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# IMPACT OF SOME CLIMATIC AND PHENOLOGICAL PARAMETERS ON THE CALLOGENESIS AND SOMATIC EMBRYOGENESIS VARIATIONS IN COCOA

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## ABSTRACT

Callogenesis and somatic embryogenesis (SE) are influenced by several factors including climate and phenology. To assess such an influence, the percentage of callogenesis and SE variations depending on five climatic and two phenological parameters was measured for 2 years. Staminodes and petals from six hybrids and two clones as controls were cultured in bulk, onto three distinct calli induction media only differing in hormonal concentrations. From the results, it emerged that sole leaves flush does not vary from year to year. Maximal temperature and flowering level are the most stably linked. Non-linear regression provides the best $R^2$-values of fitted curves. This shows that the link among climate, phenology, callogenesis and SE is not linear. In the first year, in control clones, climatic and phenological parameters explain 52.80% callogenesis variations, against 31.50% for SE. Therefore, climate and phenology significantly influence callogenesis, but not SE. For further industrial production of secondary metabolites such as butter, theobromin and chocolate aroma from calli, it would be desirable also to identify the favourable periods for calli production. Nevertheless, somatic embryos will continue to be produced all the year irrespective of period.

*Key Words*: Côte d'Ivoire, petals, staminodes

## RÉSUMÉ

La callogenèse et l'embryogenèse somatique (ES) sont influencés par plusieurs facteurs dont le climat et la phénologie. Pour évaluer une telle influence, le pourcentage de callogenèse et d'ES expliqué par 5 paramètres climatiques et 2 paramètres phénologiques a été mesuré durant 2 années. Les staminodes et les pétales prélevés sur 6 hybrides et 2 clones témoins ont été cultivés en vrac, sur 3 milieux distincts d'induction de la callogenèse se différenciant par leurs concentrations hormonales. Il est ressorti des résultats que seul le rythme des poussées foliaires ne varie pas significativement d'une année à l'autre. La température maximale et le niveau de floraison sont les plus stablement corrélés. Le modèle non linéaire fournit les meilleurs coefficients de détermination $R^2$. Ceci montre que le lien entre le climat, la phénologie, la callogenèse et l'ES n'est pas linéaire. La première année chez les 2 clones témoins, les paramètres climatiques et phénologiques expliquent 52,80 % des variations de la callogenèse, contre 31,50 % pour celles d'ES. En conséquence, le climat et la phénologie influencent significativement la callogenèse, mais non l'ES. Pour la production industrielle ultérieure de métabolites secondaires tels que le beurre, la théobromine et l'arome de chocolat à partir des cals, il serait souhaitable d'identifier également des

périodes favorables à la production des cals. Néanmoins, les embryons somatiques continueront d'être produits toute l'année sans tenir compte de la période.

*Mots Clés*: Côte d'Ivoire, pétales, staminodes

## INTRODUCTION

Chocolate tree (*Theobroma cacao* L.) is a perennial, cross-pollinated and diploid plant. It provides some substantial incomes to producing countries (Gray, 2000). In Côte d'Ivoire, 6 million people depend directly or indirectly on income from cocoa and represent 30% of the working population (Anon., 2004). Cocoa provides 30% of global export incomes and approximately contributes to 15% at gross domestic product of the country (ICCO, 2000). Its average yields in merchant cocoa in the order of 250-500 $kgha^{-1}$ obtained in fields are relatively low (Mossu, 1990), compared with 1-2.5 $tha^{-1}$ obtained in research stations (Clement *et al*., 1996). One of the ways to increase these yields is the diffusion by farmers of cloned superior genotypes by means of rooted cuttings and grafting.

In cocoa tree, the clonal propagation by rooted cuttings and grafting is unsatisfactory (Bertrand andAgbodjan, 1989; Bertrand and Dupois, 1992; Figueira and Janick, 1993). As an alternative, SE was proposed (Li *et al*., 1998; Tan and Furtek, 2003). Indeed, it provides some plantlets which behave like seed-derived plants (Tan and Furtek, 2003; Issali *et al*. 2011a). Yet, SE is vulneable to variations not only of intrinsic factors such as genotype, nature of explant, phenology among others, but also to extrinsic factors such as culture media, climate among others.

