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High night temperature promotes downy mildew in grapevine via attenuating plant defence response and enhancing early *Plasmopara viticola* infection

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Abstract: The night temperature is one of the critical environmental factors affecting the grape berry quality. The objective of this study was to clarify whether a high night temperature promotes downy mildew on grapevines. The high night temperature conditions suppressed the gene expression of the pathogenesis-related proteins in the grapevine cultured cells and grapevine seedlings compared with the control night temperature conditions. The *Plasmopara viticola* colony formation on the leaves of the seedlings exposed to the control night temperature conditions became slightly noticeable on day 5 after inoculation, whereas a large number of colonies were clearly observed on the leaves of the seedlings exposed to the high night temperature conditions. On day 10 after inoculation, the leaf defoliation and withering were marked in the *P. viticola* infected seedlings exposed to the high night temperature conditions. The high night temperature conditions promoted the *P. viticola* zoospore germination. These results suggest that the high night temperature promoted the grape downy mildew by attenuating the constitutive plant defence response as well as enhancing the *P. viticola* early infection. Viticulturists should be vigilant regarding pest management strategies against the *P. viticola* primary infection of grapevines when high night temperatures continue for a long time.

Keywords: grape downy mildew; PR protein; zoospore germination

The global warming problem has persisted for a long time. Since scientists disclosed the Earth's atmosphere problem in the 1970s, global warming countermeasures have been strengthened through atmospheric environment improvement efforts and the introduction of renewable energy. Nevertheless, carbon dioxide emissions have continued to increase and global warming has not been resolved yet. Global warming is accelerating in major wine-producing countries. A simulation model has predicted an average temperature increase of 2 °C in viticulture regions over the next 50 years (Jones et al. 2005). That prediction has suggested that future warming may give rise to a shift in wine-producing areas and the transplantation of grapevine cultivars cultivated in European and New World viticulture regions. Similarly, a meteorological observatory in Japan has announced that the average temperature in the Yamanashi Prefecture, the major producer of white and red wines in Japan, has risen by 0.042 °C every year from 1961 to date (Saito et al. 2008).

Night temperature is one of the critical environmental factors affecting grape berry quality. A high night temperature inhibits the anthocyanin accumulation in the grape berry skin. The coloration of a Cabernet Sauvignon grape berry exposed to 30 °C night temperature was markedly reduced compared to that of a grape berry ripened at 15 and 20 °C night temperatures (Kliewer & Torres 1972). On the other hand, a low night temperature (10–11 °C) enhanced

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the anthocyanin accumulation in the grape berry (Gaiotti et al. 2018), suggesting that the anthocyanin synthesis and accumulation in the grape berry is sensitive to temperature changes at night. However, so far, there have been few studies on the other physiological alterations of grapevines stressed by a high night temperature. Although it is known that the synthesis of stilbene synthase in the grape berry (Wang et al. 2008), the accumulation of phospholipase D in the grape berry (Wan et al. 2007), and the increase of salicylic acid transport from the roots to the shoots and leaves in the grapevine (Liu et al. 2008) were induced during heat acclimation, further research of the effects of a high night temperature on the physiological status of the grapevine, particularly the berry quality and yield, is required.

The objective of this study was to clarify whether a high night temperature promotes downy mildew, caused by *Plasmopara viticola*, in a grapevine. Here, we report that a high night temperature down-regulates the gene expression of pathogenesis-related proteins (PR proteins) in grapevines. We also demonstrate that a high night temperature promotes *P. viticola* zoospore germination.

MATERIAL AND METHODS

Plant materials. Grapevine seedlings (*Vitis vinifera* cv. Koshu), approximately 8 weeks old after budding, were cultivated in a growth chamber at 27 °C under light irradiation (11.8 W/m^2/16 h/day). Grapevine cultured cells prepared from meristems of *V. vinifera* cv. Koshu were maintained on a modified Gamborg's B5 medium at 27 °C in the dark (Fujita et al. 2018).

Weather data. The weather data of the experimental farm, the University of Yamanashi, Kofu city, Yamanashi Prefecture, Japan (35°36'14.0688" N; 138°34'41.9016" E; 250 m a.s.l.) were recorded by a Meteorological Observation System (FieldMini-FMC-WJ-01, FieldPro, Japan) from 2016 to 2019 growing seasons. The average temperatures, and maximum and minimum temperatures from April 1 through October 31 were summarised monthly for each growing season.

High night temperature treatment of grapevine cultured cells. The grapevine cultured cells were grown at 27 °C for 2 weeks on a modified Gamborg's B5 medium. Three cell masses (approximately 5 mm in diameter) were transferred to a new medium and grown in a growth chamber with a control night temperature condition in the dark for 3 days. After acclimatisation for 3 days, the grapevine cultured cells were transferred at 10 AM to a growth chamber whose temperature was kept at 30 °C from 5 AM to 9 PM under light irradiation (11.8 W/m^2) and at 25 °C from 9 PM to 5 AM in the dark (high night temperature conditions; supplementary Figure S1). For the control experiments, the grapevine cultured cells were transferred at 10 AM to a growth chamber whose temperature was kept at 30 °C from 5 AM to 9 PM under light irradiation (11.8 W/m^2) and at 15 °C from 9 PM to 5 AM in the dark (control night temperature conditions; Figure S1). After incubation for 3 and 5 days, the cell masses were frozen immediately in liquid nitrogen and stored at –80 °C for a real-time RT-PCR (polymerase chain reaction). Three independent experiments were performed.

