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Influence of light intensity on the photochemical activity of the vanilla plant (*Vanilla planifolia* Andr.)

Influencia de la intensidad de la luz en la actividad fotoquímica de la planta de vainilla (*Vanilla planifolia* Andr.)

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ABSTRACT

The objective of this study was to document the photochemical activity of vanilla plants, grown in the different light regimes created by host trees in different plantations, in three phenological stages; pre-flowering, flowering and post-flowering. Three natural hosts were chosen: *Citrus sp*., *Erythrina sp*. y *Gliricidia sp*., in addition to a further cultivation system utilizing artificial hosts and shade-mesh. The parameters evaluated were initial fluorescence (Fo), maximum fluorescence (Fm) and the Fv/Fm ratio. Analysis of variance showed the existence of statistically significant interaction between host type and phenological stage in terms of Fo, Fm and Fv/Fm index. The results indicated that vanilla plants grown with deciduous host trees exhibit changes in the actual and potential quantum efficiency of PSII due to the Fv/Fm index values.

Key words: Photoinhibition, chlorophyll *a* fluorescence, high light stress, *Vanilla planifolia.*

RESUMEN

El objetivo de este estudio fue documentar la actividad fotoquímica de plantas de vainilla, que se cultiva en los diferentes regímenes de luz creados por árboles hospederos en diferentes plantaciones, en tres etapas fenológicas; prefloración, floración y post-floración. Tres huéspedes naturales fueron elegidos: *Citrus* sp, *Erythrina* sp.. y *Gliricidia* sp., además de un sistema de cultivo utilizando tutores artificiales y malla-sombra. Los parámetros evaluados fueron la fluorescencia inicial (Fo), la fluorescencia máxima (Fm) y la relación Fv / Fm. El análisis de varianza mostró la existencia de una interacción estadísticamente significativa entre el tipo de tutor y la etapa fenológica en términos de Fo, Fm y el índice de Fv / Fm. Los resultados indicaron que las plantas de vainilla crecido con los cambios de los árboles hospederos de exhibición de hoja caduca en la eficiencia cuántica real y potencial de PSII debido a los valores del índice de Fv / Fm.

Palabras clave: Fotoinhibición, clorofila y fluorescencia, estres lumínico luz, *Vanilla planifolia*.

INTRODUCTION

Plants are naturally subject to different types of stress caused by variations in environmental conditions or resource availability. Stress caused by high levels of incident light can cause photoinhibition in a wide range of plants (Hamilton *et al*., 1995; Demmig-Adams and Adams, 1992). Level of irradiance is the environmental factor that most affects the growth and development of plants, which respond to different levels of solar radiation by phenotypic acclimatization and genetic adaptation; for example, leaves under shade conditions may present reduced photosynthetic capacity and nocturnal respiration per unit area, as well as a reduced electron transport capacity relative to light acclimated leaves (Lambers *et al*., 1998). Stress scenarios showing low and high levels of resources and certain environmental conditions cause plant responses such as photoprotection and shade tolerance (Hietz and Briones, 2004).

Light is one of the key environmental factors for plants, which have developed numerous biochemical and evolutionary responses to optimize its use in photosynthesis, and therefore growth, of these organisms. Daily and seasonally, most plants receive more light than can be used in photosynthesis, so that regulation of the light-gathering process is necessary in order to balance the absorption, utilization and dissipation of light energy and minimize the potential for photooxidative damage (Müller *et al.* 2001). Excessive light can cause photoinhibition or photodamage that produces a decrease in the capacity of the photosynthetic apparatus or the redistribution of the absorbed energy into non-photochemical processes, as a photoprotection mechanism (Martin *et al*., 1999). *Guzmania lingulata*, a bromeliad species that grows in the shade, has a quantum efficiency of 0.78 before dawn and reduced CO_2 fixation when exposed to 33.6 $molm^{-2}d^{-1}$ of solar radiation, compared with *Vriesea jonghei*, a bromeliad species that grows exposed to solar radiation with a quantum efficiency of 0.82 and high CO_2 fixation when exposed to the same amount of solar radiation (Griffiths and Maxwell, 1999).

Interception of light by the leaves causes it to diminish in intensity within the canopy. Plants acclimated to the shade of the canopy tend to have higher specific leaf areas than those acclimated to light, a feature that allows them to better utilize the amount of light available to them (Lambers *et al*., 1998)

Vanilla is a perennial plant that requires shading to 50% of ambient light levels for most of the year, and up to 70% during intense sunshine, in order to maintain suitable levels of both soil and air moisture (Curti, 1995; Hernández, 2009). It is grown in a shade agroforestry system and requires a host tree the primary function of which is to provide support to the vanilla plant, although live hosts also provide the vanilla with a supply of organic matter (Challenger, 1998; Curti, 1995; Dressler 1990; Hernández, 2009).

