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Detection of G143A mutation in *Erysiphe necator* and its implications for powdery mildew management in grapes

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ABSTRACT

Quinone outside inhibitor (QoI) fungicides are used worldwide for the management of *Erysiphe necator* but with associated problem of resistance development in the pathogen. Twenty nine *E. necator* isolates were collected during 2015-2016 from different geographical regions of India. In leaf disc bioassay using azoxystrobin, the EC_{50} of four isolates from research farms was <1 µg/ml, while 25 isolates from commercial vineyards had EC_{50} more than 115 µg/ml. The 256 fold resistance factor indicated G143A mutation. All the resistant isolates produced a 100 bp PCR product with G143A mutant allele specific primer which was not produced by the four sensitive isolates. A primer pair was designed for partial amplification of cytochrome b gene (*Cyt b*) and used for amplification of the gene from two resistant and two sensitive isolates. Alignment of amino acid sequences showed that the QoI resistant isolates harboured a G143A mutation, which was absent in the sensitive isolates. The two haplotypes of *Cyt b* gene from a resistant isolate, SAA2, and a sensitive isolate, HP1, have been deposited in GenBank under accession numbers KY418049 and KY418048, respectively. This is the first report of presence of QoI resistant isolates of *E. necator* from India. Studies point out the need for developing resistance management strategies by interspersing bio-control agents with judicious use of fungicides.

Keywords: *Vitis vinifera,* leafdisc bioassay, *Cytochrome b* gene, quinone outside inhibitor, fungicide resistance.

INTRODUCTION

Grapevine powdery mildew caused by the obligate pathogen, *Erysiphe necator* (Schwein.) Burrill (earlier *Uncinula necator*), is one of the most widespread diseases of grapevines in India and can be seen for most part of the year on green tender parts (Chadha and Shikhamany, 4; Sawant *et al.*, 19). It decreases vine productivity and diminishes fruit quality impacting marketable yield and shelf-life of the produce (Ashtekar *et al.*, 1). Powdery mildew is controlled mainly by fungicides, such as demethylation inhibitors, sulphur and strobilurins. The strobilurin fungicide azoxystrobin belongs to the quinone outside inhibitors [QoI] group and has preventive and curative activity against several fungal pathogens.

The QoI fungicides disrupt electron transport during cellular respiration at the ubiquinol oxidation centre (Qo site) of the cytochrome bc1 enzyme complex (complex III). This ultimately results in depletion of adenosine triphosphate (ATP) and disrupts spore germination due to lack of energy (Grasso *et al.*, 13). Being single site inhibitors they are included in the high risk group for development of resistance against them in more than 30 phytopathogeneic species including grapevine powdery mildew. *E. necator* is also classified as a pathogen with medium and high risk of development of resistance to fungicides by FRAC and EPPO respectively (FRAC, 10).

Within a few years of the introduction of QoI fungicides in disease management, field resistance to these fungicides was reported in cucurbit and cereal powdery mildew. Resistance in *E. necator* to QoI fungicides occurred in New York and Pennsylvania, Hungary, Austria, France, Virginia (Baudoin *et al.,* 2; Dufour *et al.*, 6; Fontaine *et al.*, 7; Miles *et al.*, 17; Fraaije *et al.*, 8; FRAC, 10). Three point mutations in *Cyt b* gene were reported as mechanism of resistance against QoI fungicides. Substitution of phenylalanine to leucine at position 129 (F129L) and substitution of glycine to arginine at position 137 (G137R) are associated with the low level of resistance usually controlled by the recommended field levels of QoIs, while substitution of glycine with alanine at position 143 (G143A) was reported for higher level of resistance (Ishii *et al.*, 15). These point mutations in positions 127-147 (cd loop) of the amino acid sequence results in peptide sequence changes that prevents the binding of fungicide.

Resistance factor (RF= effective dose to control 50% of resistant strain/ effective dose to control 50% of sensitive strain) caused by G143A mutation is generally greater than 100 and shows complete resistance. RF associated with mutation F129L and

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G137R are in the range of 5 to 15 and rarely in some cases it is up to 50 (FRAC, 9). Severe losses in disease control are always seen in populations where G143A predominates and QoIs are used alone.

Recently, grape growers reported low disease control in vineyards where QoI fungicides were used in powdery mildew management pointing at the possibility of development of QoI fungicide resistance in *E. necator*. Therefore, the present study was undertaken to monitor the vineyards for presence of resistant isolates of *E. necator* using biological and molecular methods.