High night temperature treatment of grapevine seedlings. Six seedlings of *V. vinifera* cv. Koshu with seven expanding leaves were used and cultivated in a growth chamber at 27 °C under light irradiation (11.8 W/m^2/16 h/day). Three seedlings were transferred at 10 AM to a growth chamber with high night temperature conditions under light irradiation (11.8 W/m^2/16 h/day). For the control experiments, three seedlings were maintained under the control night temperature conditions under light irradiation (11.8 W/m^2/16 h/day). At 5 and 7 days after transfer under the high night temperature conditions, the third to the fifth leaves were detached and frozen immediately in liquid nitrogen and stored at –80 °C for a real-time RT-PCR to analyse the transcription profiles of the PR proteins. Three independent experiments were performed.

Real-time RT-PCR. The grapevine cultured cells and grapevine leaves were ground with an SK mill (SK-200, Tokken, Japan) according to the manufacturer's instructions. The total RNA was isolated from the pulverised cell mass using a NucleoSpin RNA Plant kit (Takara, Japan) and purified using a ruit-mate for RNA Purification reagent (Takara, Japan). The first-strand cDNA was synthesised from 1 μg of the total RNA using a PrimeScript RT Master Mix (Perfect Real Time, Takara, Japan). A real-time PCR was performed using an SYBR Premix Ex Taq II (Perfect Real Time, Takara, Japan) with a Thermal Cycler Dice Real Time System (Takara, Japan).

The PCR conditions were as follows: incubation at 95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s and at 60 °C for 45 seconds. The primers

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used for the amplification are shown in Table 1. β-Actin primers were used as the reference gene for the normalisation of each gene expression because the actin was stable in the *P. viticola*-grapevine interaction (Polesani et al. 2010; Figueiredo et al. 2012). Using a standard curve method and the Thermal Cycler Dice Real Time System Single Software (version 3.00) (Takara, Japan), the gene expression levels were determined as the number of amplification cycles needed to reach a fixed threshold. The data were expressed as relative values to β-actin.

Disease assessment. To determine whether the high night temperature promotes grape downy mildew, the disease severity and incidence of the downy mildew on the leaves of the grapevine seedlings exposed to the high night temperature conditions were assessed. The seedlings of *V. vinifera* cv. Koshu with eight expanding leaves were used and cultivated in a growth chamber at 27 °C under light irradiation (11.8 W/m^2/16 h/day). The field-isolated *P. viticola* was maintained on the leaves of the potted Koshu seedlings in a growth chamber under light irradiation (11.8 W/m^2/16 h/day) at 22 °C (Aoki et al. 2019). To obtain fresh zoosporangia, the zoosporangia of *P. viticola* were washed off with sterile water from the symptoms of the seedlings and inoculated on the *V. vinifera* cv. Neo Muscat leaves. The leaves were maintained at 22 °C in a plastic box (30 × 20 × 3.5 cm) containing a moistened paper towel to achieve high humidity. *P. viticola* zoosporangia were washed off with H_2O from the *P. viticola*-infected leaves and adjusted to a concentration of 50 000 zoosporangia/mL. Eight seedlings were inoculated with 5 mL of the zoosporangia suspension using an atomiser. Each seedling was wrapped in a plastic bag (65 × 80 cm) to sustain a high humidity and kept at 22 °C in the dark for 24 hours. The plastic bags were removed and four seedlings were transferred at 10 AM to a growth chamber (relative humidity of 70%) with the control night temperature conditions or the high night temperature conditions. On days 4, 5, 7, and 10 after inoculation, the disease severity and incidence of the third to the sixth leaves from the bottom of the shoot were evaluated. The downy mildew sporulation on the leaves were scored on a scale of 0–5 as follows: 0 – no symptoms; 1 – occupying up to an 1/8 of a leaf; 2 – occupying up to a 1/4 of a leaf; 3 – occupying up to a 1/3 of a leaf; 4 – occupying up to a 1/2 of a leaf; 5 – occupying more than a half of a leaf. At same time, the number of *P. viticola* colonies on each leaf was counted with the naked eye. The means of the symptom scores or colony numbers on the third to the sixth leaves of the four seedlings were calculated to represent the disease severity and disease incidence, respectively.

