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DETECTION OF GRAPEVINE (*Vitis Vinifera*) RESISTANT VARIETIES TO POWDERY MILDEW DISEASE BASED ON SSR-PCR MARKER.

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ABSTRACT

Powdery mildew is a fungal disease caused by *Erysiphe necator* considered to be most important fungal diseases and causing loss of grapevine production. 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. This study has been conducted at Scientific Research Caenter/College of Science/University of Duhok during the period (July 2018 to August 2019). Simple Sequence Repeat (SSR) Marker has been used to detect the powdery mildew by applying ten SSR markers for twenty cultivars of *Vitis vinifera* collected from **Duhok vineyard and Directorate of Horticulture in Duhok City. Considered the useful markers as the guidelines for detecting the gene responsible for the resistance to powdery mildew, in this study all the varieties and land races that had been used to detect such a gene were some tested varieties (Chav Ga, Zaytoni, Tahlik, Tlifishak, Kishmish1, Kishmish2, Kishmish3, Payizi, Doshawi, Baytmoni, Bagerat2, Miskat Aswad, Ashqar Basrah) contain an 80, 90, 94,114 base pairs as a single band. At the same time not all varieties are powdery mildew due to absence of all alleles which make them a susceptible for powdery mildew infection.**

KEY WORD: Grape vine, Powdery mildew, SSR Marker, Vitis vinifera

INTRODUCTION

Grape (*Vitis vinifera* L.) is an important and an economic fruit among the horticultural crops production grown worldwide, because of the numerous uses of its fruit benefit, producing wine, juice table grapes dried fruit and organic compounds and it is one of the oldest plants known to man grows virtually in most country(Aigrain, 1999; Alsaidi, 2000). The grapevines are widely planted in Duhok, Erbil and Sulaymaniyah governorates, where it grows due to suitable climatic condition and soils (Abdul-Qader & Alsaidi, 2006; Al-Atrushi, 2009).

Powdery mildew is a common fungal disease of many monocotyledonous and dicotyledonous plant species and consider as the most widespread and destructive disease of grapevines (Gadoury *et al.*,., 2003). In a moderately temperate and humid climate, these ascomycete fungi cause severe yield losses in a wide range of crops (Panstruga *et al*., 2002). *Erysiph necator* the most known causative agent that causes this disease (Ficke *et al.*,, 2002). This came from studies of the disease on cultivars of the European grape, *Vitis vinifera*, which bears leaves and fruit that are highly susceptible to this disease .(Gadoury *et al*., , 2001; Scott *et al*.,, 2010).

A microsatellites are the favorite type of DNA markers, due to their properties enabling a wide range of applications, from cultivar identification and discrimination, to phylogenetics, parentage testing and pedigree reconstruction, for the

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management of germplasm collections(Laucou *et al*., 2011; Sefc *et al*., 2000). Simple sequence repeat (SSR) markers are based on repeated sequences of one- to six-base core sequences (typically two to four), found interspersed in the genome, and it is particularly useful because of their; abundance in genomes, high degree of variability in the repeat sequence, and high reproducibility, so the use of microsatellite for grapevine genetics includes; the identification of cultivars , the relatedness of cultivars and the analysis of the parentage of crosses (Sefc, *et al.*, 1997; Fundyga *et al*, 2002; This *et al*., 2004; Goto-Yamamoto, *et al*.,2006). The objective of the present study is to Screen grape yards varieties and land races characters for the resistance to Powdery Mildew and using SSR - PCR markers as molecular technique for identification of Powdery Mildew resistance genes.

MATERIALS AND METHODS

Samples collection:

The leaves of twenty local cultivars of the genus *Vitis* (gawre xate, chav ga, zaytoni, tahlik, tli fishak, kishmish1, kishmish2, kishmish3, payizi, doshawi, bagerat2, miskat aswad, ashqar basrah, baytamoni, haft bar, Shahi Hindi, Dirawishi, Baladi, Kirkirana, Salami) were collected during (July-September 2018) from Duhok vineyard and Directorate of Horticulture in Duhok.

DNA extraction:

Genomic DNA was isolated from leaf samples according to CTAB (cethyltrimethyl ammonium bromide) extraction method described by (Weigand *et al*., 1993). Genomic DNA of all samples were run on 1% Agarose gel electrophoresis. The concentration and purity of DNA was determined using Nano drop spectrophotometric.

Simple Sequence Repeats (SSR)

In order to genotype the grapevine cultivars, ten primer pairs for microsatellite

(SSR) loci were used with their sequences and annealing temperature, were initially selected to perform this research as shown in (Table 1) (Veikondis, 2014).