MATERIALS AND METHODS

During July 2015 to April 2016, twenty nine powdery mildew infected leaf samples were collected from vineyards in Maharashtra, (six from Solapur, four from Sangli, and two each from Pune and Yavat); Tamil Nadu (13, Theni); and Himachal Pradesh (two, Kinnaur). Except the research vineyards of ICAR-National Research Centre for Grapes (ICAR-NRCG) at Pune and Dr. Yashwant Singh Parmar University of Horticulture and Forestry at Kinnaur, all other samples were collected from commercial vineyards with low levels of disease control. Each sample was collected in individual polypropylene bag and was incubated at 23°C for 24h to encourage fresh sporulation. Presence of conidia was confirmed by observation under stereomicroscope (Leica M 165C).

Five to six month old plants of the susceptible *Vitis vinifera* cultivar, Thompson Seedless, were maintained in greenhouse under disease free condition. Leaves from the fifth and sixth node from the apex of a growing shoot were used for the studies. The conidia from each lesion were transferred on surface-sterilized leaves using camel hair brush. Leaves were placed directly on 1.5% agar medium in Petri dishes (90 mm) with petioles immersed in agar. Plates were incubated in growth chamber (Binder KBWF 720) with a 12 h photoperiod at 23°C for 14 days to encourage growth and sporulation of *E. necator*. This culture was used as inoculum for bio-assay and also for DNA extraction.

Sensitivity to QoI fungicide was determined by leaf disc assay using technical grade azoxystrobin (98.2% active ingradient, a.i) at 0, 0.1, 1.5, 15, 50, 115 µg/ml of active ingredient. Leaf discs of 15 mm were cut from freshly detached leaves and surface sterilized using sodium hypochlorite (4% available chlorine) for 60 s. Four discs were floated in each fungicide concentration for 1 h; air dried and then placed adaxial side up on 2% water agar plates (50 mm). The discs were left overnight in closed Petridishes on laboratory bench at 25 ± 2°C. The discs were then inoculated by dusting *E. necator* conidia from the mass multiplied culture with the help of a camel hair brush and were incubated at 23°C in plant growth chamber with alternating periods of 12 h light and darkness for 10 days. After incubation the discs were assessed under stereomicroscope for powdery mildew development using a rating scale of 0 to 5; where, 0, no visible mildew development; 1, upto 5%; 2, 6 to 25%; 3, 26 to 50%; 4, 51 to 75%; and 5, >76% of disc surface covered with mildew (Ishii *et al*., 15). The percent inhibition at each fungicide concentration was calculated and EC_{50} was calculated by regressing percent inhibition on the log-transformed fungicide concentration using Microsoft Excel 2007 (Colcol *et al*., 5). Minimum inhibitory concentration (MIC) was also calculated. Isolates with EC_{50} <1 µg/ml indicated their sensitivity to azoxystrobin, while isolates with EC_{50} > the field dose of 115 µg/ml indicated high resistance.

The DNA from each of the 29 *E. necator* samples was extracted using REDExtract-N-Amp Tissue PCR Kit (Sigma-Aldrich, USA). Fungal mycelium and conidia were collected from 14 d old leaf culture using a 1-cm^2 office cello tape, transferred to 1.5 ml sterile microcentrifuge tube, and was kept overnight at -20°C. Then 100µl of extraction solution and 2 to 3 glass beads of 2 mm size were added to the sample. Sample was vortexed for 30s and incubated at 95°C for 10 min. After brief cooling 100µl dilution solution was added and vortexed again for 30s. The obtained DNA solution was used directly or kept at -20°C until further use.

For detection of wild type and mutant G143A allele in *Cyt b* gene, amplification refractory mutation system (ARMS) PCR primers (Baudoin *et al*., 2) were used with traditional PCR (Miles *et al*., 17). PCR reactions were carried out in a volume of 25 µl, containing 10 µl of REDExtract-N-Amp PCR ready mix, 1.25 µl of each of 10µM primers, 4 µl DNA template and sterile nano pure water to make final volume. PCRs were performed with an initial denaturation of 15 min at 95°C; 40 cycles with a denaturation step at 94°C for 15 s, annealing at 60°C for 30 s, extension at 72°C for 30 s; and final extension of 10 min at 72°C (Miles *et al*., 17). PCR product was resolved in 1.2% Tris-acetate-ethylene diamine tetra acetic acid (TAE) agarose electrophoresis gel and visualized using gel-documentation system (Alpha Ease FC™version 4.0.1, Alpha Innotech Corporation). Tests with both wild type and mutant allele primers were repeated for confirmation.

