The effect of chilling on the photosynthetic activity in coffee (*Coffea arabica* L.) seedlings. The protective action of chloroplastid pigments

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Coffea arabica is considered to be sensitive to low temperatures, being affected throughout its entire life cycle. Injury caused by chilling (low temperatures above zero degree centigrade) is characterized primarily by inhibition of the photosynthetic process. The objective of this work was to evaluate the role of photosynthetic pigments in the tolerance of coffee (*C. arabica* L.) seedlings to chilling. The evaluation the photosynthetic activity was made by emission of Chl *a* fluorescence at room temperature (25 °C) *in vivo* and *in situ*, using a portable fluorometer. The pigment content was obtained by extraction with 80 % acetone, while estimation of membrane lipid peroxidation was determined by measuring the MDA content in leaf tissue extracts. The results indicated a generalized reduction in the quantum yield of PSII when the seedlings were maintained in the dark. The reduction occurred in the seedlings submitted to chilling treatment as well as in the control ones. This demonstrates that not only chilling acts to cause an alteration in PSII. It is possible that the tissue storage reserves had been totally exhausted, with the respiratory rate exceeding the photosynthetic rate; the later was nil, since the seedlings were kept in the dark. The efficiency in the capture, transfer and utilization of light energy in PS_{II} photochemical reactions requires a sequence of photochemical, biochemical and biophysical events which depend on the structural integrity of the photosynthetic apparatus. However, this efficiency was found to be related to the protective action of chloroplastid pigments, rather than to the concentration of these pigments.

Key words: chlorophyll *a* fluorescence, coffee, lipid peroxidation, photosynthetic pigments.

Efeito do resfriamento na atividade fotossintética em plântulas de café (C*offea arabica* L.): a ação protetora dos pigmentos cloroplastídicos: A espécie *Coffea arabica* é considerada sensível às baixas temperaturas, podendo ser afetada em todo o seu ciclo. A injúria pelo frio (temperaturas baixas acima de zero) se caracteriza, principalmente, pela inibição do processo fotossintético. Este trabalho teve como objetivo avaliar a participação dos pigmentos fotossintéticos na tolerância de plântulas de cafeeiro (*C. arabica* L.) à temperatura de resfriamento. A avaliação da atividade fotossintética foi feita através da emissão de fluorescência da clorofila *a* à temperatura ambiente (25 °C) *in vivo* e *in situ*, usando um fluorímetro portátil. O conteúdo dos pigmentos foi obtido a partir da extração com acetona 80 % e a peroxidação lipídica estimada a partir das medidas do conteúdo de MDA em extratos do tecido foliar. Os resultados indicaram uma redução generalizada na eficiência quântica do PSII quando as plântulas foram mantidas no escuro. A redução ocorreu tanto nas plântulas submetidas ao tratamento de resfriamento, quanto no material controle. Isso demonstra que não apenas a temperatura de resfriamento estaria atuando como causa dessas alterações na atividade do PSII. É provável que tenha ocorrido um esgotamento das reservas do tecido, com a taxa respiratória excedendo à fotossintética, já que essa era nula, uma vez que a energia de excitação esteve comprometida pela permanência das plântulas no escuro. A eficiência na captura, transferência e utilização da energia luminosa em reações fotoquímicas no PSII, requer uma seqüência de eventos fotoquímicos, bioquímicos e biofísicos intimamente dependentes da integridade estrutural do aparelho fotossintético. Sendo que essa eficiência se mostrou mais dependente da ação protetora dos pigmentos cloroplastídicos do que propriamente da concentração desses.

Palavras-chave: café, pigmentos fotossintéticos, fluorescência da clorofila *a*, peroxidação de lipídios.

INTRODUCTION

The economic exploitation of coffee plants, *Coffea arabica* L., is limited to regions where the average annual temperature varies between 18 and 22 °C (Bauer et al., 1985). This species is considered to be sensitive to low temperatures (Levitt, 1980; Bauer et al., 1985), presenting decreased growth and stunting (Smille et al., 1988); coffee plants are also prone to injuries caused by low temperatures (Levitt, 1980). Studies involving the mechanisms that modulate tolerance or susceptibility to chilling in coffee are rare (Bauer et al., 1985; Queiroz et al., 1998).

The ability of plants to tolerate chilling stress has been studied as an important ecophysiological adaptation phenomenon and is the subject of intense research in the field of plant biology with many agricultural applications (Bauer et al., 1985; Smillie et al., 1988; Alonso et al., 1997; Queiroz et al., 1998).

In general, chilling injury is characterized by inhibition of the photosynthetic process (Hodgson and Raison, 1989), by alteration of cellular functions (Graham and Patterson, 1982) and, in extreme conditions, by deterioration of these functions, which may result in plant death. The activity of certain antioxidant enzymes may be intensified in leaves acclimatized to low temperatures (Schöner and Krause, 1990). There could also be an alteration in the composition of leaf pigments conferring greater capacity to tolerate the stress effects. Some chilling injuries present evident symptoms, such as leaf chlorosis, suggesting destruction of photosynthetic pigments (Muruyama et al., 1990).

