ウィルス感染により誘導されるブドウ遺伝子の探索

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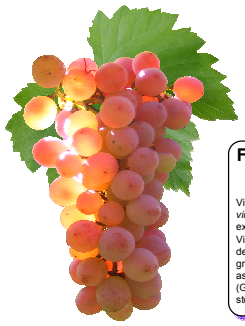
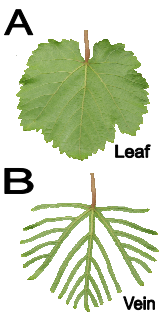
ブドウにウィルスが感染すると果実の品質や収穫量の低下を引き起こすため、適切な防除法の開発が期待される。本研究ではウィルス防除に有効な遺伝子ツールを得るため、ウィルス感染により特異的に誘導されるブドウ遺伝子の探索を行った。RT-PCRを応用したDifferential Display法により、一つの候補遺伝子を得た。この遺伝子はウィルス4種(Grapevine leafroll associated-virus 3, GLRaV-3; Grapevine virus A, GVA; Grapevine virus B, GVB; *Rupestris* stem pitting associated virus, RSPaV)に複合感染した甲州ブドウ樹(Virus+)で劇的に増加した(Fig. 3)。本遺伝子は未同定のブドウ遺伝子であったことから、*Vitis vinifera* virus-induced grapevine protein (*vigg*)と名付け、さらなる解析を行った。Virus+ブドウの茎頂点組織培養で*vigg*の発現解析を行った結果、ウィルスフリー化した植物体では*vigg*の発現は認められなかった(Fig. 5)。また、Virus-ブドウに各種ストレスを与えた場合も*vigg*の発現は誘導されなかった(Fig. 6, 7, 8)。以上の結果から、*vigg*はウィルス感染により特異的に誘導されるブドウ遺伝子であることが強く示唆された。GVAが単独感染したブドウで*vigg*の発現が確認されたが、他のGLRaV-3、GVB、RSPaVの単独感染では発現が認められなかった(Fig. 9)。GVAと他のウィルスによる二重感染のブドウでは*vigg*のサイレンシングが起こっていると考えられた。しかしながら、4種のウィルス(GLRaV-3、GVA、GVB、RSPaV)が感染したVirus+ブドウでは*vigg*が発現していることから、*vigg*のサイレンシングとウィルス感染との相関関係は複雑であると推定された。*vigg*アミノ酸配列から、*vigg*の細胞内局在はミトコンドリアと推定された。ミトコンドリアにおける*vigg*の局在性と機能を現在解析中である。



Vitis vinifera
cv. Koshu

Fig. 1
Plant materials
Virus-infected (Virus+) and virus-free (Virus-) *Vitis vinifera* cv. Koshu were collected from an experimental vineyard at University of Yamanashi. Virus RNA was detected by a RT-PCR-based detection method (Saitoh *et al.* unpublished). Virus+ grapevine was infected by Grapevine leafroll-associated virus 3 (GLRaV-3), Grapevine virus A (GVA), Grapevine virus B (GVB) and Rupestris stem pitting-associated virus (RSPaV).



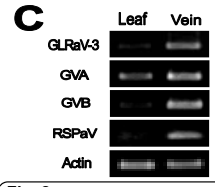


Fig. 2
Localization of viruses in grapevine leaf
(A) Fresh and symptomless leaf of Virus+ grapevine.
(B) Vein cut from **A**.
(C) RT-PCR was performed using total RNA isolated from total leaf or vein. Viruses were mainly detected in vein of grapevine leaf. β-actin primers (Actin) were used as an internal control for RT-PCR.

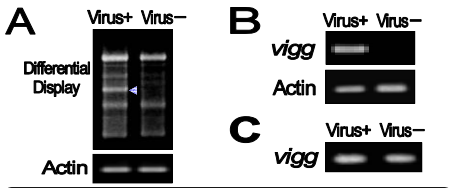
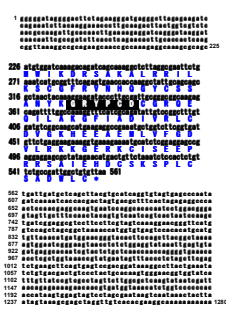


Fig. 3
***vigg* expression in virus-infected grapevine**
(A) RT-PCR-based differential display was performed using total RNA isolated from vein of Virus+ or Virus- grapevine leaf. Arrowhead, *vigg*.
(B) RT-PCR analysis was performed using vigg-specific primers. *vigg* was expressed in Virus+.
(C) Genomic DNA was extracted from the Virus+ or Virus- garapevine. PCR analysis was performed using *vigg*-specific primers. *vigg* was never any transcripts from viruses. β-actin primers (Actin) were used as an internal control for RT-PCR.



vigg

Theoretical pI: 8.91

Theoretical Mw: 12667.66

Fig. 4
Nucleotide and deduced amino acid sequences of *vigg*
Putative mitochondria targeting sequence was shaded black. The sequences of *vigg* have been deposited in the GenBank (accession number EF212291) by Katoh *et al.* (2007).