Partial amplification and sequencing of the *Cyt b* gene was performed to confirm the mutation. Primers were designed based on the conserved sequence of *Cyt b* gene of *E. necator* (Taksonyi

et al., 22) and of several ascomycetous fungi available in the NCBI GenBank. These were *Erysiphe alphitoides* (JN980979, JN980973, JN981005, JN980990, JN980980, JN980997), *Erysiphe polygoni* (KF925326, KF925325), and *Podosphaera fusca* (EF137830, EF137831, EF137833, EF137834). Primer designing was carried out using Primer 3 Plus software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). The primer pair ENCBF4 (5′GTATGAACAATAGGTGTTGTAA3′) and ENCBR3 (5′CATTGGGTTAGCCATAATAT3′) was syntheiszed (IDT, USA) and successfully used to amplify a 456-bp *Cyt b* gene fragment from *E. necator*. Alignment with the amino acid sequences of *Cyt b* gene from *Blumeria graminis* f. sp. *tritici* retrieved from Genbank (AAK26622.1) showed that the fragment covered amino acid residues from position 86 to 230 which harbor the earlier reported mutations at F129L, G137R and G143A amino acid positions. PCRs were performed in 25 µl reaction volume containing 2.5 of 10 × *Taq* buffer, 2 µl dNTPs (2.5 mM each), 1 µl each of forward and reverse (10µM) primers, 1 µl of 1U *Taq* polymerase enzyme (Bangalore Genei, India), 1 µl DNA template and 16.5 µl of sterile nano pure water. Thermal cycling was performed with initial denaturation of 4 min at 94°C; 35 cycles with a denaturation step at 94°C for 30 s, annealing at 52°C for 1 min, extension at 72°C for 1 min; and final extension of 7 min at 72°C. PCR product was resolved in 1.4% TAE agarose electrophoresis gel containing ethidium bromide and visualized using gel-documentation system (Alpha Ease FC™version 4.0.1, Alpha Innotech Corporation).

The PCR products from two resistant and two sensitive isolates were sequenced directly in both senses using ENCBF4 and ENCBR3 primer pairs. The obtained forward and reverse sequences of each sample were aligned to generate consensus sequences using BioEdit Sequence Alignment Editor. Assembled sequences were used for similarity search using BLASTN against NCBI database. Open reading frame (ORF) was determined for partial *Cyt b* gene from *E. necator* using NCBI ORFfinder tool (https://www.ncbi.nlm.nih.gov/orffinder) using yeast mitochondrial genetic code. Putative *Cyt b* protein was determined using SmartBLAST avaliable at ORFfinder by comparing with GenBank database. Amino acid sequence of resistant and senstive isolates of *E. necator* were aligned along with *Cyt b* protein sequences reported from other fungi. Multiple sequence alignment was performed using CLUTSALW.

RESULTS AND DISCUSSION

The fungicide sensitivity assay demonstrated two distinct groups of sensitivity in *E. necator*, a sensitive and a highly resistant group (Table 1). The four isolates collected from Kinnaur, Himachal Pradesh and from ICAR-NRCG, Pune had EC_{50}<1 µg/ml of indicating their sensitivity to azoxystrobin. No QoI fungicides were ever used in the vineyard at Kinnaur and hence there was no exposure of *E. necator* to QoI fungicides in this region. At ICAR-NRCG, QoI fungicides are used sparingly in powdery mildew management and no application was made in 30 months period prior to sampling. At this site, QoI fungicides were only used in the plant pathology research block where they were found to be effective in controlling powdery mildew.

A number of QoI fungicides are registered for use in grapes in India and in the commercial vineyards 4-5 applications of these fungicides is a common practice. The QoI group of fungicides is known to exhibit cross-resistance (FRAC, 11) seriously compromising disease management. The twenty-five isolates collected from different commercial vineyards showed less than 50% inhibition at 115 µg/ml of azoxystrobin and hence the EC_{50} could not be calculated. Eleven isolates were not inhibited

Table 1. Sensitivity of grapevine *E. necator* isolates to azoxystrobin.

