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Semi-lethal high temperature and heat tolerance of eight *Camellia* species

Temperatura alta semi-letal y tolerancia al calor de ocho especies de *Camellia*

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Abstract. Annual leaf segments of eight *Camellia* species were used to study the heat tolerance by an electrical conductivity method, in combination with a Logistic equation to ascertain the semi-lethal high temperature by fitting the cell injury rate curve. The relationship between the processing temperature and the cell injury rate in *Camellia* showed a typical "S" shaped curve, following the Logistic model. The correlation coefficient was above 0.95. The semi-lethal high temperature LT_{50} of the eight *Camellia* species, determined by the inflection point on the curve, varied from 50 to 57 LT_{50} / °C, following the descending order: *Camellia oleifera* > *C. japonica* > *C. polyodonta* > *C. semiserrata* > *C. nitidissima* > *C. gigantocarpa* > *C. nanyongensis* > *C. vietnamensis*. The semi-lethal high temperature of *C. oleifera* was 56.8 °C, and that of *C. vietnamensis* was 50.6 °C. Results showed *C. oleifera* appeared as the best suitable species for introduction in high-heat zones. *Camellia vietnamensis* appeared more suitable for planting in temperate regions at lower temperatures. These results can provide theoretical basis for breeding and introduction of heat-tolerant oil-tea cultivars.

Keywords: *Camellia*; Logistic equation; Semi-lethal high temperatures; Heat tolerance.

Resumen. Se utilizaron segmentos foliares anuales de ocho especies de *Camellia* para estudiar la tolerancia al calor por un método de conductividad eléctrica, en combinación con una ecuación logística para dilucidar la temperatura alta semi-letal ajustando la curva de tasa de daño celular. La relación entre la temperatura y la tasa de daño celular en *Camellia* mostró una curva con forma de "S" típica, siguiendo el modelo logístico. El coeficiente de correlación estuvo por encima de 0,95. La temperatura alta semi-letal LT_{50} de las ocho especies de *Camellia*, determinada por el punto de inflexión en la curva, varió entre 50 y 57 LT_{50} / °C de acuerdo con el siguiente orden descendente: *Camellia oleifera* > *C. japónica* > *C. polyodonta* > *C. semiserrata* > *C. nitidissima* > *C. gigantocarpa* > *C. nanyongensis* > *C. vietnamensis*. La temperatura alta semi-letal de *C. oleifera* fue 56,8 °C, y la de *C. vietnamensis* 50,6 °C. Los resultados mostraron que *C. oleifera* parece ser una especie más adecuada para introducir en zonas donde se registren altas temperaturas. *Camellia vietnamensis* parece más adecuada para ser plantada en regiones templadas, a temperaturas más bajas. Estos resultados pueden proveer una base teórica en programas de cruzamiento genético e introducción de cultivares de aceite-té tolerantes al calor.

Palabras clave: *Camellia*; Ecuación logística; Temperaturas altas semi-letales; Tolerancia al calor.

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INTRODUCTION

Camellia, mainly distributed in south China, and a small amount distributed in South Asia, is a species with a production value of edible oil, which belongs to *Camellia* spp. of Theaceae. *Camellia* is a small evergreen tree or shrub, which is one of the world's four major woody oil plants together with olive, oil palm and coconut. The planting area of *Camellia* in China is about 400 million hm^2, which accounts for more than 80% of our cultivation area of edible-oil woody species (Zhuang, 2008). *Camellia oleifera*, the most common *Camellia* species, is shown in Figure 1.

Fig. 1. Plant of *Camellia oleifera.*
Fig. 1. Planta de *Camellia oleifera.*

Camellia includes 238 species of plants. More than 50 species contain high amounts of seed oil. *Camellia oleifera* is the most widely distributed species, and *C. nitidissima*, *C. vietnamensis*, *C. semiserrata*, *C. gigantocarpa*, *C. polyodonta*, *C. japonica* and *C. nanyongensis* are cultivated in some regions in China, and have cultural history values. *Camellia* species have significantly different growth habits in different distribution areas due to different climatic conditions. This paper evaluated the heat resistance of *Camellia* by a conductivity method with the Logistic equation to provide a theoretical basis for introduction of *Camellia* to different areas.