In the vanilla growing region of Veracruz, Mexico, various vanilla cultivation methods are currently practiced; one involves the installation of host trees such as of *Citrus* sp., *Gliricidia sp*. and *Erythrina sp.*, while another system that is normally utilized on a smaller scale utilizes artificial hosts (made of cement and/or wooden poles) and a shade-mesh. These cultivation methods differ mainly in terms of soil type, light intensity, density of host trees, number of vanilla plants per host tree and host tree species, among other characteristics (Castro-Bobadilla and García-Franco, 2007; Hernández, 2009).

The objective of this study is therefore to document the physiological response of vanilla plants to variations in the levels of incident light generated by these different host types, through evaluation of the induced fluorescence of the chlorophyll.

MATERIALS AND METHODS

Plant material and cultivation conditions: The study area, comprising four delimited sites for each treatment, was established in La Union, in the municipality of Tihuatlán in the north of Veracruz State, Mexico (18°27' N and 96°21' W; 60 masl). The climate is warm-regular and the area has an average temperature of 22 °C with abundant rain in summer and early autumn.

Measurement of light radiation: Light incidence monitoring was carried out in each different cropping system over the period of one year. Measurements were taken at a height of 1.5 m every three days and monthly averages calculated from these values. All measurements were taken with a light meter (Sper Scientific Mod. 840 020) between 11:00 and 13:00 hrs, as this was considered to be the period of greatest light incidence.

Induced chlorophyll fluorescence: The study was conducted during the period mid-February to late April 2008. To determine the response of plants to treatments, variables related to the induced fluorescence of chlorophyll were recorded using a portable plant efficiency analyzer (PEA, Hansatech, King's Lynn, UK). The chlorophyll fluorescence parameters considered were: initial fluorescence (Fo), maximum fluorescence (Fm) and the Fv/Fm ratio. Measurements were taken every three days during the different phenological stages over the experimental period.

Experimental design and statistical analysis: The experiment was conducted following a completely randomized design, with a factorial arrangement of treatments and four replications. The factors (and levels) comprised cultivation system (shade-mesh, *Citrus sp*., *Gliricidia sp*., and *Erythrina sp*.) and phenological stage (pre-flowering, flowering and post-flowering). Each replication consisted of four plants.

The data were subjected to analysis of variance (ANOVA) and multiple comparisons of means by Tukey's method; these tests were performed with the SAS statistical program for personal computers (SAS Institute, ver. 8).

RESULTS

Cultivation systems that feature *Erythrina sp*. and *Gliricidia sp*. as hosts generate more heterogeneity, in terms of light incidence, thus subjecting the vanilla plants to highly contrasting light regimes throughout the year. By contrast, plantations with *Citrus sp*. and shade-mesh create greater stability and homogeneity in the incidence of light reaching the vanilla plants, with less variation observed between the months of highest and lowest incidence.

Plantations of vanilla featuring *Erythrina sp*. as the host present higher light incidences; in ranges from 38 to 54 µmol m^{-2} s^{-1} with an average of 49 µmol m^{-2} s^{-1} over a period of 5 months, with a period of low incidence ranging from 28 to 36 µmol m^{-2} s^{-1} for 7 months, and a difference of 48 % recorded between the highest and lowest monthly mean light incidence values. Unlike *Erythrina sp.*, the use of *Gliricidia sp*. as a host produces a period of 8 months with a high incidence of light ranging from 38 to 54 µmol m^{-2} s^{-1} and only 4 months of low incidence, ranging from 29 to 34 µmol m^{-2} s^{-1}, with a difference of 46 % between the minimum and maximum monthly incidence values, and high light incidence occurring for 67 % of the year. On the other hand, utilizing *Citrus* sp. and artificial hosts with shade-mesh produces a more homogeneous light incidence throughout the year: With *Citrus sp*., there are eight months of high light incidence ranging from 42 to 47 µmol $m^{-2}s^{-1}$, with a low incidence range of 38.9 to 39.3 µmol m^{-2} s^{-1} and a difference of 16 % between the months of lowest and highest incidence. While the artificial host and shade-mesh system presents seven-months of high incidence ranging from 40 to 42 µmol m^{-2} s^{-1} and a low incidence range of 36 to 37.6 µmol m^{-2} s^{-1}, with a 14 % difference between the highest and lowest mean monthly incidence of light (Figure 1).