Zoospore germination. A zoosporangia suspension of *P. viticola* (10 000 zoosporangia/mL) was prepared as mentioned above. Five hundred

Table 1. Sequences of the primers for the real-time PCR

Name	Sequence	GenBank accession
Class IV chitinase (forward)	5'-CAATCGGGTCCTTGTGATTC-3'	U97522
Class IV chitinase (reverse)	5'-CAAGGCACTGAGAAACGCT-3'	U97522
β-1,3-glucanase (forward)	5'-GAATCTGTTCGATGCCATGC-3'	DQ267748
β-1,3-glucanase (reverse)	5'-GCATTATCAACCGTAGTCCC-3'	DQ267748
Pathogenesis-related protein 10.1 (forward)	5'-CCGCTATCCTCGATTCTGAC-3'	AJ291705
Pathogenesis-related protein 10.1 (reverse)	5'-CCCGGTGTGTCATGCTTTTG-3'	AJ291705
Pathogenesis-related protein 10.2 (forward)	5'-CAAGCTATCAAGTGCGTGG-3'	AJ291704
Pathogenesis-related protein 10.2 (reverse)	5'-CACAGTGTAGCAGAATGAG-3'	AJ291704
Thaumatin-like protein (forward)	5'-CCTGGAGCCTCAATGTGAAT-3'	DQ406688
Thaumatin-like protein (reverse)	5'-GTGTACCATAGGCCGTGCAT-3'	DQ406688
Phenylalanine ammonia-lyase (forward)	5'-GCGCCACATATCCACTGATG-3'	EF192469
Phenylalanine ammonia-lyase (reverse)	5'-CCTCGAATGCCCCTATTTTTT-3'	EF192469
MybA1 (forward)	5'-ATCCCAGAAGCCCACATCAA-3'	AB111101
MybA1 (reverse)	5'-GCAAGCCTCAGGACAGAAGAA-3'	AB111101
β-actin (forward)	5'-CAAGAGCTGGAAACTGCAAAGA-3'	AF369524
β-actin (reverse)	5'-AATGAGAGATGGCTGGAAGAGG-3'	AF369524

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microlitres (5 000 zoosporangia) of the zoosporangia suspension was incubated in a microtube at 30 °C for 4 h under light irradiation (11.8 W/m^2) and then transferred to a growth chamber at 25 °C (high night temperature conditions) or 15 °C (control night temperature conditions) for 8 h under darkness. The suspension was stained with 0.05% aniline blue in 0.0067 M K_2HPO_4 pH 9 as described previously (Gómez-Zeledón & Spring 2018). After staining, the zoospore germination was observed using a fluorescence microscope (BX51, Olympus, Japan). The zoospore germination rate was calculated using the following formula:

$$\text{germination}(\%) = \frac{\text{No. of germinated zoospores}}{\text{No. of total zoospores}} \times 100 \quad (1)$$

The third to the sixth leaves were collected from healthy seedlings. Three leaf disks measuring 15 mm in diameter were cut out from each leaf using a cork borer and placed upside down on a moistened filter paper in rectangular Petri dishes (140 × 100 mm). A zoosporangia suspension of *P. viticola* (10 000 /mL) was prepared as mentioned above. Ten microlitres (100 zoosporangia) of the zoosporangia suspension (10 000 zoosporangia/mL) was dropped on the abaxial surface of the leaf disks. The Petri dishes containing the inoculated leaf disks were placed in a plastic box containing a moistened paper towel to achieve approximately 100% humidity. The box was incubated in an incubator at 30 °C for 4 h under light irradiation (11.8 W/m^2) and then transferred to agrowth chamber at 25 °C (high night temperature conditions) or 15 °C (control night temperature conditions) for 8 h under darkness. The leaf disks were fixed with 70% ethanol at room temperature and then treated with boiled 1 M KOH for 1 hour. After the leaf disks were stained with 0.05% aniline blue in 0.0067M K_2HPO_4 pH 9 as described previously (Gómez-Zeledón & Spring 2018), the zoospore germination and *P. viticola* infection on the leaf disks was observed under a fluorescence microscope. The number of germinated zoospore per 1 cm^2 of leas disk was counted.

Statistical analysis. The data are presented as the means ± standard deviations of the biological replicates. A statistical analysis was performed by the Student's *t*-test using Excel Statistics software (version 2012).

RESULTS

Weather data. The average temperatures and maximum and minimum temperatures at the experimental farm in Kofu city, Yamanashi Prefecture, Japan were not significantly different among the growing seasons (Table 2). The maximum air temperatures in June were approximately 30 °C and the minimum temperatures in July were more than 20 °C. The growing degree days (GDD) from April 1 to October 31 in each growing season, calculated in our previous study (Kobayashi et al. 2020) demonstrated that the experimental farm belonged to Region V on the Winkler Index. In the present study, 25 and 15 °C from 9 PM to 5 AM in the dark were used as the high and control night temperature conditions, respectively (Figure S1).

Suppression of gene expression of PR proteins in grapevine under high night temperature condition. The high night temperature conditions in the present study suppressed the expression of *PAL* and *MybA1*, which are related to anthocyanin synthesis, in the grapevine cultured cells on day 5 post-incubation (Figure 1A). This suggested that our experimental method is reasonable in evaluating the effect of a high night temperature on the grapevine.