The influence of genotype, explant nature and calli induction media was evidenced by several workers (Alemanno, 1995; Tan and Furtek, 2003; Issali *et al*., 2008a). Also, thirteen genotypes were characterised according to their callogenic and embryogenic abilities (Issali *et al*., 2008a). Likewise, the relationship between three phenological parameters and SE was analysed in Issali *et al*. (2008b). Such an analysis showed that in hybrids, the period stretching out from August to October, including the month of February was favourable to SE. In contrast, in control clones, time interval spreading out from February to December was revealed propitious to SE. In the same way, in both control clones, period of temperature gaps stretching out from January to September enhanced SE (Issali *et al*., 2010). It seems that variations of climatic and/or phenological parameters significantly act on those of callogenesis and/or SE. To date, no study has reported the separate or simultaneous analysis of the impact of climatic and phenological parameters on the callogenesis and/or SE variations in *Theobroma cacao*. This analysis could allow the use of both climatic and/or phenological periods which are favourable to SE for optimising purposes. Indeed, recently Issali *et al*. (2010) identified some climatic periods favourable to SE.

The objective of this work was to quantify the part of variations of callogenesis and SE due to climatic and phenological parameters through callogenesis/SE optimisation.

## MATERIALS AND METHODS

Six hybrids (L120-A2, L126-A3, L231-A4, L232-A9, L233-A4 and L330-A9) and two control clones (C151-61 and SCA6) were used in the study (Table 1). They were planted at the Station Research of Centre National de Recherche Agronomique, located at Bingerville at Abidjan in Côte d'Ivoire. The callogenic and embryogenic abilities of L232-A9 and L233-A4 were characterised as weakly and fairly callogenic, respectively; whereas L231-A4, L120-A2, L330-A9 and L126-A3, as well as both control clones C151-61 and SCA6 were classified as strongly callogenic. Regarding embryogenesis abilities, L232-A9 was identified as lowly, while L330-A9, L233-A4, L126-A3, L231-A4 and L120-A2 were characterised as fairly embryogenic. Both control clones C151-61 and SCA6, were found to be highly embryogenic (Issali *et al*., 2008a).

In the first year, the experiment ranged from September 2002 to August 2003, while in the second year it stretched from January to December 2004. Due to contaminations recorded

TABLE 1. Origin and the characteristics of each used genotype in cocoa tree

| Genotypes | | Origin | Characteristics |
|---|---|---|---|
| Hybrids | L120-A2 | crossing descendent hybrid Pa13 x IMC67 | Half sib of L232-A9, L126-A3 and L231-A4. Precocious and vigorous. Good shape and size of pods; good yield; good rate of fat. |
| | L126-A3 | Crossing descendent hybrid Pa121 x IMC67 | Full sib of L231-A4, half sib of L233-A4 and L120-A2. Precocious and vigorous. Good shape and size of pods; good yield; good rate of fat. |
| | L231-A4 | Hybrid descended of the crossing Pa121 x IMC67 | Full sib of L126-A3, half sib of L233-A4, and L120-A2. Precocious and vigorous. Good shape and size of pods; good yield; good rate of fat. |
| | L232-A9 | Crossing descendent hybrid Pa13 x Pa150 | Half sib of L120-A2 and L330-A9. Precocious and vigorous. Good shape and size of pods; good yield; good rate of fat. |
| | L233-A4 | Crossing descendent hybrid Pa121 x Pa150 | Half sib of L231-A4, L126-A3, L330-A9 and L232-A9. Precocious and vigorous. Good shape and size of pods; good yield; good rate of fat. |
| | L330-A9 | Crossing descendent hybrid P19A x Pa150 | Half sib of L233-A4 and L232-A9. Precocious and vigorous. Good shape and size of pods; good yield; good rate of fat. |
| Control clones | C151-61 | Clonal material created in Venezuela. BC1* came from the cross ICS1 x (ICS1 x SCA6) | Very elevated fruit set rate. More sensitive to pod rot, to Mirideses and to malformations of pods caused by wilt. |
| | SCA6 (SCAVINA 6) | Collected by Pound in upper Amazon near Sabina hacienda (Ecuador) | One of the ten best parents; very tolerant to witches' broom disease, resistant to Phytophthora, pod rot, but produces tiny beans; good yield; vigorous. |