Recent studies indicate that cold stress is expressed when peroxidation of cellular membrane lipids is triggered, causing alterations in fluidity (Merzlyak, 1994; Alonso et al., 1997; Queiroz et al., 1998). The most important biochemical events associated with modifications in membrane fluidity include changes in enzyme activity and loss of cell compartments. The degradation of polyunsaturated fatty acids by peroxidation produces not only peroxide ions but also MDA. Therefore, changes in the MDA concentration have been used as lipid peroxidation indicators and, consequently, of the chloroplastidic membrane integrity (rich in polyunsaturated fatty acids) in plants submitted to stress (Dhinsda et al., 1981; Hodgson and Raison, 1991; Jouve et al., 1993; Qiu and Liang, 1995).

The photosystem II (PS_{II}) reaction centers are obviously well protected against adverse environmental effects (low temperature, excess light, water and nutritional deficits, etc.) and are only affected when this protection is overcome. The stress effect on photosynthetic activity is manifested in many different ways, leading to efficiency loss in the photosynthetic apparatus, such as decreased use of photons for NADPH, ATP and carbohydrate production.

Recent proposals to study the reaction of plants submitted to extreme temperature conditions consider the use of less invasive techniques that allow the extrapolation of results to natural conditions. In this research, the effects of chilling on the photosynthetic activity of coffee seedlings were studied by fluorescence induction of Clo *a, in vivo*, which yields information about the functioning of the photosynthetic apparatus (Schreiber, 1983; Krause and Weis, 1984, 1991; Krause, 1994; Govindjee, 1995). The use of this technique has contributed greatly to the understanding and monitoring of environmental stress effects on photosynthesis (Govindjee, 1995) or even for the selection of cultivars less susceptible to photoinhibition induced by low temperatures (Bjorkman and Demming, 1987).

The objective of this study was to evaluate the participation of photosynthetic pigments in the tolerance of coffee seedlings (*C. arabica* L.) to chilling.

MATERIAL AND METHODS

Plant material and treatments: C. arabica L. seedlings cv. 'Catuaí Amarelo' were used. The seeds were provided by the Agronomic Institute of Campinas-IAC. Seedlings with expanded cotyledons were transplanted into plastic pots (500 mL) and transferred to a growth chamber under low light intensity (80 $\mu mol.m^{-2}.s^{-1}$), with an 8 h photoperiod. The temperature was controlled (25 °C / 20 °C, day/night) until the application of the chilling treatments (3 weeks). One week after transfer, the seedlings started to accumulate chlorophyll pigments; after three weeks the leaves were completely green. During this period, the seedlings were fertilized once a week with nutrient solution (Hoagland and Arnold, 1951) and soil humidity was maintained by daily watering. The position of the plastic pots in the growth chamber was changed once a week, to allow equal light interception by the cotyledons and to minimize any differences in PPFD on the plants.

Seedlings with one pair of cotyledons were then transferred to growth chambers (Forma Scientific Inc., model 24) previously adjusted to the treatment temperature (10 ± 3 °C – chilling treatment and 25 ± 3 °C – control). The seedlings were kept in darkness for the duration of the test (twenty days).

Chl *a* fluorescence kinetics, pigment content and lipid peroxidation were evaluated by sampling at two and four day intervals, respectively, for both treatments, with four replications represented by four independent assays.

Chlorophyll a fluorescence kinetics: Chl *a* fluorescence was determined at room temperature (25 °C) *in vivo* and *in situ*, using a portable fluorometer PEA (Plant Efficiency Analyser, Hansatech Instruments Ltd.). Seedlings were pre-adapted in the dark for 30 min before fluorescence determination. This 30 min period was sufficient for the oxidation of the entire photosynthetic electron transport system (Oliveira, 1995). Following exposure to saturating light (2,400 $\mu mol.m^{-2}.s^{-1}$) and complete "opening" of the system, the fluorescence peak emitted was considered as maximum fluorescence (F_M). The following variables of Chl *a* fluorescence were evaluated: initial fluorescence (F_o), F_M, variable fluorescence (F_V), the area above the fluorescence curve between the values F_o and F_M and the ratios F_V/F_M and F_V/F_o.

A mathematical relationship with the equations of the curves was made to express, by means of curves, the effects of the chilling treatment proportionally to the control. The resulting equation expresses the response of each variable after the application of the chilling treatment as a percentage of the control. An example of the mathematical relationship used is given below.

Control treatment $\Rightarrow Y_{co} = a_{co} + b_{co}x + c_{co}x^2$ (Eq. 1)

Chilling treatment $\Rightarrow Y_{ch} = a_{ch} + b_{ch}x + c_{ch}x^2$ (Eq. 2)

Chilling treatment as a percentage of the control

$$\Rightarrow Y_{\%} = (Y_{ch} / Y_{co}) \cdot 100 \qquad \text{(Eq. 3)}$$

Equation 3 was used to obtain the points plotted in the figures expressing the response of the variables in relation to the control (% of control). The curves were appropriately adjusted from these points.

