Photosynthesis and associated metabolism during development of a *Theobroma cacao* hybrid with the lethal factor *Luteus-Pa*

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Abstract

The recessive lethal character *Luteus-Pa*, expressed as a yellowing of leaves of young seedlings and followed by death approximately 60 d after emergence, presents a 3:1 segregation in crosses and/or selfpollinated plants. We evaluated quantitatively the fluorescence emission of chlorophyll (Chl), gas exchange, and chemical composition of normal and recessive homozygous cacao seedlings of the cross Pa 121×Pa 169. The characteristics of Chl fluorescence kinetics were studied in stages B_2, B_3, C, D, and E of leaf development, corresponding to plant ages of 9 to 12, 13 to 15, 16 to 20, 21 to 30, and >30 d, respectively. Gas exchanges were measured in mature leaves of both seedlings. In regular intervals of 3 d beginning at 33 d after emergence, the seedlings were separated into roots, stems, leaves, and cotyledons to determine the contents of saccharides (SAC) and free amino acids (FAA) and variation of the leaf Chl content. The Chl distribution in complexes of the photosynthetic apparatus was analysed by SDS-PAGE in mature leaves of both normal and recessive 32-d-old seedlings. There were variations in Chl fluorescence, gas exchanges and chemical composition of different parts of both types of seedlings. However, no significant differences were found in the Chl distribution through photosynthetic complexes of 32-d-old normal and recessive homozygous seedlings. After that period a decrease in the Chl concentration was observed in the recessive seedlings, and only minimum fluorescence (F_0) was found. The F_0 values were higher in the recessive seedlings than in the normal ones. The net photosynthetic rate of mature leaves was negative in agreement with low conductance, transpiration rate, and high internal CO_2 concentration. These factors might have contributed to a depletion in SAC in different plant parts. Although F_0 partially reflects the Chl concentration in leaf tissue, the increase in its value was probably due to a damage in reaction centres of photosystem 2. Therefore, the growth and development of recessive homozygous seedlings depended exclusively on cotyledon reserves, the depletion of which leads to death.

Received 5 November 1996, *accepted* 17 February 1997.

Additional key words: amino acids; cacao; chlorophyll; fluorescence induction; net photosynthetic rate; pigment-protein complexes; plant phylogeny; saccharides; transpiration.

Introduction

Compatibility investigations among clones of the Parinari (Pa) family imported from Peru showed that some seedlings from the crosses Pa 30×Pa 169, its reciprocal, and Pa 121×Pa 169 presented the total leaf chlorosis and consequent plant death *ca.* 60 d after emergence (Yamada *et al.* 1982). These authors observed a similar effect in the F2 progenies resulting from selfing of F1 plants of Pa 121×SIC 802 and Pa 121× Pa 169 grown at the Experimental Station of the Cacao Research Center (CEPEC), Bahia, Brazil. The same senescence character was also found in S1 seedlings resulting from the selfing of the Pa 30 and Pa 121 self-incompatible clones. The S1 seedlings were obtained using a technique in which a mixture of pollen from the clones and from plants of the *Herrania* genus was used (Bartley 1969).

Crosses among the Pa clones indicate no statistical difference between the cross Pa 30×Pa 169 and its reciprocal (Bartley *et al.* 1983). The senescence character segregates in a 3:1 proportion in seedlings obtained by selfing or crossing. These results are in agreement with the expected segregation of progenies, coming from the crosses of heterozygous individuals. The resulting seedlings are mutations the character of which is genetically controlled by a recessive gene the homozygous form of which is not viable due to the lethal effect (Bartley *et al.* 1983).

The objective of this work was to monitor and evaluate quantitatively the fluorescence emission of Chl, gas exchanges, variation, and distribution of Chl in the complexes of the photosynthetic apparatus, and chemical composition of normal and recessive homozygous cacao seedlings of the cross Pa 121×Pa 169.