	Origin of sample	No. of samples	Percent growth inhibition		EC_{50}	RF	MIC	Sensitivity to QoI fungicide
			115 µg/ml	230[a] µg/ml				
1	Kinnaur	2	100 ± 0.0	-	0.21-0.39	-	1.5	Sensitive
2	ICAR-NRCG	2	100 ± 0.0	-	0.58-0.62	-	<15	Sensitive
3	Sangli	4	0-20 ± 5.0	0	>115	>256	>115	Resistant
4	Solapur	6	0-45 ± 7.3	0	>115	>256	>115	Resistant
5	Yavat	2	0-45 ± 22.5	0	>115	>256	>115	Resistant
6	Theni	13	0-47± 5.0	0	>115	>256	>115	Resistant

[a]The eleven isolates (4 from Sangli, 2 from Solapur, 1 from Yavat; and 4 from Theni) which showed 0% inhibition at the field dose of 115 µg/ml were tested at the higher concentration of 230 µg/ml

at the labeled field dose of 115 µg/ml azoxystrobin for grapes and showed no inhibition at double the field dose also. The resistance factor of all resistant isolates was 256 fold indicating high level of resistance to azoxystrobin.

In molecular analysis with ARMS PCR primers, the sensitive isolates did not produce a 100-bp PCR product when amplified with mutant G143A allele primer (Fig. 1) while all the resistant isolates produced the 100-bp PCR product (Fig. 2). Partial alignment of amino acid sequences from two resistant and two sensitive isolates showed that both the QoI resistant isolates harbored a G143A mutation as a result of GGT to GCT change, which was absent in the sensitive isolates (Table 2). The two haplotype of *Cyt b* gene sequences from a resistant and a sensitive isolate have been deposited in GenBank under accession numbers KY418049 and KY418048 respectively.

Fig. 1. Amplification of *Cyt b* gene from four sensitive *E. necator* isolates using mutant G143A allele primer (lane 1 to 4; note absence of band) or wild type allele primer (lane 5 to 8; note presence of band). Samples in lanes 1, 2 and 5, 6 are from Pune; lanes 3, 4 and 7, 8 are from Kinnaur. N denotes water control, M denotes 50 bp Ladder.

The high RF value seen in the bio-assay and the amplification with mutant G143A allele primer both confirm that all the 25 isolates from commercial vineyards, which are several hundred km from each other, had high level of resistance to QoI fungicide azoxystrobin, resulting in low level of disease control in these vineyards. The results of this study show the need for large scale monitoring for fungicide resistance in commercial vineyards in India and development and implementation of effective resistance management strategies.

This is the first report of presence of QoI resistant isolates of *E. necator* from India. In vineyards in India, during flowering to fruit set period, infection of both *Plasmopara viticola* causing downy mildew and *E. necator* occurs and use of strobilurin fungicides was in vogue as it used to give excellent control of both the diseases. Reduced efficacy of strobilurins against *P. viticola* is also reported recently (Sawant *et al*., 21).

The study brings out the need for strict monitoring for resistance in vineyards in India as is being done in many other countries (FRAC, 11; Ma, 16; Beresford *et al.,* 3; Hajjeh, 14) and development of mitigating strategies. Studies under controlled conditions have shown that in the absence of QoI fungicide selection pressure, the QoI resistant population in the vineyards tends to decline (Genet *et al*., 12). However contrasting results were obtained where even after four years of disuse of QoI fungicides the *E. necator* resistant population was found to persist in vineyards (Rallos *et al*., 18). In many cases, vineyards with persistent QoI resistance also had significant DMI resistance, which could

Fig. 2. Amplification of mutant G143A allele of *Cyt b* gene from eighteen *E. necator* isolates collected from Sangli (lanes 1 to 4); Solapur (lanes 5 to 8); Yavat (lanes 9 and 10) and Theni (lanes 11 to 18). M denotes 50 bp Ladder.

Table 2. Alignment of the deduced amino acid sequences of *Cyt b* gene amplified from the QoI sensitive and resistant *E. necator* isolates with other fungi from literature. The resistant *E. necator* isolates harbor glycine to alanine change at position 143 (G143A) which is responsible for high level of resistance to QoI fungicides. Mutation F129L and G137R were not present.