MATERIALS AND METHODS

Experimental materials. *Camellia* material sources and planting sites are shown in Table 1. These *Camellia* species were all planted in 2003 in Nanning, Guangxi.

Table 1. Source and planting sites of *Camellia* species.
Tabla 1. Origen y sitios de plantación de especies de *Camellia*.

Camellia species	Place of origin	Planting sites
Camellia oleifera	South of China	Nanning of Guangxi
Camellia nitidissima	Fangcheng of Guangxi	Nanning of Guangxi
Camellia vietnamensis	Luchuan of Guangxi	Nanning of Guangxi
Camellia semiserrata	Guangning of Guangdong	Nanning of Guangxi
Camellia gigantocarpa	Bobai of Guangxi	Nanning of Guangxi
Camellia polyodonta	Wantian of Guangxi	Nanning of Guangxi
Camellia japonica	Japan	Nanning of Guangxi
Camellia nanyongensis	Zhaoping of Guangxi	Nanning of Guangxi

Experimental methods. This study was conducted in July 2011. A selected number of consistent, healthy, pest-free annual functional leaves from the tested *Camellia* species were washed with deionized water. Leaves were first dried with absorbent paper, and small, uniform 0.5 cm^2-size discs were subsequently cut, avoiding the main vein. They were accurately weighed and 0.5 g were placed into a test tube. Twenty five mL of deionized water were added into the tubes so that leaves remained immersed. Thereafter, the leaf discs were put into a water bath at 30 °C, 35 °C, 40 °C, 45 °C, 50 °C, 55 °C, 60 °C, 65 °C or 70 °C, respectively, for 20 min. Then, they were taken out of the water bath and cooled at room temperature for 2 h. Conductivity (R) ($\mu s \cdot cm^2$) was then measured by a HI-9033 conductivity Meter. Then, all leaves were placed into a boiling water bath for 15 min to kill the plant tissue. Leaves were taken out and allowed to cool. Conductivity (R_0) was then determined in the same way at 25 °C. All studies were repeated three times. The control (CK) was the conductivity of the leaf discs at 25 °C (room temperature).

The relative electrolyte leakage was calculated following Zhang et al. (1989) as follows:

Cell injury rate (%) = (R-CK) / (R_0-CK) × 100‰

Statistical analysis. The leaf cell injury rate of *Camellia* species at the different temperatures was based on the Logistic Equation by SPSS 17. The logistic regression equation was $y = K / (1+ae^{-bx})$, where (1) y represents the rate of cell damage, (2) x represents temperature, (3) K represents the maximum rate of cell damage saturation capacity, due to elimination of background interferences in this experiment, so it takes the 100 K value, and (4) a and b are the equation parameters. According to regression analysis, the turning point of temperature in the equation is precisely the high-temperature, half-lethal temperature of *Camellia*.

RESULTS AND DISCUSSION

Effect on leaf cell injury rate of *Camellia* species at different temperature. The membrane system is the center of resistance to heat injury and heat; the thermal stability of the cell membrane reflects the ability of plant heat-resistance (Wang et al., 2010). When plant cells are subjected to stress injury, cell membrane structure is destroyed, and cell membrane permeability is increased. This results into leakage of the intracellular solution; the greater the degree of cell membrane damage, the greater the conductivity of the electrolyte leakage.