According to the analysis of variance, there is statistically significant interaction between the cropping system (host type) and phenological stage evaluated for initial (Fo), and maximum (Fm) fluorescence and for the Fv/Fm ratio (Figure 2).

The Fv/Fm ratio behaved equally in plants grown under shade-mesh and with *Citrus* sp. as a host, being highest in the pre-flowering stage, decreasing during flowering and increasing in the last phenological stage. In vanilla plants grown with *Erythrina sp*. and *Gliricidia sp*., the behavior of the Fv/Fm ratio was less homogeneous during the flowering and post-flowering stages, but not during the flowering stage, where the values were higher than those reached by the same stage of plants grown under shade-mesh and with *Citrus sp* (Table 1).

Initial fluorescence (For) was relatively constant among the different phenological stages and host types with which the vanilla plants were grown. However, it is notable that the value of this parameter was higher in plants grown with *Gliricidia* as the host in all three phenological stages analyzed.

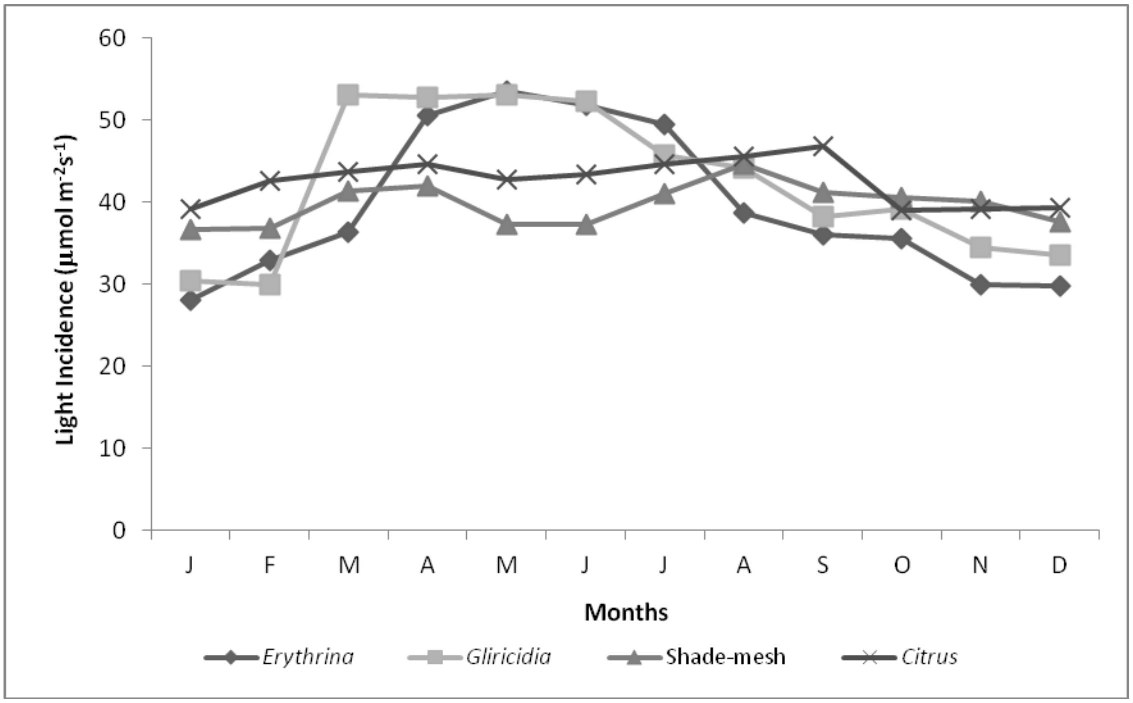

Figure 1. Light intensity evaluated over a year under different cultivation systems of the vanilla plant (*Vanilla planifolia* Andrews).

For maximum fluorescence, plants grown under shade-mesh and *Citrus* sp. presented more homogeneous behavior compared to the two other host tree species. The Fm value was greatest in plants grown below shade-mesh and with *Citrus sp*., followed by those grown with *Gliricidia* in the pre-flowering stage. In the next two phenological stages, the vanilla plants grown with *Gliricidia* presented the highest Fm values.

DISCUSSION

This result could indicate the existence of a biophysical difference in the photosynthetic apparatus of plants grown under a constant rate of light incidence. This difference could be related to the ability to develop under the shade conditions dictated by the vegetation canopy typical of their habitat (Berrocal *et al.*, 2002).

