, 15(1): 1-8.

This P, Jung A, Boccacci P, Borrego J, Botta R, Costantini L, Crespan M, Dangl G, Eisenheld C and Ferreira-Monteiro F. Development of a standard set of microsatellite reference alleles for identification of grape cultivars. *Theoretical and Applied Genetics*, 2004, 109: 1448–1458.

This P, Lacombe T and Thomas MR. Historical origin and genetic diversity of wine grapes. *Trends Genet*, 2006a, 22: 511-519.

Thomas M Rand Scott NS. Microsatellite repeats in grapevine reveal DNA polymorphism when analyzed as sequence-tagged sites (STSs). *Theoretical and Applied Genetics*, 1993, 86(8): 985-990.

Tuite J. Plant Pathological methods in fungi and bacteria. *Burgress Publishing, Minneapolis,* 1969, p 239.

Upadhyay A, Saboji MD, Reddy S, Deokar K and Karibasappa GS. AFLP and SSR Marker Analysis of Grapevine Rootstocks in Indian Grape Germplasm. *Sci. Hort*, 2007, 112: 176-183.

Varshney RK, Chabane K, Hendre PS, Aggarwal RK and Graner A. Comparative assessment of EST-SSR, EST-SNP and AFLP markers for evaluation of genetic diversity and conservation of genetic resources using wild, cultivated and elite barleys. *Plant Sci,* 2007, 173: 638–649.

Velasco R, Zharkikh A, Troggio M, Cartwright DA and Cestaro A et al. A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. *PLoS One*, 2007, 2: e1326.

Venkat R, Narayanaswamy P and Srinivasa Murthy BN. Genetic Diversity Analysis in Grape (*Vitis vinifera* L) Germplasm using Microsatellite Markers. *American International Journal of Research in Formal, Applied and Natural Sciences*, 2014, 6(1), pp. 12-18.

Vos P, Hogers R, Bleekar M, Reijans M, Vandele T, Hornes M, Fritjers A, Pot J, Pelemman J, Kuiper M and Zabeau M. AFLP: A new technique for DNA finger printing. *Nucleic Acid Research*, 1995, 23:4407-14.

Voytovich KA. New complex resistant table grape cultivars and methods for breeding. Kartya Moldovenyaske, Kishinev, Moldova, 1987, p 225.

Wan Y, Schwaninger H, He P, and Wang Y. Comparison of resistance to powdery mildew and down mildew in Chinese wild grapes. *Vitis*, 2007a, 46 (3): 132-136.

Wang Y. Genetic studies on resistance to powdery mildew *Uncinula necator* of wild Chinese *Vitis* species. Ph.D. Thesis, Northwestern Agriculture University Yangling, Shaanxi PR, China, 1993, p. 85.

Weising K, Nybom H, Wolff K and Kahl G. DNA fingerprinting in plants: principles, methods and applications: *CRC Press Taylor and Francis Group*, 2005.

Wilcox W. Grape Disease Control. Dept. of Plant Pathology, Cornell University, NY State Agric. Expt. Station, Geneva, NY, 2005.

Wilcox W. Grapevine Powdery Mildew. Dept. of Plant Pathology, Cornell University, NY State Agric. Expt. Station, Geneva, NY, 2003.

Wu ZL, Fang LY, Wang J and ShenYJ . Analysis genetic diversity of *Vitis* by using ISSR Markers. *J Fruit Sci*, 2006, 23: 605–608.

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

Zoghlami N, Riahi L, Laucou V, Lacombe T, Mliki A, Ghorbel A and This P. Origin and genetic diversity of Tunisian grapes as revealed by microsatellite markers. *Scientia Horticulturae*, 2009, 120: 479–486.

Zulini L, Fabro E and Peterlunger E. Characterization of the grapevine cultivar Picolit by means of morphological descriptors and molecular markers. *Vitis*, 2005, 44 (1): 35–38.