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Table(1): list of SSR primers used in this study.

Primer name	Primer sequence	Tº Annealing
VVIV16	(F) 5'- ACA AAA GCG GAA ACG ATC GAA T -3' (R) 5'- GAG AAG ACC TAT TTT TCC TGT GG -3'	49 ℃
VVIM93	(F) 5'- CAA CGT TTA TTG TAA GAG CCT C -3' (R) 5'- GCT TAG CTT GCT AGA AAC TTG A -3'	48 ℃
VMC1A5	(F) 5'- TCA CAC AAT TCT CCC ATG AAA TAG -3' (R) 5'- GAA CAA GTT GGC ATG TTG GTT A -3'	48 ℃
VMC3E5	(F) 5'- GAT TTG TCT TTA CAA GGC GTT C 3' (R) 5'- GCC AGG AGA CTT GCT TTG TAT TT -3'	49 ℃
UDV047	(F) 5'- TGT ATG ATA ATC CAT AAT TGT C -3' (R) 5'- GTA GGC ATG CTT GAC TTA TTC -3'	45 ℃
VMC8F4.2	(F) 5'- GCG TAA AGC ATA TTC AAG CAT T -3' (R) 5'- GAA GTT AGC GCA GAT GAA AGA T -3'	48 ℃
VVIN16-CJVH	(F) 5'- CCC GCC CTT CCT ATT TGT A -3' (R) 5'- GAA GCC AAT GAA AGA AGA ATT AAC A -3'	48 ℃
VMC3E5(F-2)	(F) 5'- GAT TTG TCT TTA CAA GGC GTT C -3' (R) 5'- GCC AGG AGA CTT GCT TTG TAT TT -3'	49 ℃
VMC8B5	(F) 5'- AAA GGA GAC ATC TGC ATC AT -3' (R) 5'- GCC TTG ATC TTC CTT CTA AT -3'	45 ℃
VVC62	(F) 5'- TGG GAT TAA CAC GGA CTT CTT -3' (R) 5'- GTG GCT AAG CTA GCC CTG TA -3'	50 ℃

Polymerase chain reaction:

The PCR mix had the following composition; 12.5 µl of master mix (GoTaq, Promega) -; 2µl of each forward and reverse primers (6 pmol/ µl); 3 µl of DNA sample (25-50 ng/ µl) the volume was completed to 25µl by adding Deionized distill water to a final volume, After the selection of the optimal temperature for each primer set (as clarify in Table 1), the PCR reactions were performed on a thermo cycler (Applied Bio systems) (Table2) (Riaz *et al.*,, 2011).

Table (2): The thermo cycler program

94 ℃ → 5 minutes		
94 ℃ → 1 minute *Tº annealing (50-60 as shown in table 1)→ 30 seconds 72 ℃ → 30 seconds		x 35 cycles
72 ℃ → 12 minutes		

*Tº Annealing temperature of primer

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The amplification product was analyzed in a 2% agarose gel, and then stained in Ethidium Bromide.

Results and discussion:

Genomic DNA isolation:

The procedure used for genomic DNA extraction in this study followed (Weigand *et al*., 1993) and was found to produce efficient yields of DNA sample for PCR amplification. The amount and concentration of isolated DNA from studied cultivars ranged from 66 - 455 ng /µl with a purity of 1.4-1.8. The molecular weight of genomic DNA sample was estimated using 1% agarose gel electrophoresis (Figure 1).

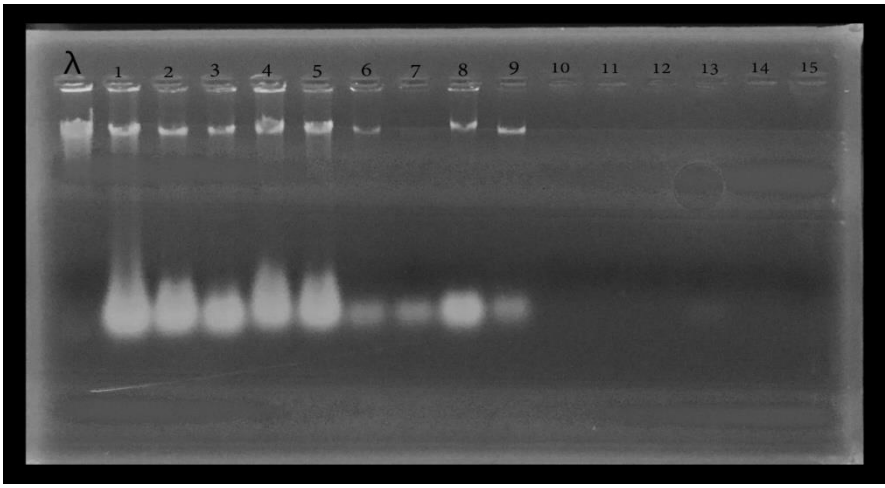

Fig.(1): Extracted genomic DNA of *Vitis* cultivars run on 1% Agarose gel electrophoresis at 3-4 v/cm.