Materials and methods

Plants: The experiment was conducted in a greenhouse (30 % of total incident radiation, temperature of 23±1 °C, relative humidity of 84±3 %), at the Cacao Research Center (CEPEC), Ilhéus, Bahia, Brazil (14°47'S, 39°16'W, 55 m a.s.l.) from August to September 1995. The seeds of the hybrid Pa 121×Pa 169 were obtained through controlled pollination and planted in polyethylene bags of 2.0 kg filled with soil (*Alfisol*, series CEPEC). The sampling began 33 d after emergence (DAE) and continued at regular intervals of 3 d. At each harvesting date, 30 disks were collected from totally expanded leaves, using a disk borer of 10 mm diameter. After collection, the disks were used to determine Chl, starch, total soluble saccharides, and total free amino acid concentrations. The seedlings were cut at the soil level and separated into stems, leaves, and cotyledons. Roots were removed from the soil and washed. The plant parts were fresh weighed on an analytical balance. Samples of stems, cotyledons, and roots were also taken to determine the contents of starch, total soluble sugars, and total free amino acids. Normal and recessive homozygous seedlings were grown on adjacent subplots in a split plot randomized

block design. There were 10 sampling dates which constituted the blocks. Each main plot and sampling date was replicated four times.

Chemical analyses: The Chl content was determined according to Arnon (1949) and Bruinsma (1961). Soluble sugars and starch were determined after extraction with 80 % ethanol, total soluble sugars were measured using the anthrone method. Starch was determined according to McCready *et al.* (1950). Total free amino acid concentrations were determined according to Moore and Stein (1948).

Chl fluorescence induction was measured during ontogeny in the stages B_2, B_3, C, D, and E of leaf development, corresponding to the ages of 9 to 12, 13 to 15, 16 to 20, 21 to 30, and >30 d, respectively (Merkel *et al.* 1993). The fluorescence measurements were done using a portable *PAM-2000* fluorometer (*Walz*, Effeltrich, Germany) [measuring radiation sources (modulation frequency 20 kHz, peak 655 nm, 0.2 μmol m^{-2} s^{-1}), actinic (peak 655 nm, 600 μmol m^{-2} s^{-1}), and saturating pulse (λ<710 nm, 15 000 μmol m^{-2} s^{-1})].

Prior to each measurement, a clip was placed on the leaf for 30 min to reflect solar radiation and decrease the leaf temperature. For the detection of fluorescence signal, a sensor of optical fiber was coupled to the clip. Afterwards, measurement and actinic radiation sources were applied to obtain modulated signals of fluorescence induction of Chl *a* at the rapid and slow phases, respectively. To determine the maximum fluorescence during actinic irradiation (F_m') and the photochemical (q_P) and non-photochemical (q_N) quenching, a saturating pulse was given at the end of the slow phase of Chl fluorescence induction.

The fluorescence signals were registered in a data acquisition system *DA-2000* which automatically calculated the minimum (F_0), maximum (F_m), variable (F_v), and terminal (F_t) fluorescences, F_m', the maximum potential quantum yield (F_v/F_m), the effective quantum yield of photosystem 2 (PS2, $\Delta F/F_m$'), and q_P and q_N. From these measurements we determined the variable fluorescence ratio [$(F_m-F_0)/F_0$], the photosynthetic electron transport rate to PS2 (Λ_{max}), the fluorescence decrease ratio [$(F_m-F_t)/F_t$], the quenching capacity (F_m-F_t), the quencher efficiency [$(F_m-F_t)/F_0$], the quantum yield of the non-cyclic electron transport system between PS2 and PS1 [$q_P(F_v/F_m)$], and the time (t) to reach the peak P of the induction kinetics of Chl fluorescence.

Leaf gas exchange, photosynthetically active radiation (PAR), and leaf temperature were measured in fully developed leaves (stage E) of normal and recessive homozygous seedlings, using a portable photosynthetic system *LICOR* model *LI-6200*.

Pigment protein complexes were separated by SDS-PAGE (Anderson 1980, Merkel *et al.* 1994) in mature leaves (stage E) of both normal and recessive seedlings.