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Fv/Fm ratio is a parameter frequently used as an indicator of the actual and potential PSII quantum efficiency of plants in the presence or absence of environmental stress (Agati *et al*., 1996). It is typically very stable in healthy leaves, where values range around 0.8 to 0.83 in vascular plants and green algae (Peña *et al*., 1999; Ospina *et al.*, 2006). When Fv/Fm values fall below 0.80 this signifies potential damage to the photosynthetic apparatus by the factors that cause inhibition of the PSII reaction centers and increased heat dissipation (Twin *et al.,* 1997; Heinz, 1999). The lower values of this ratio may be due to a decrease in Fm: a reduction that persists for several days is a sign of damage to the PSII due to a stress factor such as excessive light or extremes of temperature. The Fv/Fm ratio is well correlated with the quantum yield of photosynthesis, measured by the production of O_2 or absorption of CO_2 at low light intensities (González *et al*., 2008).

Chlorophyll fluorescence is inversely related to the rate of photosynthesis (Krause and Weiss, 1984; Lichtenthaler, 1987; Sanclemente and Peña, 2008). When the process of photosynthesis is subject to stress, levels of variable fluorescence (Fv) decrease.

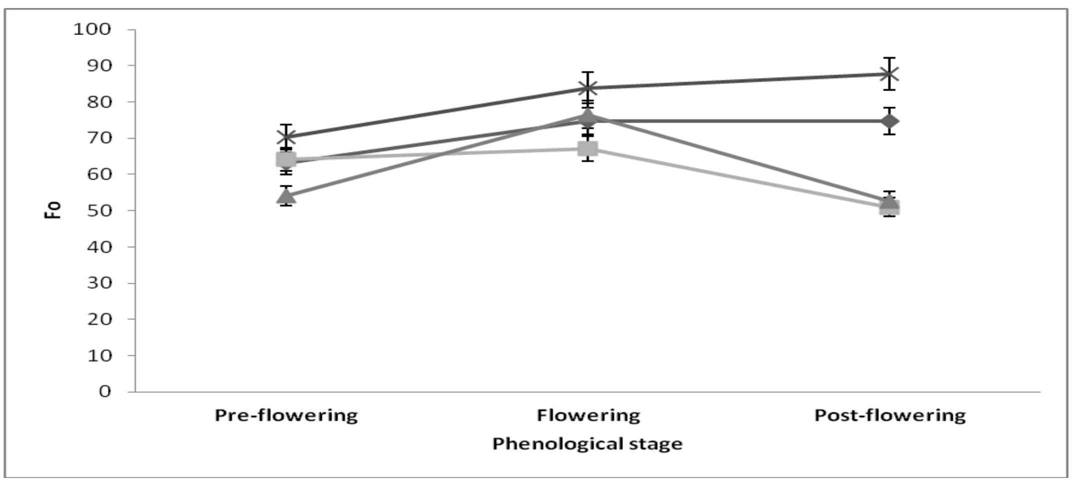
Table 1. Parameters of induced chlorophyll fluorescence determined in three phenological stages (pre-flowering, flowering and post-flowering) of vanilla plants grown with different host types (Artificial host + shade-mesh, *Citrus sp*., *Gliricidia sp*. and *Erythrina sp*.).

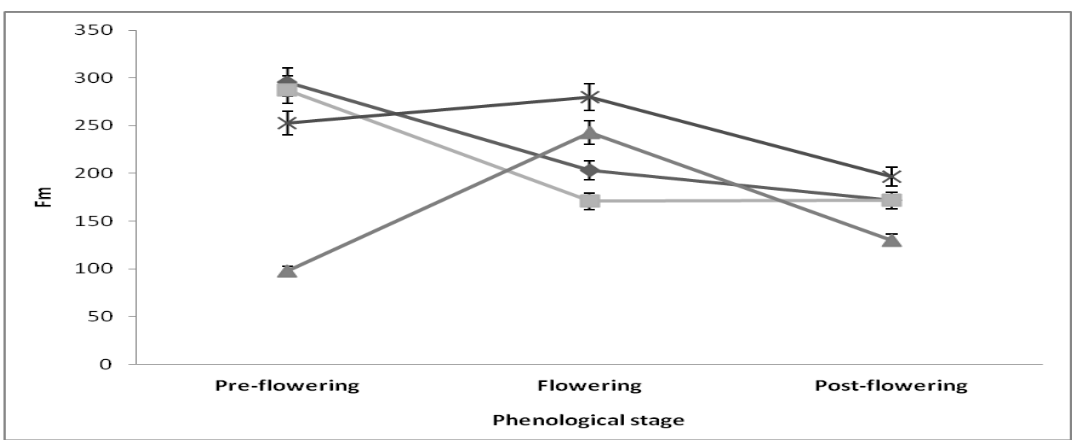
Host type	Phenological Stage	Fo	Fm	Fv/Fm
Artificial host + shade-mesh	Pre-flowering	63.066 c	295.666 a	0.773 a
	Flowering	74.733 b	203.200 c	0.599 c
	Post-flowering	74.733 b	171.621 d	0.679 b
Citrus sp.	Pre-flowering	64.133 c	287.800 a	0.771 a
	Flowering	67.133 c	170.866 d	0.586 c
	Post-flowering	51.000 e	171.724 d	0.684 b
Erythrina sp.	Pre-flowering	54.000 d	97.666 f	0.422 e
	Flowering	76.470 b	243.000 b	0.671 b
	Post-flowering	52.656 e	130.187 e	0.586 c