To determine whether the high night temperature suppresses the plant defence response, the gene expression of the PR proteins in the grapevine cultured

Table 2. Temperatures from April 1 through October 31 in Kofu city of the Yamanashi Prefecture, Japan (°C)

Month	Average				Maximum				Minimum			
	2016	2017	2018	2019	2016	2017	2018	2019	2016	2017	2018	2019
April	15.1	13.2	15.6	13.2	22.4	21.0	23.9	20.8	8.9	6.8	8.5	5.9
May	19.9	19.4	19.0	18.9	27.8	27.5	26.7	27.3	12.5	12.4	12.6	11.2
June	22.5	21.9	22.4	21.9	29.2	29.5	29.0	28.6	17.0	15.8	16.9	16.8
July	26.2	27.3	28.1	24.7	33.3	34.6	34.9	31.1	21.2	22.1	22.8	21.0
August	26.9	27.2	27.7	27.6	34.3	34.3	35.3	34.9	21.9	22.6	22.2	23.1
September	23.9	22.3	22.4	24.7	29.9	29.0	28.3	32.1	19.7	17.2	18.7	19.5
October	17.9	16.2	17.6	18.3	24.1	21.3	25.0	23.9	13.3	12.0	12.0	14.2

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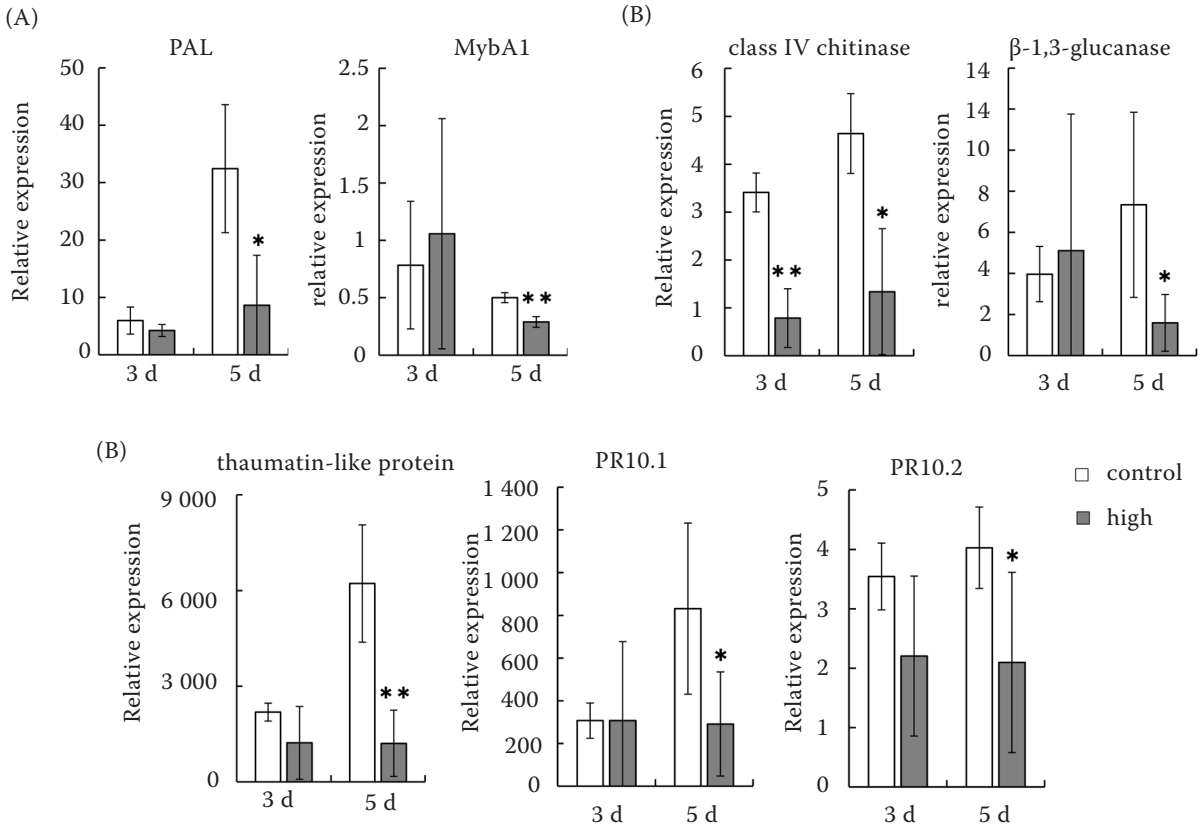

Figure 1. Transcription profiles of the anthocyanin-synthesis-related proteins and PR proteins in the grapevine cultured cells under the high and control night temperature conditions; (A) transcription of the anthocyanin-synthesis-related proteins and (B) transcription of the PR proteins

*,**$P < 0.05$, 0.01 compared with the control, respectively; control – control night temperature conditions; high – high night temperature conditions; the transcription levels of the indicated genes in the grapevine cultured cells on days 3 and 5 after incubation under the high or control night temperature conditions were estimated by a real-time PCR; the data were calculated as the gene expression relative to the actin gene expression; the bars indicate the means ± standard deviations of three cell masses in three independent experiments

cells was compared between the high and control night temperature conditions. The high night temperature suppressed the class IV chitinase gene expression in the grapevine cultured cells on day 3 post-incubation (Figure 1B). The downregulation was maintained up to day 5 post-incubation. The gene expression of β-1,3-glucanase, a thaumatin-like protein, PR10.1, and PR10.2 was also suppressed on day 5 post-incubation under the high night temperature conditions (Figure 1B).