BC1*: Back cross 1 for which the donor parent is SCA6 and the recurrent one is ICS1

in the month of April 2003 in the first year of the study, its data were not taken into consideration. Unopened flower buds (4 to 5 mm in length), harvested once a week early in the mornings, were used as source of explants. Sterilisation of buds, preparation of the culture media and initiation of cultures were conducted basing on the adapted method from Li *et al*. (1998). Such an adaptation of the protocol concerned the hormonal concentrations of the primary callus growth media (Table 2). A maximum of seven flower buds were cultured in each petri-dish in all of experiments.

A modified completely randomised design with 8 x 2 x 3 factorial scheme was used. Such modifications concerned the association of staminodes and petals in co-culture.The genotype, explant and culture medium were the factors analysed. The factorial combination was organised as follows: for each genotype (eight in all), two explants (staminodes and petals) were cultured in bulk on three distinct primary callus growth media (PCG1, PCG3 and PCG4). The latter were characterised by the same hormonal balance, but some different hormonal concentrations (Table 2). A treatment was constituted of petals and staminodes of a genotype cultured onto one culture medium. Each treatment was set up in triplicates. The explants contained in one petri-dish bearing one culture medium represented the experimental unit.

Climatic data were collected by the meteorological department of CNRA, located at Bingerville. Minimum and maximum temperature, rainfall, sunshine and relative humidity were measured (Table 3). On account of lack of variation of relative humidity, and similarity of behaviour between mean and maximal temperatures on the one hand, relative humidity and mean temperature on the other hand, mean temperature and relative humidity were eliminated from the study (Issali, 2011b).

The phenological data were collected on the day of harvest of flower buds on each of eight cocoa trees. Flowering level and leaves flush were estimated by visual observation from a scale of five percentages, namely 0, 25, 50, 75 and 100% (Table 3). These values corresponded to the cover degree of the trunk and branches in flower buds and new leaves flush on cocoa tree.

The measure of fructification was performed by exhaustive counting of cherelles, immature and mature pods borne by cocoa trees (Table 3). In order to normalise the distributions of climatic and phenological parameters and equalise the variances of analysed populations, some transformations were applied to them (Table 3).

At the end of each culture cycle of three months, five variables were measured on each genotype: (i) callogenic explants number (NCAL), (ii) embryogenic explants number (NEXEMB), (iii) embryos number per embryogenic explant (NEMB), (iv) average number of embryos per embryogenic explant (MEXEMB), and (v) the percentage of embryogenesis (PE). Square root transformation was applied to the first four variables, while the percentage of embryogenesis was subjected to arcsin $\checkmark$ x transformation.

The Statistical Package for Social Sciences (SPSS) version 12.0.1 and Xlstat version 7.5.2 softwares were used to analyse the data as a whole. Averages and reliability coefficients were calculated to appreciate the central trend and variability, respectively. In order to identify the best parameters of yearly climatic and phenological variations, their averages were separate by Student's Z test at 5% threshold. Such an identification allowed the elimination of the least variable parameters.

To analyse the relationship between five climatic parameters and two phenological parameters, Pearson's correlation coefficients at either 5 or 1% significance level were used. To

TABLE 2. Hormonal concentrations of the tissue culture media used

| Culture media | | Hormonal concentration[a] |
|---|---|---|
| PCG | PCG3 | [2,4 D] / [TDZ] : 4.52 $\mu$M / 11.35 *n*M |
| | PCG1 | [2,4 D] / [TDZ] : 9.04 $\mu$M / 22.70 *n*M |
| | PCG4 | [2,4 D] / [TDZ] : 18.08 $\mu$M / 45.40 *n*M |
| SCG | | [2,4 D] / [Kinetin] : 9.04 $\mu$M / 1.394 $\mu$M |
| ED | | Hormone free |

Hormonal concentration*: Medium PCG3 was the least concentrated than three. Medium PCG1 was twofold as concentrated as PCG3. As regards induction medium PCG4, it was fourfold as concentrated as PCG3