Results and discussion

Contrary to the normal plants in which information about different Chl fluorescence parameters was obtained (Table 1), in the recessive homozygous seedlings only the

values of F_0 (Fig. 1) were measured during leaf ontogeny: they were much higher than those of the normal seedlings (Fig. 1). In both seedling types, the values increased between the B_2 and D stages, and decreased from the D to the E stage of leaf development (Fig. 1). Similar changes were found for F_v, F_m, F_m-F_t, F_t, and F_m' of normal plants, but the values of A_{max}, $[(F_m-F_t)/F_t]$, $[(F_m-F_t)/F_0]$, $[(F_m-F_0)/F_0]$, and F_v/F_m showed a tendency to increase from the B_2 to the E stage (Table 1). On the other hand, the $\Delta F/F_m'$ and q_P values were more or less constant during the leaf ontogeny. Values of $q_P(F_v/F_m)$ were constant from B_2 to B_3 and increased from B_3 to E. The t values were similar from B_2 to C and from D to E, but increased from C to D. The q_N showed a tendency to increase, mainly at the C stage when the values were high (Table 1). The rapid phase of Chl fluorescence induction is related to the primary processes of PS2. In this phase, there is no interaction between the processes in thylakoid membranes and in stroma (Bolhàr-Nordenkampf and Öquist 1993).

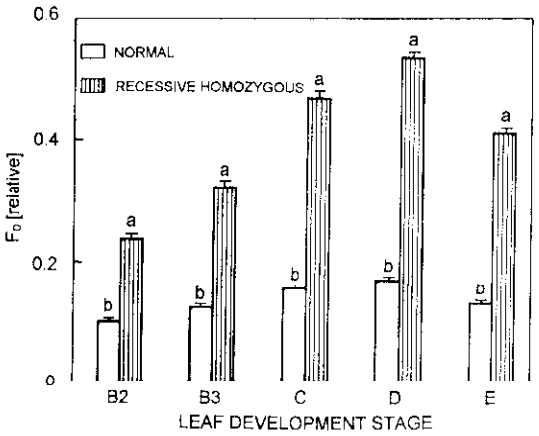

Fig. 1. Minimum fluorescence (F_0) of chlorophyll at the stages B_2, B_3, C, D, and E of leaf development of normal and recessive homozygous seedlings of *Theobroma cacao* resulting from the cross Pa 121×Pa 169. Mean (±s) values of 10 repetitions.

The increase in F_0 values implies a decrease in the excitation energy transfer from the reaction centres (RC) of PS2 (Demmig and Winter 1988). Probably, this increase is due to a damage in the RCs of PS2 (Krause 1988). On the other hand, constancy in the values of F_0 and F_m implies a perfect distribution of energy in the light-harvesting complex of PS2 (Stuhlfauth *et al.* 1988). Furthermore, it indicates that the molecules of Chl of PS2 are intact and that their radiation absorption capacity remains constant (Stuhlfauth *et al.* 1988).

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Table 1. Parameters of fluorescence of chlorophyll at the B2, B3, C, D and E stages of leaf development of normal seedlings of the *Theobroma cacao* cross Pa 121×Pa 169. Mean (± s) values of 10 repetitions

Parameters [relative]	Leaf development stage B2	B3	C	D	E
F_v	0.150±0.005	0.170±0.010	0.330±0.019	0.460±0.012	0.380±0.006
F_m	0.250±0.005	0.290±0.015	0.480±0.022	0.630±0.015	0.510±0.008
F_v/F_m	0.600±0.007	0.590±0.010	0.690±0.009	0.730±0.002	0.750±0.003
$[(F_m-F_0)/F_0]$	1.500±0.040	1.420±0.080	2.200±0.080	2.710±0.030	2.920±0.040
A_{max} [mV ms^{-1}]	46702±1557	56223±3680	97096±5661	183178±5362	189319±4942
F_t	0.110±0.003	0.140±0.006	0.170±0.005	0.230±0.008	0.150±0.004
$[(F_m-F_t)/F_t]$	1.270±0.040	1.070±0.050	1.820±0.100	1.740±0.050	2.400±0.070
F_m'	0.210±0.006	0.240±0.009	0.270±0.010	0.450±0.013	0.320±0.010
q_P	0.860±0.010	0.880±0.020	0.860±0.020	0.780±0.010	0.890±0.010
q_N	0.260±0.030	0.290±0.030	0.590±0.050	0.380±0.020	0.500±0.020
(F_m-F_t)	0.140±0.004	0.150±0.011	0.310±0.020	0.400±0.011	0.360±0.007
$[(F_m-F_t)/F_0]$	1.400±0.050	1.250±0.070	2.070±0.090	2.350±0.040	2.770±0.050
$\Delta F/F_m'$	0.450±0.008	0.440±0.008	0.360±0.020	0.490±0.010	0.530±0.008
$[q_P(F_v/F_m)]$	0.520±0.006	0.520±0.010	0.590±0.010	0.570±0.010	0.670±0.008
t [ms]	1.180±0.020	1.180±0.040	1.090±0.040	1.780±0.050	1.760±0.030