Sr. No	Fungi	Amino acid sequence of Cyt b gene	Reference
1.	*Erysiphe necator* (sensitive)-Isolate HP1	MMATA**F**LGYVLPY**G**QMSLW**G**ATVITNTMSAMPWVGQD	This study
2.	*Erysiphe necator* (sensitive)-Isolate PUN1	MMATA**F**LGYVLPY**G**QMSLW**G**ATVITNTMSAMPWVGQD	This study
3.	*Erysiphe necator* (resistant)-Isolate SAA2	MMATA**F**LGYVLPY**G**QMSLW**A**ATVITNTMSAMPWVGQD	This study
4.	*Erysiphe necator* (resistant)-Isolate SLA5	MMATA**F**LGYVLPY**G**QMSLW**A**ATVITNTMSAMPWVGQD	This study
5.	*Erysiphe necator* (sensitive)	MMATA**F**LGYVLPY**G**QMSLW**G**ATVITNLMSAIPWIGQD	Hajjeh 2012
6.	*Erysiphe graminis* (sensitive)	MIVTA**F**LGYVLPY**G**HMSHW**G**ATVITNLMSAIPWIGQD	Fraaije *et al*. 2000
7.	*Erysiphe graminis* (resistant)	MIVTA**F**LGYVLPY**G**HMSHW**A**ATVITNLMSAIPWIGQD	Fraaije *et al*. 2000
8.	*Podosphaera fusca* (sensitive)	---------**F**MGYGLPW**G**QMSLW**G**ATV----------------------------	Ishii *et al*. 2001
9.	*Podosphaera fusca* (resistant)	---------**F**MGYGLPW**G**QMSLW**A**ATV----------------------------	Ishii *et al*. 2001
10.	*Venturia inaequalis* (sensitive)	------------------------Y**G**QMSLW**G**ATVITNLMSAI---------------	Fontaine *et al*. 2008
11.	*Venturia inaequalis* (resistant)	------------------------Y**G**QMSLW**A**ATVITNLMSAI------	Fontaine *et al*. 2008
12.	Amino acid position	124 129 137 143 160	

explain the repeated occurrence of powdery mildew infection despite application of DMI sprays and the withdrawal of QoIs (Rallos *et al*., 18). It is also important to determine if resistance in *E. necator* to other chemistries used in the vineyard is present. Earlier studies have shown that interspersing of fungicides with bio-control agents and chemicals with safer profiles enhances disease control and shelf-life of grapes (Ashtekar *et al*., 1; Sawant *et al*., 20) and would be a good strategy for effective disease control and resistance management.

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REFERENCES

1. Ashtekar, N.D., Ghule, S.B., Saha, S., Sawant, I.S. and Sawant, S.D. 2017. Spray positioning of fungicides with biocontrol agents to mitigate resistance development in *Erysiphe necator* in commercial vineyards. In. National Symposium on 'Diagnosis and Management of Plant Diseases: Integrated approaches and recent trends' January 09-11, 2017 at ICAR Regional Complex for NEH region, Umiam, Meghalaya.

2. Baudoin, A., Olaya, G., Delmotte, F., Colcol, J.F. and Sierotzki, H. 2008. QoI resistance of *Plasmopara viticola* and *Erysiphe necator* in the mid-Atlantic United States. *Plant Health Progress*, (February), 0211-02, doi:10.1094/PHP-2008-0211-02-RS.

3. Beresford, R.M., Follas, G. B., Hagerty, G.C., Manson, P., Manktelow, D. and Lupton, T. 2015. Fungicide resistance management strategy for grapevine powdery mildew (Erysiphe necator) in New Zealand. http://resistance.nzpps.org/index.php?p=crops/grapes_powderymildew. Accessed on 13 September 2017.

4. Chadha, K.L. and Shikhamany, S.D. 1999. The Grape: Improvement, Production and Post-harvest Management Malhotra Publishing House, New Delhi, 579 p.

5. Colcol, J.F., Rallos, L.E. and Baudoin, A.B. 2012. Sensitivity of *Erysiphe necator* to demethylation inhibitor fungicides in Virginia. *Plant Dis.* **96**: 111-16.

6. Dufour, M.C., Fontaine, S., Montarry, J. and Corio-Costet, M.F. 2011. Assessment of fungicide resistance and pathogen diversity in *Erysiphe*

necator using quantitative real-time PCR assays. *Pest Manag. Sci*. **67**: 60-69.