Then, the transcriptional profiles of the PR proteins in the leaves of the grapevine seedlings was compared between the high and control night temperature conditions. The high night temperature suppressed the class IV chitinase, β-1,3-glucanase, a thaumatin-like protein, PR10.1, and PR10.2 gene expression in the grapevine leaves on day 5 post-incubation under the high night temperature conditions (Figure 2). The downregulation of the β-1,3-glucanase, PR10.1, and PR10.2 gene expression was maintained up to day 7 post-incubation under the high night temperature conditions.

Taken together, these results suggest that a high night temperature might suppress the constitutive defence response in grapevines.

High night temperature promotes downy mildew in grapevine. To investigate the effect of a high night temperature on the grape downy mildew, the *P. viticola* infection behaviours were monitored under the high night temperature conditions or the control night temperature conditions. The *P. viticola* colonies on the leaf abaxial surface were counted with the naked eye and a number of colonies per leaf were used to represent the disease incidence (Figure 3A). On day 4 after the *P. viticola* inoculation, sporulation was observed on the leaves of the seedlings exposed

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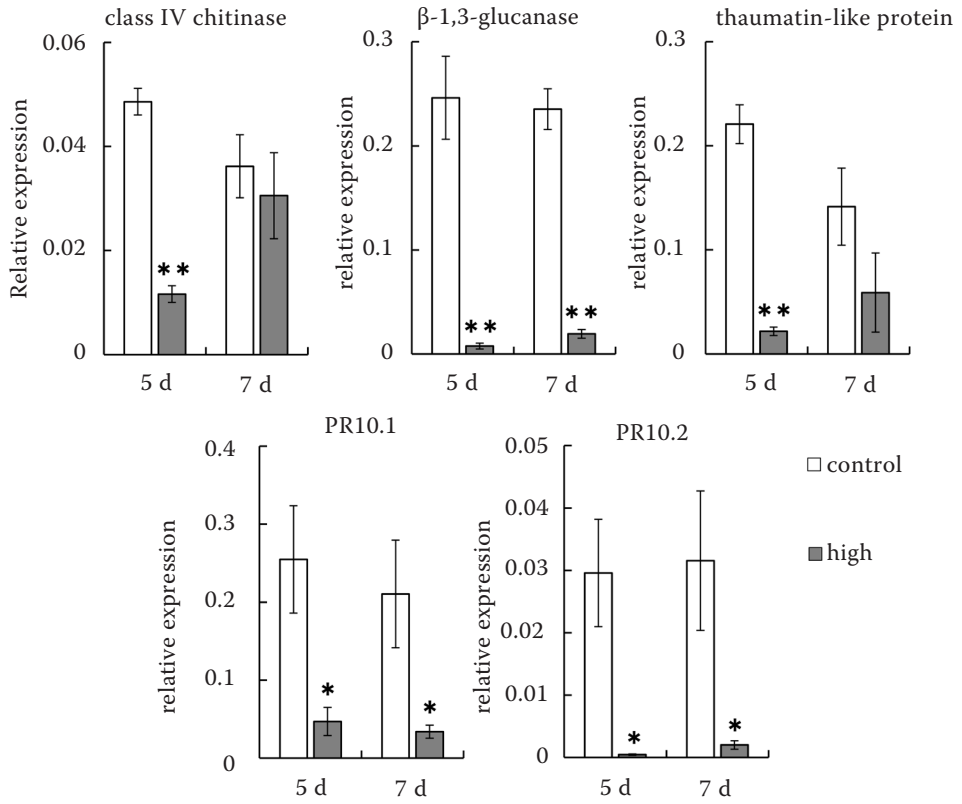

Figure 2. Transcription profiles of the PR proteins in the grapevine under the high and control night temperature conditions

*,**P < 0.05, 0.01 compared with the control, respectively; control – control night temperature conditions; high – high night temperature conditions; the transcription levels of the indicated genes in the grapevine leaves on days 3 and 5 after incubation under the high or control night temperature conditions were estimated by a real-time PCR; the data were calculated as the gene expression relative to the actin gene expression; the bars indicate the means ± standard deviations of three leaves in three independent grapevine seedlings

to the high night temperature conditions, suggesting that *P. viticola* can sporulate at 25 °C or more. On day 5 after the *P. viticola* inoculation, the colony formation became slightly noticeable on the leaves exposed to the control night temperature conditions, whereas a large number of colonies were clearly observed on the leaves exposed to the high night temperature conditions (Figure 3A). The disease severity, which was presented by the symptom score, showed a similar tendency to the disease incidence (Figure 3A). The symptom scores on days 4 and 5 after the *P. viticola* inoculation under the high night temperature conditions were significantly higher than those under the control night temperature conditions.