TABLE 3. Used climatic and phenological parameters, their nature, applied transformations and abbreviation corresponded to each of them

| Climatic and phenological parameters | Nature of parameter | Subjected transformation* | Abbreviation |
|---|---|---|---|
| Minimal temperature rainfall | Monthly mean of weekly mean minimal temperatures | log(x) | Tmin |
| | Monthly mean of weekly pluviometrical total | log(x+1) | Rain |
| Maximal temperature | Monthly mean of weekly mean maximal temperatures | log(x) | Tmax |
| Temperature gaps | Monthly mean of weekly mean temperature gaps | log(x) | Etm |
| Sunshine | Monthly mean of weekly mean sunshine | log(x+1) | Sun |
| Floweringlevel | Monthly mean of weekly mean flowering level | arcsin ✓ percentage | Nivflo |
| Fructification level | Monthly mean of weekly mean fructification level | Square root | Nivfru |
| Leaves flushrhythm | Monthly mean of weekly mean leaves flush rhythm | arcsin ✓ percentage | Rythfl |

Subjected transformation*: log is the abbreviation of decimal logarithm, while arcsine"percentage is that of arc sine of square percentage root

quantify the impact of climatic and phenological parameters on the variations of callogenesis and SE, several models of linear and non-linear regressions were tested. The best retained model was the one which provided the highest correlation coefficient of fitted curve termed $R^2$. The equations of modelling of variations of callogenesis and SE as well as the $R^2$-values which are associated with them were compared, from year to year.

## RESULTS

For the eight climatic and phenological parameters, sole leaves flush did not vary from year to year. It was, thus, eliminated from the study. Therefore, rainfall, maximum and minimum temperatures, temperature gaps, sunshine, flowering level and fructification level were identified as the best climatic and phenological parameters on which the study continued. Variability of observations around each of averages of the measured parameters stretched from 0.00 to 1.81% (Table 4).

Regarding flowering level, in hybrids from year to year, its link with maximum temperature was very stable (Table 5). Indeed, their correlation coefficient was same sign and both parameters were very significantly and favourably correlated.

Concerning fructification level, its relationship with rainfall was very stable regardless of the year. Also, their correlation coefficient was of the same sign and the two variables were unfavourably correlated. It was approximately the same relating to the link between fructification level and maximum temperature. Here, the link between the two parameters was less stable. In the first year, they were just significantly and positively correlated, while in the second year they were very significantly and positively correlated (Table 5).

In control clones, flowering level and maximum temperature showed a very stable link, from year to year. Both correlation coefficients recorded the same sign. Concerning fructification level, sole relationship with sunshine was not stable enough. Indeed, in the first year, they were only significantly, but unfavourably correlated, whereas in the second year they were significantly, but very unfavourably correlated (Table 5). Indeed, in the first year, they were only unfavourably correlated, whereas in the second year they were very unfavourably correlated (Table 5).

Parabolas of fourth and third degrees were identified as the best model describing the fluctuations of callogenesis and SE, respectively in the first and second years. In the first year, the equation of the model is spelt:

TABLE 4. Classification of averages of climatic and phenological parameters as a function of years of the study for the analysis of their impact on callogenesis and SE in cocoa tree

| Climatic and phenological parameters* | Year | Transformed average* | RC (%)* | Untransformed average* |
|---|---|---|---|---|
| Rain | Year 1 | 0.829 a | 1.81 | 5.745 mm |
| | Year 2 | 0.987 b | 1.42 | 8.705 mm |
| Tmax | Year 1 | 1.488 a | 0.00 | 30.761 °C |
| | Year 2 | 1.481 b | 0.00 | 30.269 °C |
| Tmin | Year 1 | 1.319 a | 0.08 | 20.845 °C |
| | Year 2 | 1.277 b | 0.08 | 18.923 °C |
| Etm | Year 1 | 0.972 a | 0.31 | 9.376 °C |
| | Year 2 | 1.027 b | 0.29 | 10.641 °C |
| Sun | Year 1 | 0.794 a | 0.50 | 5.223 °C |
| | Year 2 | 0.705 b | 0.57 | 4.070 °C |
| Nivflo | Year 1 | 0.775 a | 0.77 | 48.96% |
| | Year 2 | 0.752 b | 0.80 | 46.66% |
| Nivfru | Year 1 | 3.278 a | 1.19 | 10.745 fruits |
| | Year 2 | 4.178 b | 0.84 | 17.456 fruits |
| Rythfl | Year 1 | 0.622 a | 1.29 | 33.95% |
| | Year 2 | 0.633 a | 1.11 | 35.00% |