Pigment content: The Chl *a* and chlorophyll *b* (Chl *b*) and carotenoid (C_{x+c}) concentration was determined using a 50 mg (FW) sample of leaf tissue collected and placed in a test tube containing 5 mL 80 % acetone. The sample was incubated at 70 °C for 20 min, cooled in the dark and then the volume was adjusted to the initial 5 mL. Absorbance was monitored by a B390 Micronal spectrophotometer, at wavelengths of 470, 646 and 663 nm. Pigment concentration was calculated according to the procedure described by Lichtenthaler and Wellburn (1983). The concentration of Chl *a* and Chl *b* and C_{x+c} was expressed in relation to the concentration of these pigments in the plant tissue at the beginning of the assay. The best fitting curves using the polynomial model were used. The rate of pigment accumulation was also calculated.

Lipid peroxidation: The membrane lipid peroxidation was estimated by measuring the malondialdehyde (MDA) content, a by-product of lipid peroxidation in leaf tissue extracts. The MDA concentration was calculated from 150 mg (FW) of leaf tissue samples, using the procedure described by Dhindsa et al. (1981), adapted from Heath and Packer (1968). The extinction coefficient (ξ) for this assay was 155 $mM^{-1}.cm^{-1}$. The MDA concentration was expressed as a function of the period of acclimatization of the treatments and of the total pigment content. For the 10 °C treatment, the data was adjusted in two straight lines, representing two distinct stages: the first one, from the beginning until the 12th day and the second starting on the 12th day until the end of the assay. For the 25 °C treatment the data were adjusted as a single straight line.

RESULTS

The plants maintained at 25 °C initially presented an increasing quantum yield of PS_{II} until the third day with a subsequent decrease. This greater initial yield also resulted in an increased flow of electrons, characterized by the initial decrease in the F_o curve (data not shown). From the third day on, the quantum yield in the control treatment decreased, apparently at a constant rate, until the end of the assay.

Plants submitted to chilling temperatures maintained the same quantum yield until the 6th day. After that, the ratio F_V/F_M decreased until the end of the experiment. The values of F_V/F_M for the 10 °C treatment plants were similar to those for the control between the 6th and 12th day, whereas after day 12 the reduction of F_V/F_M in the plants at 25°C was greater, differing from the results for the plants treated at 10°C (figure 1A).

The relative quantum yield (% relative to control) of seedlings submitted to chilling temperatures, after an initial decrease, increased from the fourth day on, surpassing the control after the 9th day (figure 1B). With a decrease in the quantum yield of the control material, the relative yield of the plants treated at 10 °C increased, surpassing, at the end of the assay, the yield of the control plants by more than 6% (figure 1B).

In relation to the F_o signal, it was observed that the increase in F_o was greater in the seedlings treated at 10°C during the first 16 days of the assay, reaching the maximum around the 8th day (figure 2A). Thereafter, the F_o increase in the seedlings submitted to the chilling temperature was lower (in relation to the increase in the control) and was exceeded by the control levels after the 16th day.

F_M, in relation to the control (figure 2B), decreased until the 3rd day, and then increased, surpassing the control values after the 6th day and reaching a maximum around the 16th day. The control treatment (25 °C) results, for the first three days, differed from the chilling treated plants in that there was an increase in F_M. This response pattern was similar to that observed for the F_o variable.

Completing these observations, the plastoquinone pool response (represented by the area traced between the signals by F_o and F_M), was analyzed as a function of exposure time to the treatments (data not shown). It was determined that the significant difference between the data for the chilling and that for the control treatment, was due the plastoquinone pool which at 10 °C remained low, while in the control treatment it increased, practically doubling the initial values. Considering the relative values (% of the control) of the plastoquinone pool (figure 2C), a constant decrease in these values was observed during the treatment at 10 °C.

The F_V/F_o ratio showed a response pattern similar to the F_V/F_M ratio but with significantly higher values. At the end of the assay (19th day), the F_V/F_o ratio for the control treatment decreased to approximately 20% of its maximum value; which was a much steeper decrease in comparison with the F_V/F_M ratio (approximately 6%). The chilling temperature treatment caused a decrease in the F_V/F_o ratio throughout the entire period of the study, with a smaller decrease in the F_V/F_M ratio, than the control treatment. Both ratios presented a high correlation regardless of the treatment (figure 2D).