SSR marker Analysis

Amplified microsatellite loci were scored in 20 *Vitis* varieties and in order to obtain reproducible results, several modifications were made to optimize PCR reaction conditions; these included annealing temperatures to increase the efficiency and eliminate some of unspecific products. PCR reactions were then prepared and successful results were obtained with the ten primers (VMC8B5, VVIM93, VVIV16, VMC1A5, UDV047, VMC3E5, VMC8F4.2, VVIN16-CJVH, VMC3E5 (F-2) and VVC62).

In the (Figure 2): illustrates that fifteen *Vitis* varieties amplified at VMC8B5 microsatellite locus shows the result of base pair size 60 to 110, by which the homozygous status of a certain SSR locus can be established.

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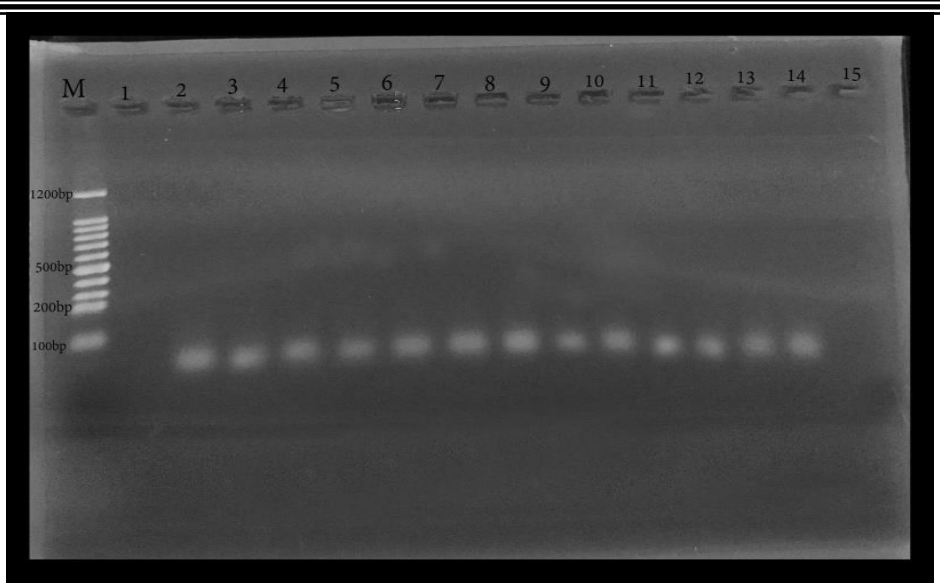

Fig. (2): Amplified PCR products obtained with microsatellite primer (VMC8B5) in *vitis vinifera* (grape) varieties, run on 2 % Agarose gel electrophoresis at 3-4 v/cm, M represents DNA Marker (100bp). 1.Gawre Xate 2.Chav Ga 3.Zaytoni 4.Tahlik 5.Tli Fishak 6.Kishmish1 7.Kishmish2 8.Kishmish3 9.Payizi 10.Doshawi 11.Bagerat2 12.Miskat Aswad 13.Ashqar Basrah 14.Baytamoni 15.Haft Bar.

In (Figure 3) clarify that fifteen *Vitis* varieties amplified at VMC1A5 microsatellite locus, shows the result of base pair size 60 to 80, by which the homozygous status of a certain SSR locus can be established.