Climatic and phenological parameters*: Rain: Rainfall. Tmax: Maximum temperature. Tmin: Minimum temperature. Etm: Temperature gaps. Sun: Sunshine, Nivflo: Flowering level. Nivfru: Fructification level. Rythfl: Leaves flush.Transformed average*: Averages bearing the same letter in column are not significantly different according to Student's Z test at 5% likelihood. Untransformed average*: Values of untransformed averages were obtained using the inverse function of the one used for their transformation

TABLE 5. Link between climatic and phenological parameters by means of Pearson's linear correlation at either 5 or 1% level

| Year | Group of genotype | Climatic parameters* | | | | | |
|---|---|---|---|---|---|---|---|
| | | Phenological parameters* | Rain | Tmax | Tmin | Etm | Sun |
| Year 1 | Hybrid | Nivflo | -0.243** | +0.098** | -0.080* | +0.097** | -0.155** |
| | | Nivfru | -0.160** | +0.083* | +0.440** | -0.248** | -0.153** |
| | Clone | Nivflo | -0.270** | +0.335** | +0.227** | +0.048 | +0.162** |
| | | Nivfru | -0.231** | -0.023 | +0.377** | -0.234** | -0.135* |
| Year 2 | Hybrid | Nivflo | -0.043 | +0.253** | +0.076* | +0.039 | +0.281** |
| | | Nivfru | -0.152** | +0.285** | -0.444** | +0.610** | -0.066 |
| | Clone | Nivflo | +0.049 | +0.281** | -0.380** | +0.548** | -0.050 |
| | | Nivfru | +0.135** | -0.285** | -0.085* | -0.074 | -0.205** |

Climatic and phenological parameters *: Values placed at the intersection of the lines and columns and bearing either one or two asterisk(s) reveal a significant link between climatic and phenological parameters after Pearson's linear correlation at either 5 or 1% probability

$$Y_1 = b_1 + b_2X_1^1 + b_3X_2^1 + b_4X_3^1 + b_5X_4^1 + b_6X_5^1 + b_7X_6^1 + b_8X_7^1 + b_9X_1^2 + b_{10}X_2^2 + b_{11}X_3^2 + b_{12}X_4^2 + b_{13}X_5^2 + b_{14}X_6^2 + b_{15}X_7^2 + b_{16}X_1^3 + b_{17}X_2^3 + b_{18}X_3^3 + b_{19}X_4^3 + b_{20}X_5^3 + b_{21}X_6^3 + b_{22}X_7^3 + b_{23}X_1^4 + b_{24}X_2^4 + b_{25}X_3^4 + b_{26}X_4^4 + b_{27}X_5^4 + b_{28}X_6^4 + b_{29}X_7^4.$$

However, concerning the second year, the equation of the best model was $Y_2 = b_1 + b_2X_1^1 + b_3X_2^1 + b_4X_3^1 + b_5X_4^1 + b_6X_5^1 + b_7X_6^1 + b_8X_7^1 + b_9X_1^2$

$+ b_{10}X_2^2 + b_{11}X_3^2 + b_{12}X_4^2 + b_{13}X_5^2 + b_{14}X_6^2 + b_{15}X_7^2 + b_{16}X_1^3 + b_{17}X_2^3 + b_{18}X_3^3 + b_{19}X_4^3 + b_{20}X_5^3 + b_{21}X_6^3 + b_{22}X_7^3$.

In these equations, $Y_1$ or $Y_2$ indicates either callogenesis or SE variable. Value $b_1$ is the regression coefficient corresponding to the ordinate at the origin when the callogenesis or SE is null. $b_2$, $b_3$……$b_{29}$ represent the partial regression coefficients once callogenesis and SE vary. Variables $X_1$, $X_2$, $X_3$……$X_7$ express sunshine, minimal temperature, rainfall, maximal temperature, temperature gaps, flowering level and fructification level, respectively.