is due to a damage in the RCs of PS2 (Krause 1988). On the other hand, constancy in the values of F_0 and F_m implies a perfect distribution of energy in the light-harvesting complex of PS2 (Stuhlfauth *et al.* 1988). Furthermore, it indicates that the molecules of Chl of PS2 are intact and that their radiation absorption capacity remains constant (Stuhlfauth *et al.* 1988).

The F_m values are proportional to the quantity of Chl *a* molecules in the leaf tissue (Miranda *et al.* 1981). Increases of F_m values are related to the phosphorylation of ATP-dependent proteins in the thylakoid membranes (Horton and Black 1983). On the other hand, the decrease in this value can be attributed to alterations in energy distribution in the interior of the light-harvesting complex (LHC), and it is also related to the reduction of the PAR absorption capacity of the chloroplast pigments (Stuhlfauth *et al.* 1988). A significant reduction in the F_m' and F_m values implies a substantial increase in fluorescence quenching of Chl (Genty *et al.* 1989).

The F_v yield is determined mainly by the oxido-reduction reactions of the primary electron acceptor (Q_A) and by the degree of energizing of thylakoid membranes (Ögren and Baker 1985). A decrease in F_v may indicate the existence of damage in the RCs of PS2 associated with the reduction of photosynthetic electron transport (PET) capacity to PS2 (Krause and Behrend 1986). This fact, however, was not evident in normal cacao seedlings between the D and E stages, since, in spite of the reduction in F_v, the A_{max} values continued to increase (Table 1).

There is a linear relationship between the decrease in F_v/F_m and the optimal photosynthetic quantum yield (Krause and Weis 1991). The increase in F_v/F_m during leaf development of *Triticum aestivum* shows the existence of a great quantity of Chl molecules unable to transfer excitation energy to the RCs of PS2 (Baker *et al.* 1984).

On the other hand, low values of F_v/F_m imply a reduction in the photochemical efficiency of PS2 (Demmig and Björkman 1987). Thermal deactivation of the antenna excitation state or the gradual loss of reactivity in the RCs of PS2 decrease the quantum efficiency of PS2, implying an increase in Λ_{max} in relation to F_v (Krause *et al.* 1990). This effect increases the t values which means closed RCs and increasing F_v values (Krause *et al.* 1990).

The fluorescence quenching of Chl, from F_m to F_t, depends on the oxido-reduction of Q_A and on the pool of plastoquinone (PQ), and it is also related to the formation of electrochemical gradient in the thylakoid membranes (Scholes and Farrar 1985). With the reduction in the PQ pool, there is a decrease in the probability of utilization of the excitation energy by PS2 and an increase in the energy dissipation of Chl by other photochemical processes increasing the fluorescence emission (Baker and Bradbury 1981).

The $[(F_m\ F_t)/F_0]$ values can be used as *in vivo* indicators of the photosynthetic capacity due to its direct relationship with PET resulting from the electric potential and the gradient in the ionic concentration around the thylakoids (Miranda *et al.* 1981). On the other hand, the recuperation of $[(F_m\ F_t)/F_t]$ implies the rehabilitation of Q_A oxidation and the increase in energizing of the thylakoid system as a consequence of the rebuilding of a secondary gradient of metallic cations (Krause *et al.* 1982).

The variations of q_P may be related to the non-cyclic photosynthetic electron transport (NCPET) (Krause *et al.* 1982). These variations are attributed to the differences between the absorbed radiation and the PET rate (Demmig and Winter 1988). In the light, there can be a slight relation between q_P and $[q_P(F_v/F_m)]$ as a consequence of the limitation in the NCPET at the RCs of PS2. Furthermore, the $[q_P(F_v/F_m)]$ can be estimated through the determination of $\Delta F/F_m'$ (Genty *et al.* 1989). However, q_N mostly comes from the thermal deexcitation of PS2, associated with the rebuilding of the transthylakoidal proton gradient (Krause *et al.* 1988). The regulation of the efficiency of PS2 by q_N can be done by reducing the excitation energy that reaches the RCs of PS2 (Weis and Berry 1987).

