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Identification of Heat Tolerance in Chinese Wildgrape Germplasm Resources

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Abstract: Global climate warming will significantly impact grapevine growth and development, and thus grape and wine industries worldwide. Heat-tolerant germplasms are very valuable for grapevine breeding programs. In this study, we assessed the thermotolerance of 247 wild grape accessions by chlorophyll fluorescence parameter (F_v/F_m) under critical high temperature according to Xu et al. in 2016, 2017, and 2018. The results showed that 36 grape accessions showed strong heat tolerance. Therefore, these accessions can be used as parents for breeding heat-tolerant grape cultivars.

Keywords: *Vitis*; high temperature; F_v/F_m

1. Introduction

Global warming is predicted to cause an increase of 2–6 °C in ambient temperature by the end of the 21st century [1]. This increase in temperature could be a rate-limiting factor affecting plant growth and development [2]. Grapevine is a globally important fruit crop [3]. Grapevine growth and development are relatively sensitive to high temperature [4], as berry ripening and yield are strongly affected if air temperature exceeds 35 °C [5]. Meanwhile, in many grape-producing areas, the maximum midday air temperature may exceed 42 °C, and in some regions, it may exceed 45 °C. The significant impact of high temperature on viticulture has been shown previously [6].

High temperatures can cause a series of changes in plant physiological and biochemical processes [7,8]. The extent of heat damage in grapevine leaves depends on the magnitude and duration of the stress [9], leaf age [10], and genotype [11]. Different research methods have been used to study grapevine responses to heat stress, such as chlorophyll fluorescence [12], electrolyte leakage [13,14], and photosynthetic O_2 evolution rate [15]. Chlorophyll fluorescence investigates the physiological state of photosystem II (PSII), and it has been widely used as a parameter for evaluating plant responses to many environmental stress phenomena on leaves [10,16,17]. F_v/F_m describes the maximal quantum efficiency of photosystem II, and is the most common method used to measure chlorophyll fluorescence; the ratio is inversely proportional to heat-damage intensity in PSII reaction centers [18,19]. In recent years, heat-stress damage was often evaluated through change of F_v/F_m values in many plants including grape [10,20–22]. Previous studies have shown that F_v/F_m values of two

grapevine cultivars decreased in response to combined drought and high-temperature stress in the presence of arbuscular mycorrhiza [23]. Similarly, high temperature (50 °C) decreased F_v/F_m in grape cultivars 'Hong Yuli' and 'Red seedless' [14]. Chlorophyll fluorescence parameter F_v/F_m is a rapid, sensitive, and convenient method for measuring heat injury in grapevine. Moreover, the results from the evaluation of germplasm with this method are highly reproducible, as shown by the correlation analysis between different years and different months [10,24].

China is one of the geographical centers of origin of wild grapevines [25]. At present, there are many studies on the cold and drought tolerance of wild grapes [26–28]; however, the heat tolerance of wild grapes has been largely unknown. Heat tolerance of *Vitis davidii* was reported in previous studies [10,14], but there are few reports on the heat tolerance of other wild grape species. The immediacy of global climate warming has recently triggered very active research efforts on heat tolerance of wild plants [29], as there is an urgent need to breed heat-tolerant commercial cultivars. The Zhengzhou Fruit Research Institute collected a large number of wild grapes as the National Grape Germplasm Repository of China. We chose 247 accessions from these resources and evaluated their heat tolerances by F_v/F_m under critical high temperature (47 °C) according to Xu et al. [10]. The germplasm identified with strong heat tolerance would be used in grape breeding programs.

2. Materials and Methods

2.1. Plant Materials

A total of 247 assessed grape accessions belonging to 28 different species were collected from Yunnan, Hubei, Hunan, Guangxi, Henan, and Shandong provinces in China (Supplementary Table S1), which were planted in the spring of 1998 in the Zhengzhou National Grape Germplasm Repository (ZNGGR) in Zhengzhou (38°48′ N, 113°42′ E; 114 m a.s.l.) in China. The average annual temperature of Zhengzhou is 14.2 °C, and the highest temperature is 43 °C from July to September. There are more than 2400 h of sunshine annually. The annual average rainfall is 660 mm and is mostly concentrated from June to October. Grapevines were planted 1.2 m apart within rows and 2.5 m apart between the rows with a north–south orientation. Grapevines were trained to bilateral cordons all under similar soil, irrigation, pruning, and disease control management.