On the other hand, on days 7 and 10 after the *P. viticola* inoculation, no difference in the disease incidence and severity was noted between the high and control night temperature conditions. However, on day 10 after the *P. viticola* inoculation, leaf defoliation and withering were observed in the seedlings exposed to the high night temperature conditions (Figure 3B). The influence of the *P. viticola* infection on the leaf shape was apparent in the seedlings exposed to the high night temperature conditions; the leaves were severely withered and shrivelled compared to those under the control night temperature conditions (Figure 3C).

Taken together, the results suggest that the early *P. viticola* infection was accelerated under the high night temperature conditions, resulting in the marked development of downy mildew.

High night temperature promotes *P. viticola* zoospore germination. To observe the early infection behaviours of *P. viticola* under the high night temperature conditions, a microscopic observation was performed. The zoospore germination was observed in the spore suspension (Figure 4A). Approximately 15% of the zoospores germinated in the spore suspension 8 h after inoculation under the control night temperature conditions, whereas

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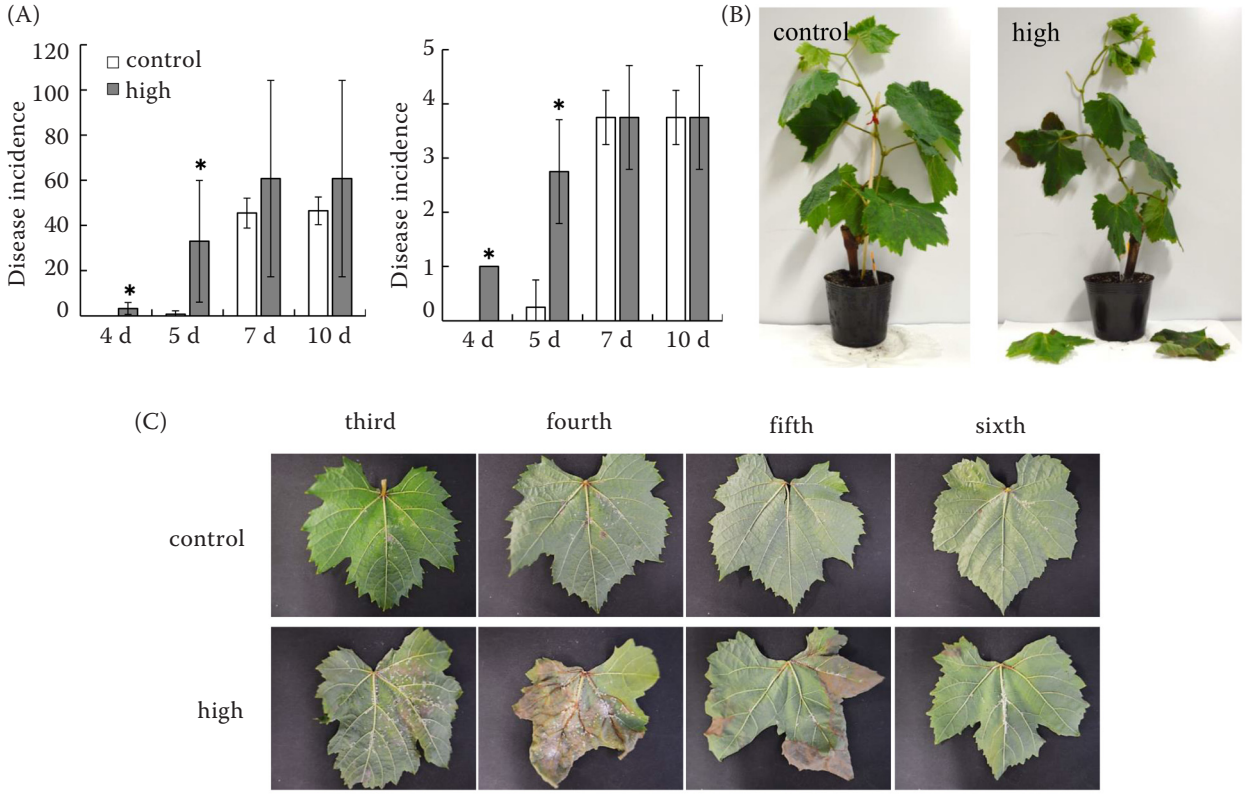

Figure 3. High night temperature promotes downy mildew in the grapevine (A) the disease incidence and severity, (B) the seedlings on day 10 after the *P. viticola* inoculation and (C) the third to the sixth leaves on day 10 after the *P. viticola* inoculation