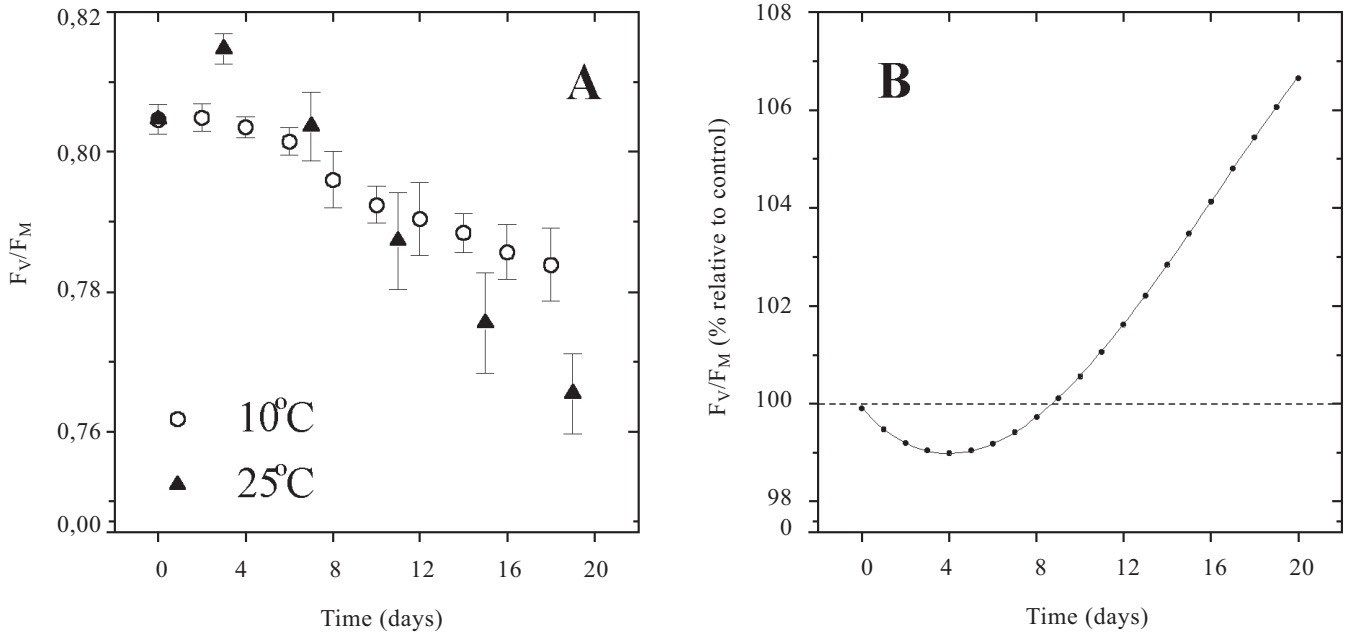

Figure 1. PS_{II} quantum yield (F_V/F_M) in coffee plant leaves, as a function of the period of acclimatization to temperatures of 10 °C and 25 °C (A). The curve in figure B corresponds to the chilling treatment with the results expressed relative to the control. Each point represents the average of four repetitions; bars represent the standard error.

For the seedlings maintained at 25 °C, the accumulation in the content of all pigments presented a similar pattern. The pigment content as a function of time was mathematically adjusted using a 3rd degree polynomial, describing three distinct phases. At the beginning of the treatment, the increase in the pigment content occurred very rapidly until the 7th day, approximately three fold for the chlorophylls or even four fold for the carotenoids, in comparison with their initial contents. In the second phase, between the 7th and the 15th day, there were small increases in the contents. The third phase of the greening process occurred from the 15th day until the end of assay (19th day) (figures 3A, 3B and 3C). The increase rates in the pigment content in this 3rd phase were greater when compared to the second phase but lower than the values observed during the first phase (table 1). During this period, the seedlings accumulated chlorophyll *a* and *b* as well as carotenoids, which were about 2/3 of the total content.

The concentration of carotenoids, which include carotenes and xanthophylls (figure 3C), presented the same pattern as the green pigments but with greater accumulation rates, especially during the 3rd phase of the process (table 1).