2.2. Heat Treatment and Measurement of F_v/F_m

The study was continuously conducted in July 2016, 2017, and 2018. Each accession was investigated with three biological replicates (three grapevines), while five leaves as technology replicates were sampled in the fifth to seventh healthy expanded leaves from base to top on the shoots in a grapevine. Leaf discs, 3 cm in diameter, were punched from detached sampled leaves; the discs were wrapped in a wet filter paper and placed in aluminum specimen boxes, and then floated in a water bath at 47 °C (25 °C for control, Supplementary Table S2) in the dark. After 40 min, the leaf discs were cooled down at room temperature for 20 min in the dark [10]. F_v/F_m value was measured using a pulse–amplitude–modulation fluorometer (IMAGING-PAM, Heinz Walz GmbH, Effeltrich, Germany).

2.3. Data Analysis

Statistic was carried out using IBM SPSS Statistics (Statistical Product and Service Solutions) program, ver. 21.0 (August 2012, IBM Corporation, Somers, NY, USA). Data in tables and figures are means and standard errors representing three biological replicates. Significant differences among means were determined by Duncan's multiple range comparison test at a probability level of $p < 0.05$.

3. Results

The F_v/F_m values were used to assess the level of heat tolerance of 247 wild grape accessions belonging to 28 species. The higher F_v/F_m values indicate stronger heat tolerance. In Figure 1, we used boxplots to show the change in F_v/F_m values. In general, the evaluation results in consecutive three years

(2016, 2017, and 2018) are consistent. The heat tolerances in *V. heyneana* Subsp *ficifolia*, *V. pseudoreticulata*, *V. davidii*, *V. heyneana*, *V. amurensis*, *V. adenoclada*, *V. wuhanensis* are strong, while *V. piasezkii*, *V. bryoniifolia*, *V. betulifolia*, *V. rotundifolia*, and *V. wilsonae* are medium, while those in *V. yunnanensis*, *V. riparia*, *V. rupestris*, and *V. romanetii* are weak. In some species, the difference in heat tolerance among accessions is very large. The top 10 grape varieties with high heat tolerance are listed in Table 1.
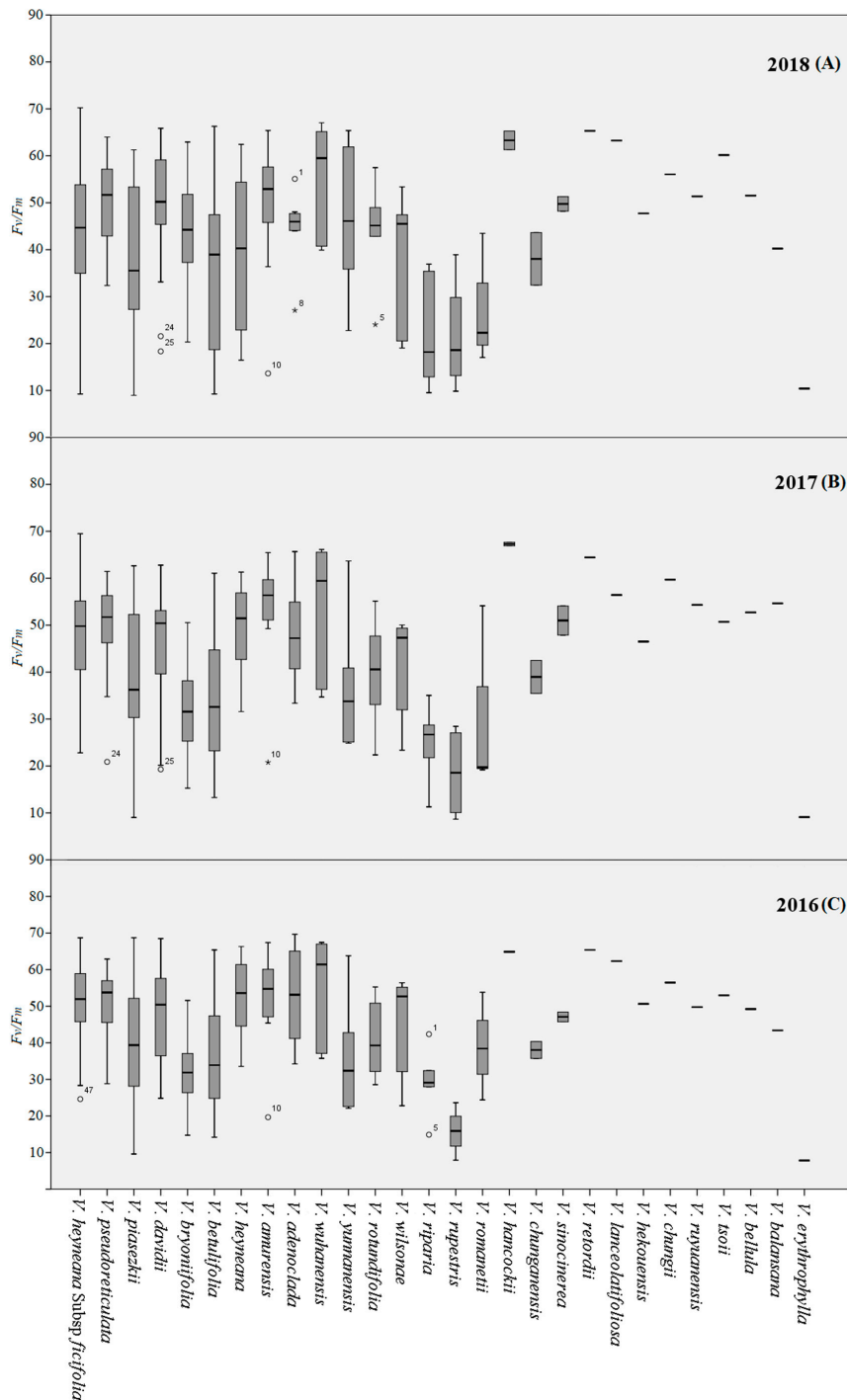