There were variations in leaf gas exchange between the normal and recessive homozygous seedlings in the E stage of leaf development (Table 2). There were no significant differences in both plant types in Chl distribution among the complexes of the photosynthetic apparatus in the E stage (Table 3). The leaves of recessive homozygous seedlings, at the E stage, showed a negative CO_2 exchange. Furthermore, these leaves showed low rates of the stomatal conductance (g_s) and transpiration (E), and a high internal CO_2 concentration (C_i) in relation to normal seedlings (Table 2). The reduction of g_s can decrease the net photosynthetic rate and, consequently, the rate of PET through PS2 (Daley *et al.* 1989). Besides, the C_i levels variations in the mesophyll conductance and/or in the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) affect the relation between photorespiration and photosynthesis (Krall and Edwards 1992). On the other hand, maximum quantum yield is not related to C_i and leaf temperature (Ehleringer and Björkman 1977).

Merkel *et al.* (1993) showed that in *T. cacao* the photosynthetic rate and the

maximum apparent photosynthetic efficiency increased during leaf development and decreased when the leaves reached the E stage. During leaf ontogeny from the A to the E stage there was a continuous increase in molar ratios of Chl *a* to Chl *b*, a decrease in the respiration rate, and changes in functional properties of the photosynthetic apparatus that may be related to the changing pigment composition. However, Baker and Hardwick (1973) did not find changes in the molar Chl *a/b* ratio during leaf development. They also observed that the photosynthetic capacity and the Chl concentration increased in parallel during leaf flush and that another factors, besides Chl, were involved in the determination of the photosynthetic capacity. Furthermore, Baker *et al.* (1975) reported an increase of plastid breath, length, number of grana per plastid, index of lamellae per plastid, and number of lamellae per granum during leaf development.

Table 2. Parameters of gas exchange [net photosynthetic rate (P_N), transpiration rate (E), intercellular CO_2 concentration (C_i), stomatal conductance (g_s)] in comparison with photosynthetically active radiation (PAR) and leaf temperature (T) of mature leaves at the E stage of normal and homozygous recessive seedlings of the *Theobroma cacao* cross Pa 121×Pa 169. Mean (±a) values of 10 repetitions. Means followed by the same letter in the same column are not statistically different by the Duncan's Multiple Range Test ($p < 0.05$).

Cross	P_N [μmol m^{-2} s^{-1}]	C_i	PAR	E [mmol m^{-2} s^{-1}]	g_s [mol m^{-2} s^{-1}]	T [°C]
Normal	2.88±0.15[a]	281±2[a]	787±48[a]	3.02±0.1[a]	0.137±0.01[a]	32.7±0.3[a]
Recessive	−0.63±0.05[b]	341±2[b]	710±23[a]	2.46±0.1[b]	0.106±0.01[b]	32.5±0.2[a]

From 33 to 63 DAE there were variations in Chl concentrations in both normal and recessive homozygous seedlings. Contrary to the response of the recessive homozygous plants, in normal seedlings the Chl concentration increased during leaf ontogeny (Fig. 2*A,B*). Only Chl *a* significantly contributed to the increase of total Chl. Independent on the genetic characteristics of the seedlings, Chl *b* concentrations were constant throughout the experimental period in the normal seedlings. From 45 DAE until the end of the experiment, a continuous decrease in the concentrations

Table 3. Pigment-protein complexes (PPC) isolated of leaves of 33 d-old normal and recessive homozygous seedlings, solubilized and fractionated based on the procedure of Anderson (1980). PS1: P700 containing PPC (CP1a + CP1) of PS1; PS2: PPC of PS2; LHC: light-harvesting complex PPC (LHCP1-3); FP: free pigments complexed with SDS. Chlorophyll content of each PPC was determined according to Arnon (1949).