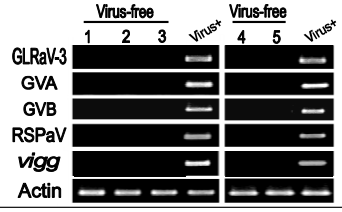


Fig. 5
***vigg* was expressed in virus-infected grapevine**
RT-PCR was perfomed using total RNA isolated from Virus+ grapevine or Virus-free meristem cultures 1, 2, 3, 4 and 5. The meristem cultures were prepared from apical meristem of Virus+ grapevine. *vigg* was expressed in Virus+ grapevine. β-actin primers (Actin) were used as an internal control for RT-PCR.

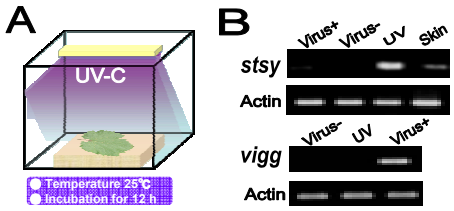


Fig. 6
***vigg* expression was not induced by UV irraliation**
(A) Virus- grapevine leaf was treated with UV-C.
(B) Total RNA was isolated from UV-irradiated leaf. RT-PCR was performed using *vigg* or stilebene synthase (*stsy*)-specific primers. *stsy* was used as a stress marker of grapevine. Skin, control of *stsy* expression. β-actin primers (Actin) were used as an internal control for RT-PCR.

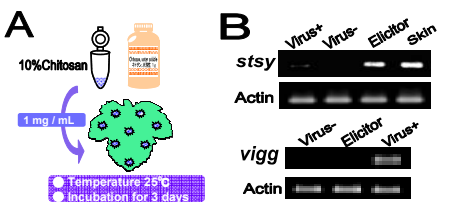


Fig. 7
***vigg* expression was not induced by elicitor treatment**
(A) Virus- grapevine leaf was treated with chitosan elicitor.
(B) Total RNA was isolated from elicitor-treated leaf. RT-PCR was performed using *vigg* or stilebene synthase (*stsy*)-specific primers. *stsy* was used as a stress marker of grapevine. Skin, control of *stsy* expression. β-actin primers (Actin) were used as an internal control for RT-PCR.

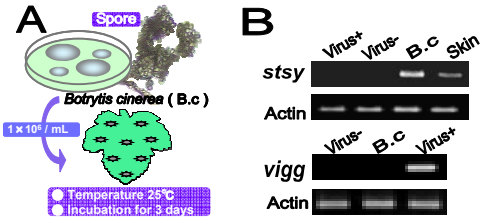


Fig. 8
***vigg* expression was not induced by fungal infection**
(A) Virus- grapevine leaf was inoculated with spores of *Botrytis cinerea*.
(B) Total RNA was isolated from *B. cinerea*-infected leaf. RT-PCR was performed using *vigg* or stilebene synthase (*stsy*)-specific primers. *stsy* was used as a stress marker of grapevine. Skin, control of *stsy* expression. β-actin primers (Actin) were used as an internal control for RT-PCR.

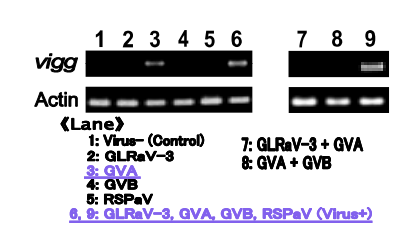


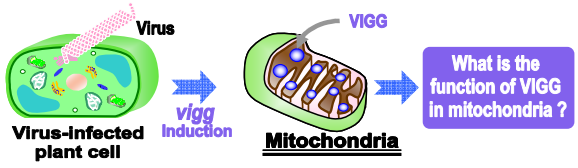
Fig. 9
GVA induces *vigg* expression in grapevine
Total RNA was isolated from Virus- grapevine (lane1), GLRaV-3 (lane2), GVA (lane3), GVB (lane4), RSPaV (lane5), Virus+ (lanes6 and 9), GLRaV-3 + GVA (lane7) or GVA + GVB (lane8)-infected grapevines. and virus+ (9). RT-PCR was performed using *vigg*-specific primers. β-actin primers (Actin) were used as an internal control for RT-PCR.

Conclusion 1

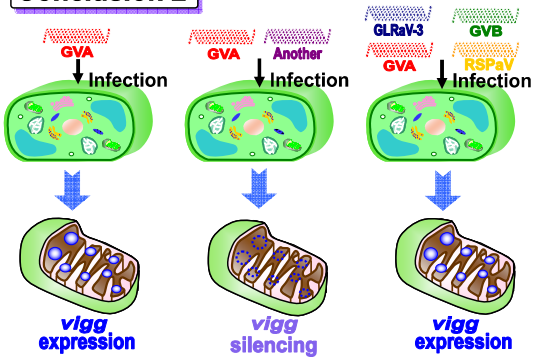
≪Localization and function of VIGG ≫

PSORT (Plant) http://psort.hgc.jp
Mitochondrial matrix space-Certainty= 69.8%

Target P (Plant) http://www/cbs/dtu.dkserrices/Targetp/
Mitochondria, mitochondrial targeting peptide-Certainty= 97.5%



Conclusion 2



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