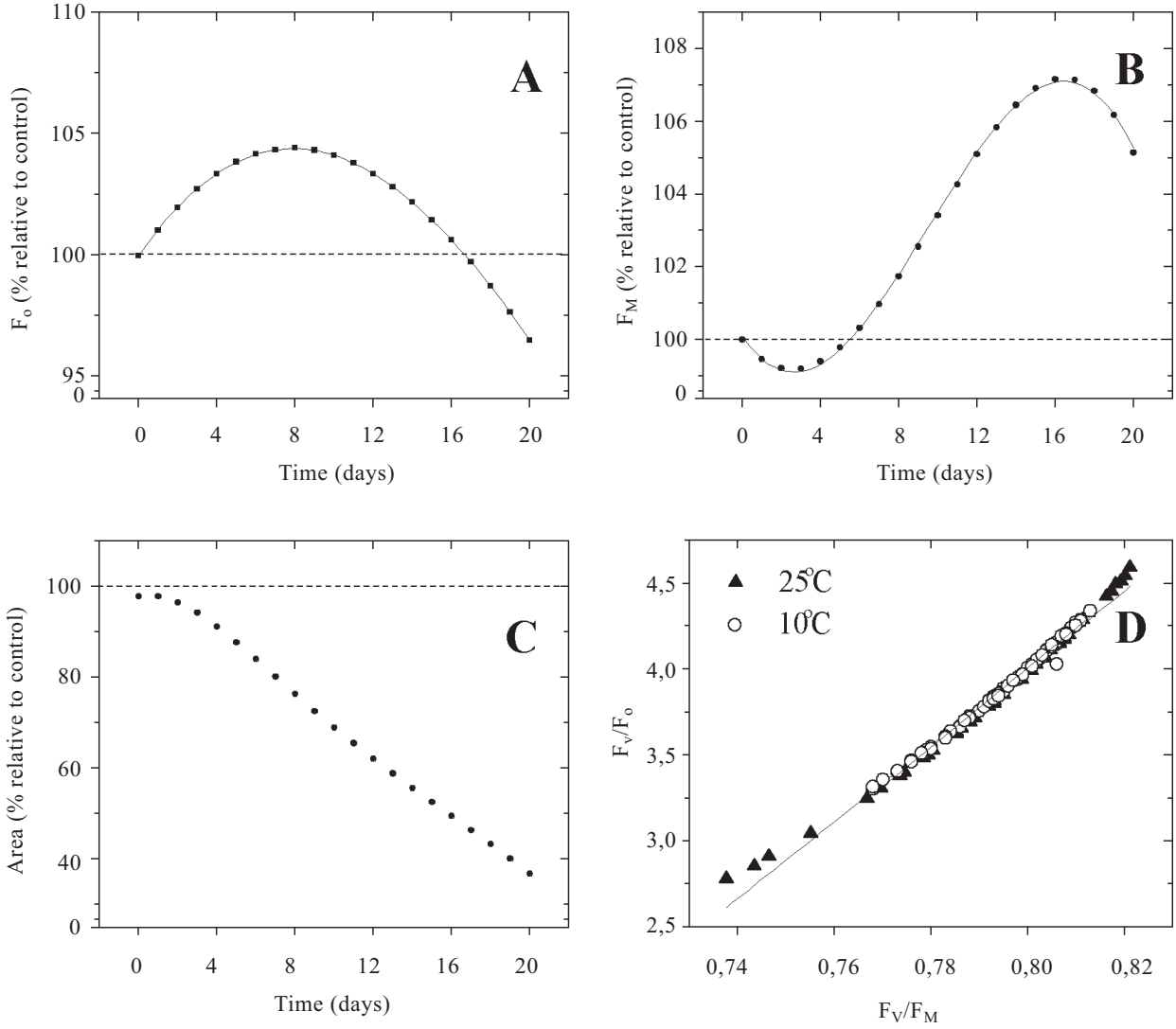

Figure 2. Initial fluorescence (F_O – A), maximum (F_M – B) and area above the fluorescence curve (C) as a function of the period of acclimatization to the temperatures of 10 and 25 °C. Figures 2A, 2B and 2C correspond to the chilling treatment (10 °C) with the results expressed relatively to the control (25 °C). Figure 2D represents the correlation between the ratios F_V/F_M and F_V/F_O measured in coffee seedlings. Each point represents the average of four repetitions.

The accumulation pattern for the chilling treatment was similar when the pigments were compared, describing an asymptotic curve, and stabilized on the 8th day (figures 3A, 3B and 3C). The pigment content during this treatment showed an intense increase in the first 8 days (with average rates around 35 % per day for the chlorophylls and 33 % for the carotenoids), and then stabilized. At the end of this phase, the increase in pigment content was around 3.7 fold the observed values at the beginning of the treatment and close to that observed for the control treatment during the same period. The second phase began after the 8th day, with reduced rates (rates of approximately 2 %), while the accumulation continued in the control seedlings, especially by the 19th day (figures 3A, 3B and 3C).

A decrease in MDA was observed in the control treatment, with decreasing rates practically throughout the entire period of the experiment. The chilling treatment resulted in a less intense decrease. In spite of the stress conditions (10 °C) to which the plants were subjected, the level of lipid peroxidation decreased during the treatment period.

The plants submitted to chilling stress presented, basically, two phases in the lipid peroxidation level (figures 4A, 4B). The first phase ended on the 12th day of treatment. During this period, the MDA concentration was practically unaltered, which corresponded to the stabilization in the levels of chloroplastidic membrane degradation. A second phase began after the 12th day, lasting until the end of the assay. In this phase, the MDA concentration in the tissues decreased; however, with higher values than the plants treated at 25°C during same period (figure 4A).