Figure 1. F_v/F_m in different grapevine species in 2018 (**A**), 2017 (**B**), and 2016 (**C**). The horizontal lines in the interior of each box are the median values. The height in a box is equal to the interquartile distance, indicating the distribution for 50% of the data. Approximately 99% of the data falls inside the whiskers (the straight lines extending from the top and bottom of the box). The data outside these whiskers are indicated by blank circles.

Table 1. The top 10 accessions or species in wild grape resource evaluated by F_v/F_m in the study.

No.	Species	Accession	Origin	2016 F_v/F_m	2017 F_v/F_m	2018 F_v/F_m	Average Value [1]	Rank [2]
1	*V. heyneana*	Shibanyan 11	Henan	0.6870 ± 0.0097a	0.6652 ± 0.0274b	0.7022 ± 0.0110a	0.6848 ± 0.0160	I
2	Subsp *ficifolia*	Shibanyan 5	Henan	0.6096 ± 0.0233de	0.6610 ± 0.0194b	0.6694 ± 0.0103b	0.6467 ± 0.0177	I
106	*V. davidii*	Meilingshan 1301	Jiangxi	0.6850 ± 0.0066a	0.6272 ± 0.0311a	0.6580 ± 0.0073a	0.6567 ± 0.0150	I
181	*V. amurensis*	Shuangyou	Unknown	0.6574 ± 0.0212a	0.6546 ± 0.0035a	0.6540 ± 0.0107a	0.6553 ± 0.0118	I
199		Dawu 1701	Hubei	0.6698 ± 0.0057ab	0.6554 ± 0.0045a	0.6704 ± 0.0021a	0.6652 ± 0.0041	I
200	*V. wuhanensis*	Jingshan 1708	Hubei	0.6744 ± 0.0038a	0.6612 ± 0.0058a	0.6406 ± 0.0043c	0.6587 ± 0.0047	I
201		Xianning 1710	Hubei	0.6602 ± 0.0047b	0.6508 ± 0.0051a	0.6516 ± 0.0090b	0.6542 ± 0.0063	I
233		Ruian 1610	Zhejiang	0.6478 ± 0.0019a	0.6764 ± 0.0067a	0.6528 ± 0.0028a	0.6590 ± 0.0038	I
234	*V. hancockii*	Lingye 945	Unknown	0.6498 ± 0.0111a	0.6692 ± 0.0129a	0.6130 ± 0.0069b	0.6440 ± 0.0103	I
239	*V. retordii*	Luocheng 1602	Guangxi	0.6538 ± 0.0038	0.6444 ± 0.0250	0.6532 ± 0.0044	0.6505 ± 0.0111	I

Note: Average value [1] is the average for three years. F_v/F_m values are means ± S.E.; different letters indicate means are significantly different at $p < 0.05$. Rank [2] is the classification of heat resistance of different grapevine varieties by SPSS.