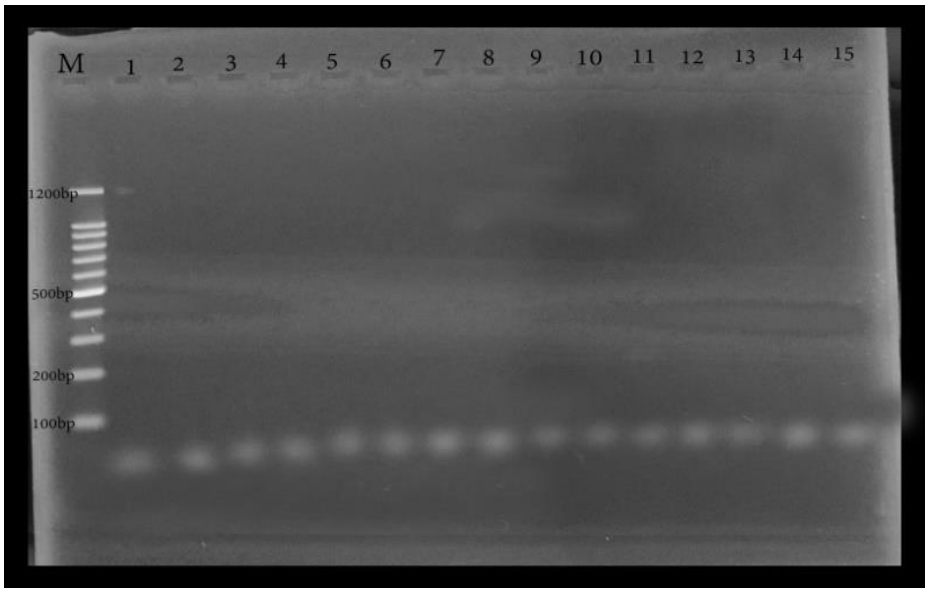

Fig. (3): Amplified PCR products obtained with microsatellite primer (VMC1A5) in *vitis vinifera* (grape) varieties, run on 2 % Agarose gel electrophoresis at 3-4 v/cm, M represents DNA Marker (100bp). 1.Gawre Xate 2.Chav Ga 3.Zaytoni 4.Tahlik 5.Tli Fishak 6.Kishmish1 7.Kishmish2 8.Kishmish3 9.Payizi 10.Doshawi 11.Bagerat2 12.Miskat Aswad 13.Ashqar Basrah 14.Baytamoni 15.Haft Bar.

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In the locus VVC62, all *Vitis* varieties used in this study do not show any bands, it lack this allele Figure (4).

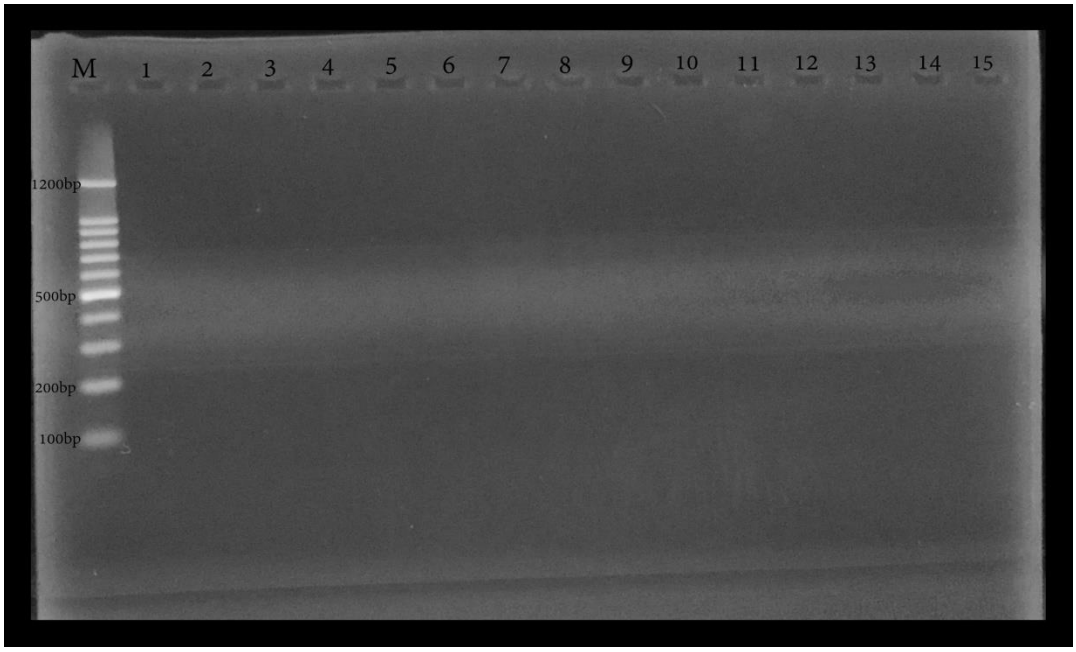

Fig. (4): Amplified PCR products obtained with microsatellite primer (VVC62) in *vitis vinifera* (grape) varieties, run on 2 % Agarose gel electrophoresis at 3-4 v/cm, M represents DNA Marker (100bp). 1.Gawre Xate 2.Chav Ga 3.Zaytoni 4.Tahlik 5.Tli Fishak 6.Kishmish1 7.Kishmish2 8.Kishmish3 9.Payizi 10.Doshawi 11.Bagerat2 12.Miskat Aswad 13.Ashqar Basrah 14.Baytamoni 15.Haft Bar.