A highly significant correlation (r = 97.1 %) was observed between the accumulation of total pigment accumulation and the MDA concentration for the control treatment (figure 4B). High pigment concentrations were seen with the lower lipid peroxidation levels (lower MDA concentration). Two phases were observed in this correlation in the chilling treatment: the first showed a smaller decrease (r = 85.8 %), followed by the second (r = 86.3 %), calculated from the values obtained from the 12th day on (second phase of the peroxidation activity), showing a greater decrease. The results indicate that chilling also causes (due to the reduction of the metabolic activity) a reduction in the peroxidation activity, although in this treatment (10 °C) the correlation between peroxidative activity and pigment concentration was lower (86.0 %) than that seen in the control treatment (97.0 %) (figure 4B).

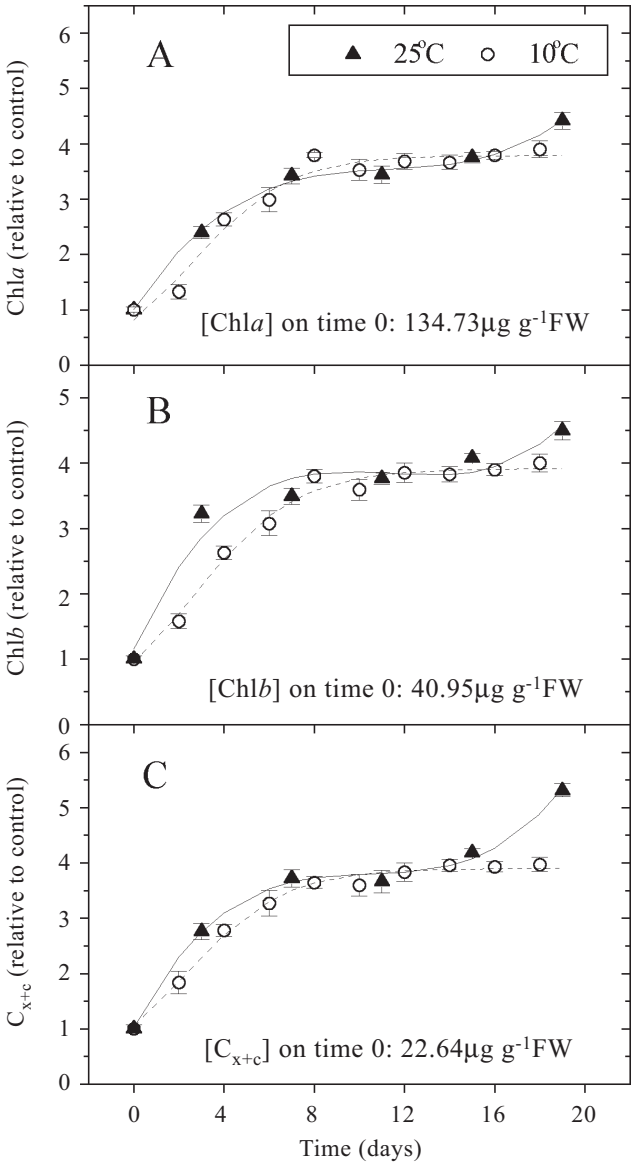

Figure 3. Alterations in the concentration of Chl *a* (A) and, Chl *b* (B) and of C_{x+c} (C) in coffee seedlings, in response to the period of acclimatization to the chilling treatments (10 °C) and control (25 °C). Each point corresponds to the average of four repetitions; bars represent the standard error.

Table 1. Rate of accumulation of chloroplastid pigments in coffee seedling cotyledons during the period of acclimatization at a temperature of 25 °C. The values correspond to the daily average rate of pigment accumulation within each phase.

Pigment	Time interval (days)		
	1st Phase (0-7)	*2nd Phase* (7-15)	*3rd Phase* (15-19)
Chl *a*	34.5	4.0	17.0
Chl *b*	35.5	7.4	10.5
Carotenoids	39.0	6.0	28.0