PPC	Chlorophyll content [%] normal	recessive
PS1	25	23
PS2	10	11
LHC	50	50
FP	15	16

of Chl *a* and *b* was observed in the homozygous recessive seedlings. Furthermore, Chl concentrations in the recessive homozygous seedlings were 50 % lower than in the normal ones. The maximum concentration value of total Chl for the normal seedlings was 4.0 g kg^{-1}(FM) at 63 DAE. The maximum concentration of total Chl for the homozygous seedlings was 2.6 g kg^{-1}(FM) found at 45 DAE; it declined to 1.0 g kg^{-1}(FM) at 63 DAE due to the decrease in Chl *a* concentration.

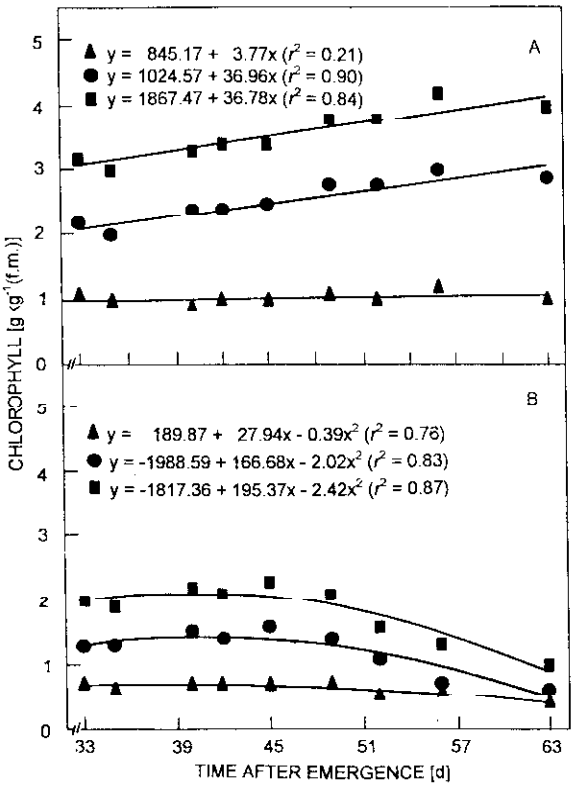

Fig. 2. Concentration of chlorophylls (*a*+*b*) (■), *a* (●) and *b* (▲) determined in leaves of normal (*A*) and recessive homozygous (*B*) seedlings of the *Theobroma cacao* cross Pa 121×Pa 169.

The reduction in Chl concentration and visible leaf chlorosis in the homozygous recessive seedlings from 45 to 63 DAE indicated the onset of senescence. Normally, the degenerative events precede death of mature cells (Beevers 1976). A progressive breakdown of the internal membrane system of the chloroplasts is observed during senescence of the primary leaves of *Phaseolus vulgaris* (Barton 1966), and major changes occur in the composition and physical properties of the thylakoid membranes (Fong and Heath 1977, McKersie and Thompson 1978). The flow of electrons out of the PQ pool, rather than the transfer of electrons into the pool through PS2, limit electron transport in senescent leaves (Jenkins *et al.* 1981). The absence of reoxidation of PQ in the senescent leaf indicates that the quenching of fluorescence was brought about by increase in thermal de-excitation or excitation transfer to PS1 (Jenkins *et al.* 1981).

From 33 to 43 DAE there was an increase in concentrations of total soluble sugars in different parts of normal seedlings (Fig. 3*A*), whereas, with the exception of leaves, decreases were found in other vegetative parts of the recessive homozygous seedlings (Fig. 3*B*). During this period, cotyledons of normal seedlings accumulated