7. Fontaine, S., Remuson, F., Fraissinet-Tachet, L., Micoud, A., Marmeisse, R. and Melayah, D. 2009. Monitoring of *Venturia inaequalis* harbouring the QoI resistance G143A mutation in French orchards as revealed by PCR assays. *Pest Manag. Sci*. **65**: 74-81.

8. Fraaije, B.A., Butters, J.A. and Hollomon, D.W. 2000. The BCPC Conference: Pests and diseases, Volume 1. Proceedings of an international conference held at the Brighton Hilton Metropole Hotel, Brighton, UK, 13-16 November 2000, British Crop Protection Council, pp. 401-406.

9. FRAC. 2006. *Mutations Associated with QoI-Resistance*. Brussels, Belgium: Fungicide Resistance Action Committee, Crop Life International. FRAC list of plant pathogenic organisms resistant to disease control agents. *http://www.frac.Info/docs/defaultsource/about-frac/mutations-associated-with-qoi-resistance december-2006*. Accessed 07 September 2017.

10. FRAC. 2013. FRAC list of plant pathogenic organisms resistant to disease control agents. *http://www.frac.Info/docs/defaultsource/publications/pathogen risk list/pathogen risk list december-2013*. Accessed 25 August 2017.

11. FRAC. 2015. QoI working group of FRAC, Minutes of the meeting all crops: December 3, 2015, Frankfurt, Germany, accessed on 20th September 2017.

12. Genet, J.L., Jaworska, G. and Deparis, F. 2006. Effect of dose rate and mixtures of fungicides on selection for QoI resistance in populations of *Plasmopara viticola*. *Pest Manag. Sci*. **62**: 188-94.

13. Grasso, V., Sierotzki H., Garibaldi, A. and Gisi, U. 2006. Characterization of the cytochrome b gene fragment of *Puccinia* species responsible for the binding site of QoI fungicides. *Pest Biochem Physiol.* **84**: 72-82.

14. Hajjeh, H.R. 2012. Observation on baseline sensitivity of *Erysiphe necator* genetic groups to Azoxystrobin. *American J. Plant Sci*. **3**: 1640-45.

15. Ishii, H., Fraaije, B.A., Sugiyama, T., Noguchi, K., Nishimura, K., Takeda, T., Amano, T. and Hollomon, D.W. 2001. Occurrence and molecular characterization of strobilurin resistance in cucumber powdery mildew and downy mildew. *Phytopathology*, **91**: 1166-71.

16. Ma, B. 2006. Azoxystrobin sensitivity and resistance management strategies of *Magnaporthe grisea* causing gray leaf spot on perennial ryegrass (*Lolium perenne*) turf. PhD thesis. Pennsylvania State University; University Park, USA.

17. Miles, L.A., Miles, T.D., Kirk, W.W. and Schilder, A.M.C. 2012. Strobilurin (QoI) resistance in populations of *Erysiphe necator* on grapes in Michigan. *Plant Dis.* **11**: 1621-28.

18. Rallos, L.E.E., Johnson, N.G., Schmale III, D.G., Prussin, A.J. and Baudoin, A.B. 2014. Fitness of *Erysiphe necator* with G143A-based resistance to quinone outside inhibitors. *Plant Dis.* **98**: 1494-1502.

19. Sawant, I.S., Ghule, S.B. and Sawant, S.D. 2015. Molecular analysis reveals that lack of chasmothecia formation in *Erysiphe necator* in Maharashtra, India is due to presence of only MAT1-2 mating type idiomorph. *Vitis*, **54**: 87-90.

20. Sawant, I.S., Wadkar, P.N., Ghule, S.B., Rajguru, Y.R., Salunkhe, V.P. and Sawant, S.D. 2017. Enhanced biological control of powdery mildew in vineyards by integrating a strain of *Trichoderma afroharzianum* with sulphur. *Biol Control*. **114**: 133-43.

21. Sawant, S.D., Ghule, M.R. and Sawant, I.S. 2016. First report of QoI resistance in *Plasmopara viticola* from vineyards of Maharashtra, India. *Plant Dis.* **100**: 229.

22. Taksonyi, P., Kocsis, L., Mátyás, K.K. and Taller, J. 2013. Investigation into effective control of powdery mildew with strobilurins in viticulture-a case study. *Mitt Klosterneuburg, Rebe und Wein, Obstb Früchteverwert.* **63**: 30-37.

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