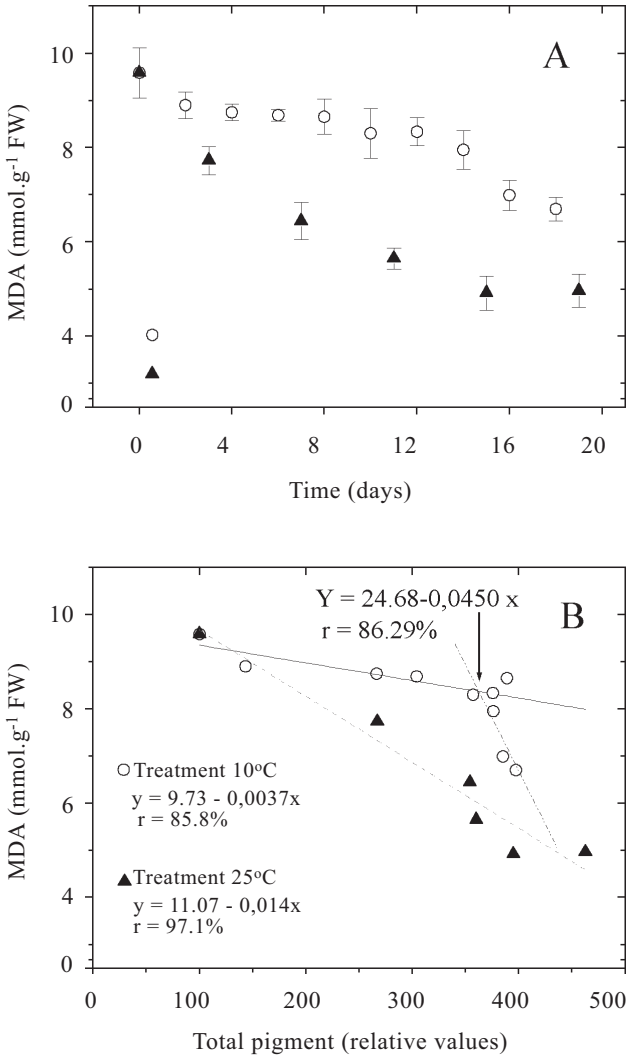

Figure 4. Alterations in the lipoperoxidative activity, expressed as MDA concentration, in coffee cotyledons, as a function of the period of acclimatization to the temperatures of 10 and 25 °C (A) and of the total pigment concentration (B). Each point corresponds to the average of four repetitions. In figure B, the arrow indicates the 12th day, when the second phase of the chloroplastid membrane lipid peroxidative activity began; the second straight line (with the equation and the coefficient r), in the treatment at 10 °C, was adjusted to the values obtained in the second phase (after the arrow).

DISCUSSION

The results of this study show a reduction in the PS_{II} quantum yield, signaled by the F_V/F_M ratio response. This reduction occurred in the plants submitted to chilling as well as in the controls. This decrease in the quantum yield may have been caused by the reduced flow of electrons through the PS_{II}, observed by an increase in F_o for both treatments. Bilger et al. (1984), Ducruet and Lemoine (1985) and Buknhov et al. (1990) described the increase in F_o as a consequence of the electron flow inhibition from Q_A to Q_B. Rosenqvist et al. (1991) attribute the increase in F_o to functional damage of P_{680} in the center of the reaction of PS_{II}. F_o signals a route of energy dissipation in the form of fluorescence through Chl *a* from the antenna complex, after capturing excitation energy, when P_{680} is in the oxidized state (Strasser et al., 1995).

According to Oquist (1983), low temperatures drastically affects the photosynthetic apparatus in cold sensitive plants. Coffee seedlings, which are cold sensitive (Levitt, 1980; Bauer et al., 1985), were also affected by cold temperatures. Low temperatures generally cause a decrease in the entire metabolism and also in the photochemical steps of photosynthesis, which are interdependent on the biochemical phase and, as expected, more directly influenced by low temperatures (Wang, 1982; Oquist, 1983). Creencia and Bramlage (1971) considered that although the first consequence may be a reduction in the F_V/F_M ratio, the damage caused by low temperature is not restricted to the photosynthetic apparatus. A relevant aspect, regarding the effect by low temperatures on the photosynthetic activity, is the physical alteration of membranes. These, distributed in organized manner as a network all over the chloroplast, support the photosynthetic electron transport (Hall and Rao, 1995). A decrease in temperature induces an increase in the predominance of the lipid domains in gel state, in the chloroplastid membranes, causing a greater rigidity of these membranes (Lyons, 1973; Murata and Fork, 1975). As a consequence, there is a decrease in the lateral diffusion of PQ, which reflects in the PQ pool and in the rate of PQ reduction (Bowyer and Leegood, 1997).

In this study on coffee seedlings, the amount of PQ decreased drastically with the chilling temperature. If there was less plastoquinone (smaller pool) to respond after its reduction, via P_{680} re-oxidization, and furtherl, a decrease in the electron flow through PS_{II}, it is probable that, as a consequence, there was less Q_A^- (quinone in the reduced state). The decrease in the Q_A reduction rate in the coffee seedlings could have been the cause of the systematic reduction in the F_M signal (data not shown). This reduction was more intense than the control treatment and was reflected in a relative increase of F_M in the 10 °C treatment (figure 2B).

According to Schreiber (1983) and Xu et al. (1989), the redox state of the primary Q_A electron acceptor and the H^+ inter-membrane gradient are the main factors that control the PS_{II} photochemical reactions. The efficiency in the capture, transfer and use of light energy in PS_{II} photochemical reactions requires a sequence of photochemical, biochemical and biophysical events (Nobel, 1991). These, however, are energy dependent, whether for the synthesis of constitutive (proteins), metabolic or structural compounds, such as thylacoid membranes. The maintenance of this photosynthetic apparatus structure in a typical and functional arrangement is also energy dependent, being directly affected by the metabolism decrease caused by chilling (Lyons, 1973; Wang, 1982).