In hybrids, in the first year, the equation of fourth degree describing the variations of callogenesis was:

NCAL = 6742362.562 – 329.435 $Sun^1$ – 691506.919 $Tmin^1$ + 0.217 $Rain^1$ + 18913775.401 $Tmax^1$ - 514.701 $Etm^1$ + 6.335 $Nivflo^1$ – 0.211 $Nivfru^1$ + 614.776 $Sun^2$ + 832030.062 $Tmin^2$ – 1.535 $Rain^2$ – 19280824.886 $Tmax^2$ + 5875.816 $Etm^2$ – 2.680 $Nivflo^2$ + 0.484 $Nivfru^2$ – 517.974 $Sun^3$ – 443872.811 $Tmin^3$ + 1.864 $Rain^3$ + 8736427.941 $Tmax^3$ – 4612.873 $Etm^3$ + 0.237 $Nivflo^3$ – 0.145 $Nivfru^3$ + 166.425 $Sun^4$ + 91183.506 $Tmin^4$ – 0.582 $Rain^4$ – 1487269.368 $Tmax^4$ + 3494.708 $Etm^4$ – 0.216 $Nivflo^4$ + 0.011 $Nivfru^4$.

However, in the second year, the model of third degree was:

NCAL = - 19704.696 – 5.198 $Sun^1$ – 11251.794 $Tmin^1$ – 1.396 $Rain^1$ + 52257.935 $Tmax^1$ – 1965.512 $Etm^1$ – 2.140 $Nivflo^1$ + 0.317 $Nivfru^1$ + 15.206 $Sun^2$ + 9976.637 $Tmin^2$ + 1.595 $Rain^2$ – 37912.390 $Tmax^2$ + 2275.036 $Etm^2$ + 2.948 $Nivflo^2$ – 0.067 $Nivfru^2$ – 10.045 $Sun^3$ – 3612.902 $Tmin^3$ – 0.437 $Rain^3$ + 9961.732 $Tmax^3$ – 1469.350 $Etm^3$ - 0.949 $Nivflo^3$ + 0.005 $Nivfru^3$.

In the first year, seven climatic and phenological parameters explained 40.30% of fluctuations of callogenesis, against 9.70% in the second year. Regardless the year, maximum temperature recorded the highest partial regression coefficient with SE, while fructification level provided the weakest one.

In both control clones, in the first year the model showing the fluctuations of callogenesis was:

NCAL = - 3366249.739 + 104.358 $Sun^1$ + 1501041.787 $Tmin^1$ – 3.193 $Rain^1$ + 7869594.617 $Tmax^1$ - 739.0211 $Etm^1$ + 8.614 $Nivflo^1$ - 0.880 $Nivfru^1$ – 215.708 $Sun^2$ - 1676530.732 $Tmin^2$ + 5.201 $Rain^2$ – 8111372.573 $Tmax^2$ + 6617.471 $Etm^2$ + 0.561 $Nivflo^2$ + 0.838 $Nivfru^2$ + 180.306 $Sun^3$ + 831216.791 $Tmin^3$ - 3.310 $Rain^3$ + 3716837.803 $Tmax^3$ - 5106.640 $Etm^3$ - 8.859 $Nivflo^3$ - 0.221 $Nivfru^3$ - 52.244 $Sun^4$ - 151498.394 $Tmin^4$ + 0.699 $Rain^4$ - 641762.913 $Tmax^4$ + 3840.199 $Etm^4$ + 3.892 $Nivflo^4$ + 0.016 $Nivfru^4$.

In contrast, in the second year the model equation was: NCAL = - 10252.767 + 3.774 $Sun^1$ – 8296.785 $Tmin^1$ – 0.470 $Rain^1$ + 29405.539 $Tmax^1$ – 1169.972 $Etm^1$ + 26.839 $Nivflo^1$ + 0.923 $Nivfru^1$ – 9.388 $Sun^2$ + 7250.707 $Tmin^2$ + 0.666 $Rain^2$ – 21427.043 $Tmax^2$ + 1356.940 $Etm^2$ – 26.057 $Nivflo^2$ – 0.183 $Nivfru^2$ + 7.034 $Sun^3$ – 2521.380 $Tmin^3$ – 0.231 $Rain^3$ + 5689.969 $Tmax^3$ – 888.419 $Etm^3$ + 8.102 $Nivflo^3$ + 0.009 $Nivfru^3$.