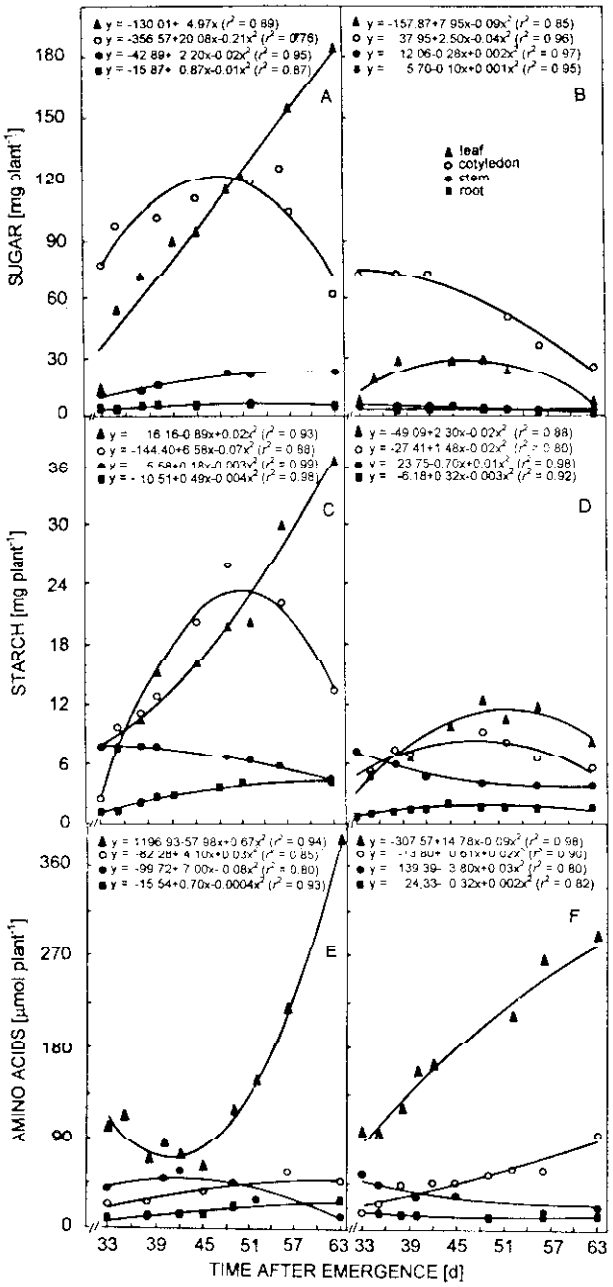

Fig. 3. Leaf (▲), cotyledon (○), stem (●), and root (■) total soluble sugar (*top*), starch (*middle*), and amino acid (*bottom*) concentrations of normal (*A, C, E*) and recessive homozygous (*B, D, F*) seedlings of the *Theobroma cacao* cross Pa 121×Pa 169.

most sugar, followed by leaves, stems, and roots. In recessive homozygous seedlings, concentration of sugars in cotyledons, stems, and root decreased from 33 to 63 DAE. After an initial increase, leaf sugar concentrations began to decrease from 48 DAE. In these seedlings, even though there was a decrease in the cotyledon sugar concentration, the concentration was greater than in any other plant part (Fig. 3*B*). In normal plants the concentration of cotyledonary sugars steadily decreased after 48 DAE until the end of the experimental period. During this period, leaf sugar concentration increased and sugar contents in stems and roots were fairly constant (Fig. 3*A*). The maximum sugar concentration value in normal plants was 180 mg per plant, found in leaves at 63 DAE. In the recessive seedlings the maximum sugar concentration (70 mg per plant) was found in cotyledons at 33 DAE.

At the beginning of growth, the seedlings depend on seed reserves for organ development. In this phase, the process of respiration uses considerable amounts of these reserves as a part of the carbon distribution system and sugar utilization. In normal seedlings, the increase in cotyledonary sugar concentration, from 33 to 48 DAE, was due to the hydrolysis of reserve saccharides into total soluble sugars. During this period the leaves were preferential metabolic sinks of photosynthates coming from the cotyledons in relation to stems and roots. Even though the leaves photosynthesized, their carbon balance was not positive. From 48 DAE, when leaf sugar concentration began to be higher than in cotyledons, there was a shift in the source-sink relationships. The leaves became sources which, in association with cotyledons, mobilized assimilates to stems and roots (Fig. 3*A*). In recessive homozygous seedlings, leaves were also preferential sinks in relation to other plant parts throughout the experimental period. Nevertheless, parts of these seedlings were maintained basically by the cotyledonary reserves, since the leaves showed a negative CO_2 balance. After 63 DAE, when the liposoluble pigments decayed and the cotyledonary reserves were exhausted, the recessive homozygous seedlings showed a general necrosis which ended in tissue decay followed by plant death.