*$P < 0.05$ compared with the control; control – control night temperature conditions; high – high night temperature conditions; the disease incidence and severity were measured on days 4, 5 ,7, and 10 after the *P. viticola* inoculation, as described in the Material and Methods; the bars indicate the means ± standard deviations of four leaves each of four independent seedlings; defoliation was observed in the seedlings under the high night temperature conditions; severe withering and the shrivelling of leaves were observed under the high night temperature conditions; the luminance, brightness and contrast were adjusted

approximately 45% of the zoospores germinated under the high night temperature conditions (Figure 4B). Since the ungerminated zoospores were burst and removed from the leaf disk during fixation and staining, the number of the germinated zoospores on the leaf disk were counted (Figure 4C). More germinated zoospores were detected under the high night temperature conditions than under the control night temperature conditions (Figure 4D). These results suggest the promotion of the *P. viticola* zoospore germination by the high night temperature.

DISCUSSION

The effect of the temperature on the early infection behaviour of *P. viticola* on the host leaves was studied in the past (Lalancette et al. 1988; Kennelly et al. 2007; Williams et al. 2007). The maximum infection efficiency occurred at 15 °C in an infection efficiency model for *P. viticola* (Lalancette et al. 1988). The report also indicated that the infection efficiency at 25 °C was approximately half that at 15 °C. In contrast, the present study demonstrated that the high night temperature conditions (25 °C in the dark) promoted the *P. viticola* zoospore germination on the host plant, resulting in a more severe downy mildew infection than the control night temperature conditions (15 °C in the dark). This inconsistent result might arise from the temperature regime differences and/or a difference in the temperature tolerance for germination among the *P. viticola* isolates. The study by Lalancette et al. (1988) was performed at a fixed temperature throughout the day, whereas the present study used day/night temperature regimes for the *P. viticola* infection. As

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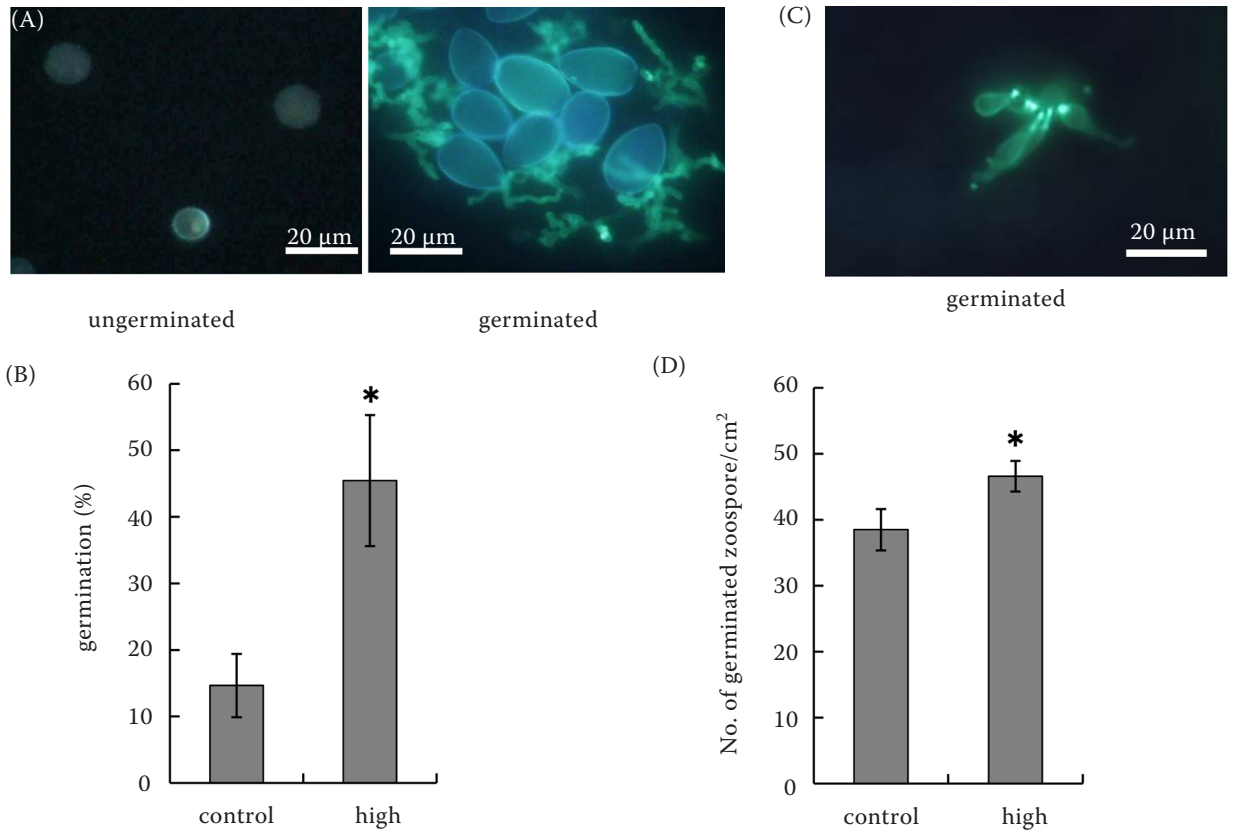