Table (3): Size of alleles at 10 microsatellites loci, in twenty grapevine cultivars

Cultivar	**SSR Primers**									
	VVIV16	VMC3E5	VMC8B5	VVIM93	VMC1A5	VMC8 F4.2	VVIN16-CJVH	UDV047	VMC3E5(F-2)	VVC62
Gawre Xate	--------	-------	-------	105/105	60/60	-------	--------	60/60	85/85	--------
Chav Ga	100/100	110/110	85/85	100/100	60/60	-------	--------	60/60	90/90	--------
Zaytoni	100/100	110/110	85/85	110/110	60/60	-------	--------	60/60	100/100	--------
Tahlik	100/100	110/110	100/100	110/110	65/65	-------	--------	60/60	95/95	--------
Tli Fishak	100/100	110/110	100/100	110/110	65/65	-------	--------	60/60	105/105	--------
Kishmish1	100/100	110/110	110/110	110/110	65/65	-------	--------	60/60	105/105	--------
Kishmish2	100/100	110/110	110/110	105/105	70/70	-------	--------	60/60	105/105	--------
Kishmish3	100/100	100/100	110/110	65/65	75/75	-------	--------	60/60	90/90	--------
Payizi	100/100	100/100	105/105	65/65	75/75	-------	--------	60/60	90/90	--------
Doshawi	100/100	100/100	110/110	65/65	75/75	-------	--------	60/60	105/105	--------
Bagerat2	100/100	100/100	90/90	65/65	75/75	-------	--------	65/65	85/85	--------
Miskat Aswad	100/100	98/98	90/90	65/65	75/75	-------	--------	60/60	100/100	--------
Ashqar Basrah	100/100	98/98	100/100	65/65	75/75	-------	--------	60/60	90/90	--------
Baytamoni	105/105	98/98	100/100	65/65	75/75	-------	--------	60/60	100/100	--------
Haft Bar	------	----------	----------	65/65	70/70	65/65	--------	60/60	90/90	--------
Shahi Hindi	100/100	95/95	60/60	65/65	80/80	65/65	65/65	60/60	75/75	--------
Dirawishi	98/98	95/95	70/70	65/65	80/80	65/65	65/65	60/60	75/75	--------
Baladi	98/98	95/95	80/80	65/65	80/80	65/65	65/65	60/60	80/80	--------
Kirkirana	98/98	98/98	90/90	65/65	80/80	65/65	65/65	65/65	85/85	--------
Salami	------	------	--------	65/65	80/80	65/65	65/65	65/65	85/85	--------

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In this study, limited SSR primers were utilized related to Powdery mildew resistance genes in Duhok Governorate and many landraces types were choose to be investigated and powdery mildew gene detection.

According to previous findings the bellow results can be summarized:

Some tested varieties (Chav Ga, Zaytoni, Tahlik, Tlifishak, Kishmish1, Kishmish2, Kishmish3, Payizi, Doshawi, Baytmoni, Bagerat2, Miskat Aswad, Ashqar Basrah) contain an 80, 90, 94,114 base pairs as a single band. These results agree with the Veikondis (2014) who mentioned that using SSR profile in grape varieties the allele of gene resistance to powdery mildew was 81, 90, 94, 114 allele.

To gain all powdery mildew resistance genes, in should inherited the multiple binding site and produce complex SSR profile Veikondis (2014), So the obtained results revealed that the genes give the partial resistance to powdery mildew.

At the same time, not all varieties are powdery mildew due to absence of all alleles which make them a susceptible for powdery mildew infection.

The identification of an overlapping resistance region from different genetic backgrounds is of significant importance to grape breeders, as well as to molecular biologists studying host–pathogen interactions, the evolution of resistance mechanisms, and comparative analysis of key resistance genes.

The genetic base of resistance to powdery mildew and other grape vine diseases originates from *Vitis* species which are natural sources of resistance, mainly deriving from North-America and more recently from the Far East. Most of them confer varying levels of partial resistance.

The severity of powdery mildew pressure could vary from year to year due to a varying composition of strains, (Montarry *et al.*, 2008) reported a strong relationship between disease severity and the genetic composition of *E. necator* populations, so investigation of the larger number of genes and more varieties and land races in order to have wider aspect for analysis and detect the resistance genes.

This work is the first kind in Kurdistan region where evaluate the gene resistance of powdery mildew among local variety of grape in Duhok. The work provides further evidence of the strong predictive power of marker assisted to powdery mildew.

CONCLUSION

In conclusion these varieties are susceptible to the powdery mildew, and for the recommendation more varieties and more primers need to be used in order to detect this gene, which is important because it effects the crop production and grape plant as well.

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