***Gliricidia* sp.**	**Pre-flowering**	**70.333 b**	**252.800 b**	**0.705 b**
	Flowering	**83.937 a**	**279.625 ab**	**0.687 b**
	Post-flowering	**87.666 a**	**196.777 c**	**0.533 d**

Values sharing a letter do not differ significantly ($P \leq 0.05$)

The present study shows that the Fv/Fm ratio remains relatively stable in plants grown under shade-mesh and with *Citrus* sp., where incident light exhibits little variation throughout the year. However, growth with the other two host types causes a reduction in the value of this parameter, indicating that the plants are exhibiting the effects of the stress caused by excessive light, among other factors.





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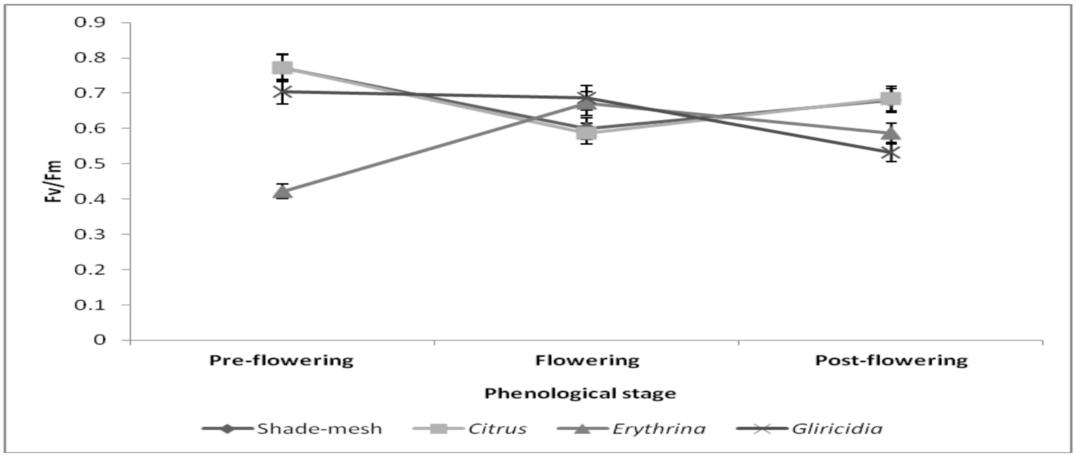

Figure 2. Interaction between host type (Artificial host + shade-mesh, *Citrus* sp., *Gliricidia* sp. and Erythrina sp.) and phenological stage (pre-flowering, flowering and post-flowering).

The phenological stage that presents the lowest Fv/Fm ratio value coincides with the period during which these host types undergo leaf senescence as part of their life cycle.

On the other hand, initial fluorescence (Fo), the reaction of which originates in the antenna pigment, increases frequently under stress conditions. This has been reported in studies conducted with certain herbicides that cause blockage of the photosynthetic electron transport, or when the reaction centers of the PSII chlorophyll are destroyed by photooxidation (Lichtenhaler and Buschmann 1984; Lichtenhaler and Rinderle, 1988).

CONCLUSIONS

Vanilla plants grown in a cultivation system where the incidence of light does not change significantly throughout the year present reduced photochemical damage: this is reflected in the photosynthetic efficiency of these plants. However, vanilla plants grown with deciduous hosts are subject to the stress caused by high light incidence, generating a photochemical alteration that has an impact on net photosynthesis.

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