From year to year, the impact of seven climatic and phenological parameters on the variations of callogenesis was 52.80 and 18.10%, respectively. Here also, taking into consideration the partial regression coefficient value, maximum temperature was the most linked with callogenesis. On the contrary, climatic and phenological parameters, which recorded the weakest partial regression coefficient with callogenesis varied from year to year. Indeed, in the first year, fructification level was the least linked with callogenesis, whereas in the second year it was rainfall.

In hybrids, in the first year the equation expressing the fluctuations of SE as a function of variations of climatic and phenological parameters was:

MEXEMB = 4441477.955 – 125.343 $Sun^1$ – 676628.833 $Tmin^1$ – 4.057 $Rain^1$ - 11408647.604 $Tmax^1$ + 56.071 $Etm^1$ + 0.897 $Nivflo^1$ + 2.504 $Nivfru^1$ + 271.506 $Sun^2$ + 748181.825 $Tmin^2$ + 6.697 $Rain^2$ + 11584077.448 $Tmax^2$ + 1198.518 $Etm^2$ + 3.993

Nivflo2 – 1.554 $Nivfru^2$ – 248.059 $Sun^3$ – 367326.250 $Tmin^3$ - 3.526 $Rain^3$ - 5225803.184 $Tmax^3$ – 901.380 $Etm^3$ – 2.659 $Nivflo^3$ + 0.302 $Nivfru^3$ + 82.352 $Sun^4$ + 68298.027 $Tmin^4$ + 0.613 $Rain^4$ + 882982.767 $Tmax^4$ + 874.493 $Etm^4$ + 0.019 $Nivflo^4$ - 0.019 $Nivfru^4$.

In contrast, that of the second year was: MEXEMB = - 7403.829 + 4.492 $Sun^1$ + 5415.731 $Tmin^1$ + 2.559 $Rain^1$ + 9414.996 $Tmax^1$ + 1046.780 $Etm^1$ – 10.113 $Nivflo^1$ + 0.094 $Nivfru^1$ – 9.305 $Sun^2$ - 5077.097 $Tmin^2$ - 2.635 $Rain^2$ – 5038.678 $Tmax^2$ - 1244.011 $Etm^2$ + 7.939 $Nivflo^2$ + 0.077 $Nivfru^2$ + 6.607 $Sun^3$ + 1990.686 $Tmin^3$ + 0.7428 $Rain^3$ + 306.119 $Tmax^3$ + 866.730 $Etm^3$ - 1.592 $Nivflo^3$ - 0.008 $Nivfru^3$.

In the first year, the percentage of variation of SE attributable to climatic and phenological parameters was 7.80, against 2.20% in the second year. Irrespective of the year, maximum temperature expressed the highest partial regression coefficient with SE. However, the weakest partial regression coefficient of SE was recorded in the first year with flowering level, while in the second year it was fructification level.

In control clones, in the first year, the curve equation showing the fluctuations of SE as a function of seven parameters was:

MEXEMB = 596022.068 + 486.461 $Sun^1$ + 2651644.531 $Tmin^1$ + 1.251 $Rain^1$ – 3930634.622 $Tmax^1$ + 1013.603 $Etm^1$ – 12.220 $Nivflo^1$ + 2.917 $Nivfru^1$ - 833.477 $Sun^2$ – 3040555.330 $Tmin^2$ – 3.0346 $Rain^2$ + 3959822.652 $Tmax^2$ - 3810.831 $Etm^2$ + 42.480 $Nivflo^2$ – 1.540 $Nivfru^2$ + 615.698 $Sun^3$ + 1549195.380 $Tmin^3$ + 1.743 $Rain^3$ - 1773537.578 $Tmax^3$ + 3119.953 $Etm^3$ – 39.125 $Nivflo^3$ + 0.252 $Nivfru^3$ - 166.350 $Sun^4$ – 296832.026 $Tmin^4$ – 0.289 $Rain^4$ + 298896.722 $Tmax^4$ – 1683.008 $Etm^4$ + 11.3647 $Nivflo^4$ – 0.013 $Nivfru^4$.