The decrease in the Q_A reduction rate and electron flow, as well as the reduction in the PS_{II} quantum yield, were observed in the coffee seedlings in both treatments. This demonstrates that not only chilling was acting as the cause of these alterations in the electron flow. It is possible that a decrease in all the energy transfer steps might have occurred, since the excitation energy was hindered due to the fact that the seedlings were kept in darkness. Probably, there was drainage of the storage tissue, with the respiratory rate exceeding the photosynthetic rate, since the latter was nil. As the metabolism was repressed in the seedlings submitted to chilling, the respiration rate was lower than in the control treatment, leading to a slower drainage of the reserves in these seedlings. This would explain the more drastic decrease in the PS_{II} quantum yield in the seedlings treated at 25 °C, compared to the ones treated at 10 °C, as observed after the 10^{th} day of treatment.

The variation in the F_V/F_o ratio, throughout the entire period of acclimatization of the seedlings in this experiment, was shown to be similar to the F_V/F_M ratio, which reflects the PS_{II} quantum yield, but with greater amplitude of values. However, the use of the latter ratio (F_V/F_M), allows a better discrimination of the small differences in the PS_{II} quantum yield, which can be used to select ecotypes more adapted to low temperatures. For Schindler and Lichtenthatler (1994), the F_V/F_o ratio also presented values with greater amplitude than F_V/F_M in studies of photoinhibition.

The results related to the alterations in the content of photosynthetic pigments showed a tendency to a sharp increase in the concentration of the accessory pigments (Chl *b* and carotenoids) when compared to Chl *a*, probably in order to increase the photon capture, essential to seedling survival. The accessory pigments are responsible for the capture and transfer of photons to the reaction center (Lichtenthaler and Calvin, 1964; Hall and Rao, 1995).

A significant increase in the carotenoid content in the seedlings was not observed, even with the low temperature stress. These pigments are involved in protection strategies against photoinhibition (Hall and Rao, 1995). Plants maintained in the dark were not subjected to the photoinhibitory process and all its damaging consequences to the photosynthetic apparatus. Without the risk of photoinhibition, there was no need for greater carotenoid accumulation in the coffee seedlings treated at 10 °C. The carotenoid group of pigments are present in the PS_I and PS_{II} antennae complex. They can be found more abundantly in the lutheine, β-carotene, neoxanthine, violaxanthene, anteroxanthene and zeanxanthine forms in PS_{II} (Bowyer and Leegood, 1997). The xanthophyll group plays an important role in the plant protection mechanism. Through the xanthophyll cycle, these pigments allow for the non-photochemical dissipation of the energy excess, protecting PS_{II} against damage caused by the photoinhibitory process in plants sensitive to certain environmental stresses, such as low temperature (Sarry et al., 1994; Thiele and Krause, 1994). Under these conditions, there is a significant decrease in the photosynthetic capacity of the plant tissue (Rosenqvist et al., 1991).

Similarly to protein degradation, lipid peroxidation also has a triggering agent, the action of free radicals. These, through a cascade reaction, lead to the formation of reactive species of oxigen (superoxide anion radical, hydroxyl radical, etc.) that are highly damaging to the integrity of the thylacoids (Barber and Anderson, 1992). However, to generate free radicals, light energy is required (Barényi and Krause, 1985; Richter et al., 1990). Since this experiment was conducted in the absence of light, the formation of these reactive species was not stimulated.

During the chilling treatment, the level of chloroplastid membrane degradation was, at the first stage, stable. However, at the second stage a sharper reduction in the peroxidative activity was observed, possibly due to the decreasing production of reactive oxygen species in response to darkness, and the greater concentration of chloroplastidic pigments.

The results demonstrated that pigment accumulation during the chilling treatment could be one of the factors responsible for the decrease in lipid peroxidative activity. Since the correlation between this activity (peroxidative) and the pigment concentration was lower (86%) in the 10 °C treatment than in the control treatment (97 %), it indicated that the chilling temperature also influences (due to the decrease in metabolic activity) the peroxidative activity. The synthesis of pigments (especially xanthophylls, from the carotenoid group) may act as a protective mechanism (Schindler and Lichtenthaler, 1994; Thiele and Krause, 1994), possibly against the formation of free radicals (Young 1991), inhibiting the peroxidation of constitutive thylacoid membrane lipids (Sarry et al., 1994). This protection against the "attack" on the integrity of the chloroplast membrane is very important for the maintenance of the photosynthetic activity as a whole (Oquist, 1983; Dwivedi et al., 1995). This was observed here in the first six days, when the PS_{II} quantum yield (F_V/F_M) of the 10 °C treatment was kept above 0.800 – a high yield.

Our results suggest that the maintenance of the integrity of the thylacoid membranes in coffee seedlings is crucial for the conservation of high PS_{II} quantum yield, whereas this efficiency was shown to be more dependent upon the protecting action of the chloroplastidic pigments than the actual concentration of these pigments. The use of the F_V/Fo ratio in detriment to the F_V/F_M ratio allows for better discrimination of small differences in the PS_{II} quantum yield which can be used for the selection of ecotypes more adapted to low temperatures.

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