During the experiment, there were also changes in the concentration of starch of both normal and recessive homozygous crosses (Fig. 3*C,D*). From 33 to 48 DAE there was an increase in starch concentration in plant parts of both genotypes with the exception of cotyledons in which the concentration decreased throughout the experimental period. In normal seedlings, starch began to accumulate from 36 DAE more in stems than in leaves which may have been, until that date, preferential sinks of cotyledonary metabolites (Fig. 3*C*). From 52 DAE until the end of the experiment, the starch concentration was higher in leaves than in stems. This period coincided with the second flushing and consequently, the sink strength was higher due to the appearance of new leaves. The root starch concentration increased in normal seedlings from 33 to 63 DAE (Fig. 3*C*). In recessive homozygous crosses, the starch concentration in stems was higher than in leaves and roots until 38 DAE. From this date leaf starch concentration increased until day 51, and decreased thereafter. Similar behaviour was found with stems and roots (Fig. 3*D*). The decline in cotyledonary starch concentration of recessive homozygous seedlings was deeper than in normal seedlings. This indicated a greater demand for cotyledonary metabolites since the photosynthate production was impaired by the onset of leaf

senescence.

Up to 50 % of the carbon fixed by photosynthesis can be used in starch and sugar formation depending upon plant species, environment, nutritional status, and developmental stage (Silvius *et al.* 1979, Huber 1983). This partitioning is important for plant growth since formation of sucrose, the main sugar transported *via* phloem in the majority of crops, determines the carbon export from photosynthesizing leaves (Huber 1983). Leaf starch, the main reserve saccharide, is hydrolyzed into sucrose when the current photosynthesis is relatively low in relation to sink demands for photosynthates. This occurs under low irradiances in darkness (Fondy and Geiger 1982), or when leaves are senescing (Franceschi and Giaquinta 1983). Sink-source studies in which the whole plant was manipulated have shown that carbon fixed by photosynthesis can be preferentially hydrolyzed into sucrose to be exported during periods of a high sink demand or retained as starch when the sink demand is low (Thorne and Koller 1974). However, in some plants the carbon export from photosynthesizing leaves does not decrease in response to rapid changes of sink demands; the exported carbon accumulates in alternative sinks (Fondy and Geiger 1980).

Independent on the genetic characteristics of crosses, total leaf free amino acid concentrations in leaves were higher than in any other plant part (Fig. 3*E,F*). During the experimental period, normal seedlings leaf, stem, and root amino acid concentrations increased. Leaf and stem amino acid concentrations of homozygous recessive seedlings also increased. However, root amino acid concentrations in these seedlings were constant throughout the experimental period. In normal seedlings, there was an increase in cotyledonary amino acid concentration up to 41 DAE, afterwards the amino acid concentration of leaves declined (Fig. 3*F*). From 41 DAE the amino acid concentration increased in leaves and decreased in cotyledons. In recessive homozygous seedlings, the behaviour was more straightforward, since the cotyledonary and leaf amino acid concentrations decreased and increased, respectively, from 33 to 63 DAE (Fig. 3*F*). In normal and recessive homozygous seedlings, the maximum leaf amino acid concentration was 390 and 285 μmol per plant, respectively, determined at the end of the experiment. The lowest value of 6 μmol per plant was found in roots of normal seedlings at 33 DAE. Stem amino acid concentration for recessive homozygous seedlings, at 63 DAE, was approximately 90 μmol per plant which was twice the normal plants stem concentration.

The initial growth of cacao seedlings is maintained by cotyledonary saccharide and protein reserves which are enough to satisfy the requirements until the photosynthetic machinery is formed. The nitrogen stored in seeds is translocated to the growing plant shoots as amino acids (Danielson 1951, McKee 1958). These amino acids, as final potential source of nitrate, can restrict nitrogen assimilation due to the inhibition of nitrate reductase activity (Filner 1966). Comparison of the stem and leaf amino acid concentrations between normal and recessive homozygous seedlings showed that there were more amino acids in stem and leaves of recessive homozygous seedlings than in normal seedlings, for most of the experimental period.

In conclusion, the leaf chloroplasts of recessive homozygous seedlings of the Pa 121×Pa 169 cross showed damage in the RCs of PS2, which impeded the transfer of

excitation energy to them. These leaves were maintained due to the respiration of cotyledonary reserves, although until 32 DAE the distribution of Chl in the complexes of photosynthetic apparatus remained intact, similar to the normal plants. From 45 DAE, when most of the cotyledonary reserves had been used, the pigment degradation started to end in death of the recessive homozygous seedlings.

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