However, in the second year the equation of model was:

MEXEMB = - 8028.581 – 4.680 $Sun^1$ + 8186.185 $Tmin^1$ + 0.281 $Rain^1$ + 9934.383 $Tmax^1$ + 239.088 $Etm^1$ + 21.674 $Nivflo^1$ – 3.364 $Nivfru^1$ + 11.841 $Sun^2$ – 6382.084 $Tmin^2$ – 0.177 $Rain^2$ – 7325.652 $Tmax^2$ – 260.208 $Etm^2$ – 22.996 $Nivflo^2$ + 0.736 $Nivfru^2$ - 8.009 $Sun^3$ + 1642.931 $Tmin^3$ + 0.069 $Rain^3$ + 1803.215 $Tmax^3$ + 82.285 $Etm^3$ + 7.769 $Nivflo^3$ - 0.049 $Nivfru^3$.

In the first year, the percentage of variation of SE due to seven climatic and phenological parameters was 31.50%, whereas that of the second year was 6.80%. From year to year, maximum temperature and rainfall were the most and least linked with SE, respectively.

## DISCUSSION

Leaves flush was eliminated from the study because it was the least variable parameter from year to year (Table 4). Such lack variation could be due to too strong sensitivity of cocoa tree to the variation of climatic parameters (Mossu, 1990). Sure enough, at each observation, there were always new leaves flush, so that no gap was detected at the calculations. A few rains were sufficient to induce the formation of new leaves and flowers at either two or three weeks after. As such, it was not easy to separate the periods of bud dormancy and waking up. Some works have reported the elimination of climatic parameters in some studies, because of their lack of variation (Issali, 2011b). Nevertheless, this leaves flush was proved to be the most linked phenological parameter with SE in Issali *et al*. (2008 b).

Regardless of genotype group, maximum temperature was the most stable climatic parameter in relation to flowering level (Table 5). Thus, an increase in maximal temperature triggers a similar increasing of flowering level. According to Heller *et al*. (1995), flowering is intrinsically influenced by gibberellins and cytokinins, which are some plant growth regulators. Extrinsically, maximum temperature might influence at first the sensitivity of cocoa tree to day/night, namely photoperiod. This sensitivity to photoperiod confers the ability of a plant to flower. Moreover, the significant link between maximal temperature and flowering level, should get us to eliminate one of the two (Table 5). They have a similar behaviour. But here, we could not do such an elimination, because each parameter has contributed to expression of $R^2$-value. In addition, both have some comparable $R^2$-values. Indeed, using the linear regression, the individual

contribution of maximal temperature to the callogenesis variation was 7.60, against 8.40% for flowering level (data not shown). In the first year, in hybrids, the elimination of flowering level in the regression equation reduces the $R^2$-value of fitted curve from 40.30 to 28.80% (data not shown). Furthermore, irrespective of the year and genotype group, maximal temperature remains the most highly and stably linked with callogenesis and SE in *Theobroma cacao*. This seems to indicate that the precursor metabolites in the expression of callogenesis and SE need high temperatures to act.

Regarding callogenesis, in the first year, the impact of five climatic parameters and two phenological parameters was important. Sure enough, as well as both in hybrids and control clones, 40.30 and 52.80% of callogenesis variations were caused by these seven parameters. These high $R^2$-values reveal a significant impact of the climatic and phenological parameters on the expression of callogenesis in *Theobroma cacao* L. Upon the same plant material, the part of variation of callogenesis explainable by only three phenological parameters was 36.90% (Data not shown). So, climate and phenology variations significantly act on callogenesesis variations expression. Therefore, it would be desirable one day, for the industrial production of secondary metabolites such as butter, theobromin and aroma of chocolate that the periods of high production are identified as for SE in optimisation purposes of callogenesis. These metabolites could be made from cell calli suspensions (Pence, 1989). In contrast for SE, these seven climatic and phenological parameters weakly influenced it in *Theobroma cacao* L. Indeed, the part of SE variations attributable to seven parameters did not exceed 50%. So, climate and phenology variations do not significantly act on SE variations expression. The somatic embryos will continue to be produced all year without regard to periods.

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