Figure 4. Promotion of the *P. viticola* zoospore germination by the high night temperature; (A) the ungerminated and germinated zoospores in the spore suspension, (B) the germination rates in the spore suspension, (C) the geminated zoospores on the leaf disk and (D) the number of germinated zoospores on the leaf disk

*$P < 0.05$ compared with the control; (B) the germination rates were calculated as described in the Material and Methods; the bars indicate means ± standard deviations of three spore suspensions; (D) the number of geminated zoospores per 1 cm^2 of the leaf disk was counted; the bars indicate means ± standard deviations of three leaf disks; control night temperature conditions; high – high night temperature conditions

P. viticola zoospores germinate over a wide temperature range from 5 to 30 °C (Williams et al. 2007), day/night temperature regimes may affect the *P. viticola* germination on the host. It was also reported that the stomatal penetration of the germinated zoospores occurred at 10 and 25 °C, but not at 30 °C, and that the zoospore germination and host penetration seldom occurred under a light condition (Williams et al. 2007). Hence, it is assumed that the application of day/night temperature regimes is essential for the evaluation of the *P. viticola* early infection behaviour on the host, and the present study is able to reflect field conditions with a high night temperature.

The present study suggests that the high night temperature attenuated the constitutive plant defence response in the grapevine cultured cells (Figure 1) and leaves (Figure 2). The relative expression level of each gene in the grapevine cell cultures was extremely higher than those in the grapevine leaves. Unfortunately, we have no idea about the difference in the gene expression levels between the grapevine cultured cells and leaves. One possibility, the cells producing PR proteins might be restricted in the leaves. Leaves consist of a variety of cell types, whereas the grapevine cultured cells originated from a shoot apex of *V. vinifera* cv. Koshu and might be homogenous (Fujita et al. 2018). In a word, the difference may stem from the difference in the homogeneity of the cells between the grapevine cultured cells and leaves. Nevertheless, the gene expression analysis suggested that the high night temperature suppressed the constitutive defence response in the grapevine irrespective of the cell types.

Temperature-dependent disease resistance in plants has been reported. Relatively high

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temperatures (25 to 35 °C) induced resistance to wheat stripe rust (Fu et al. 2009). In contrast, a high temperature (32 °C) suppressed the PR protein gene expression, the increase of salicylic acid, and hypersensitive response in tobacco (Malamy et al. 1992). Thus, the high temperature condition might positively or negatively affect the expression of the disease resistance in a plant depending on the pathosystems. When wheat plants were transferred to a day temperature of 18 from 25 °C, the incidence of wheat stripe rust increased by as much as twofold (Bryant et al. 2014). Conversely, wheat plants grown at a day temperature of 18 °C and then transferred to a day temperatures of 25 °C were effectively resistant to wheat stripe rust. Therefore, exposure to varying temperatures might affect the disease resistance in a plant. In the present study, compared with the control night temperature, the high night temperature suppressed the PR protein gene expression in the grapevine cultured cells and the grapevine leaves without the *P. viticola* inoculation. As the optimum temperature for the growth of the grapevine cultured cells was 27 °C (Fujita et al. 2018), it appears that the constitutive and basal expression levels of the PR protein genes in the cells exposed to the high night temperature conditions were lower than those in the cells exposed to the control night temperature conditions. Whether the PR proteins tested in the present study functionally work for protection of grape downy mildew or not has yet to be confirmed. As the early transcriptional changes of class IV chitinase and PR10 genes, which are related to the expression of the resistance traits, during the *P. viticola* early infection in grapevine are a make-or-break situation (Figueiredo et al. 2012), the low expression of the PR protein genes under the high night temperature conditions might be one of the main causes of the acceleration of the *P. viticola* early infection as well as the promotion of the *P. viticola* zoospore germination. However, so far, we cannot understand the mechanisms underlying the suppression of the PR protein gene expression under the high night temperature conditions. Future study employing a comparative transcriptome using RNA-seq is expected to reveal part of the mechanisms regarding the temperature-sensitive transcription of the PR protein genes.

The air temperature is one of the driving forces in pathosystems. A future downy mildew epidemic in grapevines as a result of an air temperature increase has been predicted using general circulation models (Salinari et al. 2006). The model simulation demonstrated that the probable increase in the air temperature in May to July, during which the *P. viticola* primary infection in grapevines occurs, might accelerate a severe grape downy mildew epidemic. The present study demonstrated that the high night temperature promoted the downy mildew in the grapevine via attenuating the constitutive plant defence response and enhancing the early *P. viticola* infection. As global warming has resulted in high night temperatures, viticulturists face a tough challenge of maintaining the grape berry quality and yield under high night temperature conditions. In response to high night temperatures, more attention should be given to pest management strategies against the *P. viticola* primary infection in grapevines.

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