When treatment temperature was below 45 °C, the leaf cell injury rate of the eight study *Camellia* species increased slowly as the temperature rose (Fig. 2). When the treatment temperature reached 50 °C, leaf cell injury rates of the eight study *Camellia* species increased sharply. However, the rate of increase varied according to the species. When the temperature was higher than 60 °C, leaf cell injury rates were stable (Fig. 2), which proved that the membrane system of each study leaf was almost completely destroyed. The whole process of electrolyte leakage with increasing temperatures was a typical "S"-shaped type of growth. The eight study *Camellia* species have the same variation of the curve (Fig. 2). Leaf cell injury rate of *Camellia* increased sharply from 50 °C to 60 °C. This proved that *Camellia* leaves were most sensitive to damage by high temperature at this temperature range. Thereafter, we can initially infer the critical temperature that might cause an irreversible damage to the membrane system of *Camellia* leaves when going from 50 °C to 60 °C.

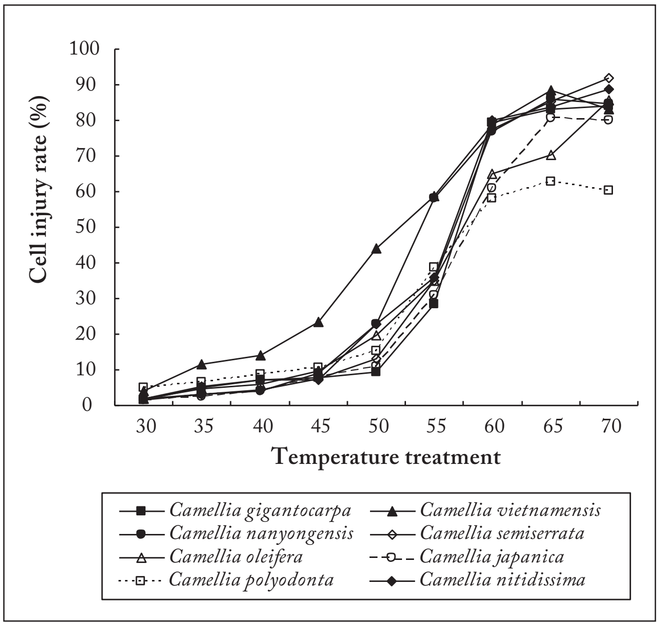

Fig. 2. Leaf cell injury rate of *Camellia* species under different temperatures.
Fig. 2. Tasa de daño de células foliares de especies de *Camellia* expuestas a diferentes temperaturas.

Determining Logistic equation parameters and the lethal temperature of *Camellia* species. Relative electrolyte leakage values of eight *Camellia* species were modeled and predicted applying nonlinear Regression using SPSS 17.0 software. The results were fitted by the Logistic regression equation. After iteration, the parameter values of the equations were obtained. The procedure is as follows: (1) solve the second derivative of the Logistic equation, and presume it is equal to zero, (2) get the curve inflection point $t = \ln a/b$, which is the semi-lethal temperature; $K/2$ is the knee injury rate W; $bW/2$ is the maximum growth rate of cell damage. Logistic equation parameters of the *Camellia* species, and its growth parameters, are shown in Table 2.

Results showed that semi-lethal temperatures of the eight *Camellia* species ranged from 50 °C to 57 °C, and correlation coefficients that fitted by the Logistic equation were above 0.95 (Table 2). The higher the semi-lethal high temperatures, the stronger the capability to resist those high temperatures, whereas the lower the half-lethal high temperatures, the weaker the capability to resist those high temperatures (Li et al., 2010). The descending order of the capacity to resist high

Table 2. Parameter estimates and growth variables of *Camellia* species.
Tabla 2. Estimaciones de parámetros, y variables de crecimiento de especies de *Camellia*.