Based on cluster analysis and according to the average values of F_v/F_m for the three years in the study (Supplementary Table S1), the 247 grape accessions were divided into five major classes—I (high heat-tolerant type), II (heat-tolerant type), III (medium heat-tolerant type), IV (medium heat-sensitive type), and V (heat-sensitive type). The results of this comprehensive evaluation from 2016 to 2018 showed that 36 wild grape accessions were highly heat-tolerant (I class). They are mainly distributed in Henan, Hubei, Jiangxi, and Fujian provinces; nearly 75 grape accessions were heat-tolerant (II), mostly distributed in Henan, Yunnan, Zhejiang, and Fujian provinces. In contrast, 29 grape accessions were sensitive types (V class), mainly distributed in Henan and Jiangxi provinces. Our study found significant intraspecific differences for heat tolerance, such as 'Shibanyan' 11 (1, F_v/F_m = 0.6848) and 'Songshan' (49, F_v/F_m = 0.2459) in *V. heyneana* Subsp *ficifolia* species, or 'Fujian' (50, F_v/F_m = 0.6211) and 'Tongmu 1 ' (78, F_v/F_m = 0.3224), both belonging to *V. pseudoreticulata*.

The F_v/F_m of the 247 grape accessions ranged from 0.0783 to 0.7022. In 2016, the highest F_v/F_m is 0.6966 and is recorded for *V. Adenoclada* (No.191, in Supplementary Table S1) and the lowest (0.0783) is for *V. erythrophylla* 'Fuliang 1605' (247). The median is the representative value of all the unit sign values determined by its position in all the sign values, which is not affected by the maximum or minimum value of the distribution series, thus improving the representativeness of the median to the distribution series to a certain extent. As shown in Figure 1 and Table 1, the median F_v/F_m differed significantly among the 28 *Vitis* species evaluated; the highest median (0.6538) was measured in *V. retordii*, and the lowest was measured in *V. erythrophylla* (0.0783). Among the 28 *Vitis* species in the study, the range of F_v/F_m values was similar for *V. piasezkii* and *V. betulifolia*, which showed the widest range of all, followed by those for *V. davidii*, *V. heyneana* subsp *ficifolia*, *V. adenoclada*, and *V. yunnanensis*. For the evaluation results in consecutive three years (2016, 2017, and 2018), the overall trend is consistent. In 2017, however, the highest F_v/F_m [Sang 943 (7), 0.6948] was recorded in V. *heyneana* subsp *ficifolia* and the lowest F_v/F_m was measured in *V. rupestris* [Shadi 1117 (228), 0.0866]. On the contrary, in 2018, the highest F_v/F_m was detected in *V. heyneana* subsp *ficifolia* [Shibanyan 11 (1), 0.7022], while the lowest F_v/F_m value was observed in *V. piasezkii* [Laojunshan 1 (105), 0.0896].

4. Discussion

With global warming, high temperature stress, as one of the main abiotic stress factors, has a direct or indirect influence on many important grape and wine producing areas in the world. The increase of temperature, on the one hand, advances the growth period of grapes and increases the sugar content of fruits, but on the other hand, it results in premature senescence of leaves and reduction of the quality of grapes or wine [30]. Using the physiological defense mechanism of plants to carry out the breeding of new heat-tolerant varieties is of great significance for plants to resist high temperature stress and solve the problem of plant production under high temperature stress, hence, there is a need for evaluation of high-temperature sensitivity of grapevine [31]. Here, we investigated differences in heat tolerance among 247 grape accessions in response to high ambient temperature by chlorophyll

fluorescence according to Xu et al. [10]. The results indicate that the method is practical, efficient, and highly accurate, and the results are consistent in the three years.

In this study, we found that *V. amurensis* originating in the Jilin province of north-eastern China shows highly heat-tolerant, while *V. erythrophylla* in Jiangxi province of south-eastern China is heat-sensitive. Therefore, we need to revise the previous thought. We speculate that, on the one hand, in a certain historical period in the north, it used to be high-temperature weather, and grapes in the north were domesticated by the high temperature. On the other hand, many plants have cross-adaptation including grapes [32]. Based on the conclusion of this study, the wild species with special excellent characters in the north can be introduced to the south for domestication and cultivation, or the wild species in the north can be used as parents to cultivate heat-resistant grape varieties, which can avoid some disadvantages of grapes in the south. The heat tolerance of most grape species varies greatly, indicating that the grapes have a large variation in the evolution process, and these resources can be used to mine molecular markers of heat tolerance. In addition, the difference of heat tolerance among different accessions in some species is very large (Figure 1), and we may utilize these resources to investigate quantitative trait locus of heat tolerance in grape.

In summary, we evaluated the heat tolerance of 247 grape accessions. The results will provide very valuable information for breeding heat-tolerant grape cultivars and the molecular mechanism of heat tolerance.

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