Species	Logistic equations	Coefficient (r)	Semi-lethal temperature (LT_{50} / °C)	Knee injury rates (%)	Maximum growth rate of cell damage (%)
Camellia oleifera	$y= 92.066/(1+29187.955e^{-0.181x})$	0.99**	56.8	46.03	4.17
C. nitidissima	$y=92.774/(1+1266601.665e^{-0.254x})$	0.98*	55.3	46.39	5.89
C. vietnamensis	$y=92.097/(1+3849.749e^{-0.163x})$	0.989*	50.6	46.05	3.75
C. semiserrata	$y=91.708/(1+7540.17e^{-0.159x})$	0.978*	56.2	45.85	3.65
C. gigantocarpa	$y=85.249/(1+290349.581e^{-0.236x})$	0.969*	53.3	42.62	3.85
C. polyodonta	$y=85.033/(1+16317.607e^{-0.172x})$	0.952*	56.4	42.52	3.66
C. japonica	$y=85.021/(1+5921823.058e^{-0.275x})$	0.993**	56.7	42.51	5.85
C. nanyongensis	$y=86.4/(1+938.235e^{-0.13x})$	0.982*	52.6	43.2	2.81

Note: * represents a significant level at $p<0.05$, ** represents a significant level at $p<0.01$.

temperatures of the eight *Camellia* species is *Camellia oleifera* > *C. japonica* > *C. polyodonta* > *C. semiserrata* > *C. nitidissima* > *C. gigantocarpa* > *C. nanyongensis* > *C. vietnamensis*. *Camellia oleifera*, *C. semiserrata*, *C. polyodonta* and *C. japonica* have similar semi-lethal temperatures which ranged from 56.2 °C to 56.8 °C. *Camellia vietnamensis* showed the weakest capacity to resist high temperatures; its semi-lethal temperature was 50.6 °C. *Camellia nitidissima* had the maximum growth rate of cell damage (5.89%) followed by *Camellia japonica* (5.85%), which showed that the extent of cell damage increased faster when temperature increased 1 °C. *Camellia nanyongensis* showed a 2.81% growth rate of leaf damage, which showed that this species had the stronger ability to resist high temperatures.

Tolerance of plants to adverse environments is one of the key factors to determine whether its introduction and cultivation will be successful or not (Zhao et al., 2003). The ability of resistance to adverse environmental conditions varies according to the habitat and the species. Different plant materials have different quantitative resistance indicators: the seeds are usually identified by germination rate (Liang & Tan, 1997), cell suspension solutions by its protein content (Jugawara & Sakai, 1978), and visual methods to survey plant survival (Yelenosky, 1979). The adaptability of plants to high temperature stress is mainly reflected by the cell membrane permeability changes (Yu & Tang, 1998). Cell membrane structure under high temperature conditions is subject to different degrees of damage; leakage of intracellular solution is caused by membrane permeability changes. Severity of the injury and high temperature tolerance of plants can be often reflected by the conductivity of the cell electrolyte leakage rate. Rajashekevar et al. (1979) fitted the relationship of cell injury rate of the wheat and Kentucky bluegrass leaves *versus* temperature using the Logistic regression equation. They reported that the inflection point of the equation determined the half lethal temperature of plants. Zhu et al. (1986) determined the temperature when the leakage rate of the cells was 50% (i.e., the half-lethal temperature: LT_{50}). Currently, conductivity methods have been widely used in *Taxus* (Liang & Tan, 1987), plums (Liu et al., 1999), apple (Gao et al., 2000), sweet cherry (Liu et al., 2005) and eucalyptus (Liu et al., 2009) to determine the plant tolerance to adverse environments. However, the heat-resistance of *Camellia* has been rarely reported. This study found that as temperature increased, the leaf cell injury of all study *Camellia* species also increased (i.e., there was a positive correlation between temperature and cell membrane permeability).

The relationship between the *Camellia* leaf cell injury rate and temperature was a typical "S" type curve, which was in line with the Logistic equation. We determined that the half-lethal temperature ranged from 50 °C to 57 °C in the eight Camellia species using the conductivity method and the Logistic equation. *Camellia oleifera* appeared as the best species for introduction into high heat zones, and *Camellia vietnamensis* appeared as more suitable for planting in temperate regions of lower temperatures.

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