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Full Length Research Paper

Relationship between five climatic parameters and somatic embryogenesis from sporophytic floral explants of *Theobroma cacao* L.

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To analyse the relationship between climatic parameters and somatic embryogenesis (SE), some favourable and unfavourable periods were identified. Likewise, to optimize SE in unfavourable periods the relationship among 2,4-D/TDZ, SE and year was analysed. Staminodes and petals of six hybrids and two clones as controls were sown in bulk onto three different calli induction media. Minimal temperature, rainfall, maximal temperature, mean temperature, temperature gaps, sunshine and relative humidity as climatic parameters were simultaneously recorded the day of the harvest of flower buds. Student-Fisher's test at 5% level, Principal Component Analysis and Pearson's linear correlation at 5%, 1% or 1‰ were used to separate the averages, identify the best climatic parameters and analyse the link between the climate and SE, respectively. The relative humidity and mean temperature were eliminated from the study. The period that spreads out from January to September favoured SE. In favourable periods, the SE variation was independent of that of concentration in 2,4-D/TDZ. This shows that these are the metabolites coming from 2,4-D/TDZ that activate the genes rather than these two compounds themselves. In unfavourable periods, in the first year, the weakest concentration in 2,4-D/TDZ of PCG3 medium favoured SE, while in the second year that is the strongest concentration of PCG4 which increased it. This could indicate an interaction among year, concentration in 2,4-D/TDZ and SE. However, the link thus established is only statistical. It did not allow the quantification of the contribution level of these climatic parameters to variations of SE.

Key words: Somatic embryogenesis variations, staminodes, petals, PCG calli induction media, favourable and unfavourable periods. 2,4-D/TDZ concentration in periods.

INTRODUCTION

Cocoa tree is a plant native of rainforest of Tropical America belonging to the Malvaceae (Whitlock et al., 2001). In Côte d'Ivoire, cocoa provides 30% of export global incomings and approximately contributes to 15% at gross domestic product (ICCO, 2000). The covered area by cocoa trees farms represents 6% of national territory.

The life of 6 millions of people directly or indirectly depends on incomings of cocoa. These peoples represent

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Abbreviation: **SE**, Somatic embryogenesis; **2,4-D,** 2,4-dichlorophenoxyacetic acid; **TDZ**, thidiazuron.

30% of the working population (Anonymous, 2004). Moreover, its average yields in merchant cocoa in the order of 250 - 500 kg/ha obtained in fields are relatively low (Mossu, 1990). However, in research stations, some yields in the order of 1 – 2.5 t/ha are obtained (Clement et al., 1996). One of the ways able to increase these yields is the diffusion by farmers some selected elite genotypes. In cocoa tree, the elite genotypes are found at the end of selection process, which classically comes true in the individuals, the families and the populations. On a level with individual, after the creation of the variability by sexual hybridization, the superior genotypes are chosen from their individual performances and then cloned. The best families, as regards them, are reproduced through the gardens at seeds consisted of two clones (Braudeau, 1969; Mossu, 1990).

They are distributed in bulk in the form of pods to farmers. At the present time, the new orientation consists to improve the populations by the means of reciprocal recurrent selection (Clement et al., 1993). Concerning the individual selection, the clones' hybrids obtained from rooted cuttings and grafting show some defaults. Indeed, rooted cuttings from plagiotropic branches are certainly numerous, but their fasciculated rooting makes them vulnerable at gusts of wind and dryness. Grafting, as for it, provides some trees with unbalanced architecture (Bertrand and Agbodjan, 1989; Bertrand and Dupois, 1992).

To remedy these defaults, SE was recommended as complementary method to rooted cuttings and grafting. However, the production of SE seems to vary not only as a function of internal factors such as genotype, phenology, nature of explant, etc, but also some external factors such as climate, culture media, etc. Recently, the variation as a function as well as both of genotype, of explant nature, of calli induction media and combination of these factors was evidenced (Li et al., 1998; Tan and Furtek, 2003; Issali et al., 2008b). In the same way, not only the relationship between three phenological parameters and SE (Issali et al., 2008c), but also the contribution of these three phenological parameters to variations of callogenesis and SE in *Theobroma cacao* (Issali et al., 2008, accepted for publication) were investigated.

To date, no study reported the analysis of relationship between climatic parameters and SE. Additionally to this, no work evaluated the relationship between concentration in 2,4-D/TDZ and SE inside favourable and unfavourable periods. The knowledge and the understanding of these relationships could allow as well as both the identification of favourable periods to SE and action mode of plants growth regulators in favourable and unfavourable periods, from year to year.

This work aimed to analyse as well as both the relationship between five climatic parameters - SE and the one between concentration in 2,4-D/TDZ-SE in favourable and unfavourable periods.

MATERIALS AND METHODS

Plant material and tissue culture

Six hybrids under the assessment (L120-A2, L126-A3, L231-A4, L232-A9, L233-A4 and L330-A9) and two control clones (C151-61 and SCA6) were used (Table 1). The callogenic abilities of L232-A9 and L233-A4 were characterized as weak and fair, respectively, whereas those of L231-A4, L120-A2, L330-A9 and L126-A3 and both control clones C151-61 and SCA6 were classified as great. Regarding embryogenesis abilities, L232-A9 was marked weakly, while L330-A9, L233-A4, L126-A3, L231-A4 and L120-A2 were characterized as fairly embryogenic. Two control clones, C151-61 and SCA6, were found to be greatly embryogenic (Issali et al., 2008b).

The first year of study spread out from September 2002 to August 2003, while the second year stretched out from January to December 2004. Due to the contaminations recorded in the month of April 2003 in the first year of study, its data were eliminated. We took into account annual sequence of months for the identification of climatic periods. Flower buds measuring 4 to 5 mm in length were harvested once a week early in the morning and used as source of explants. Sterilization of buds, preparation of the culture media and initiation of cultures were conducted basing on the adapted method of Li et al. (1998). Such an adaptation of the protocol concerned the hormonal concentrations of the primary callus growth media. Seven flower buds were sowed to the maximum in a single Petri dish during the experimentation. A modified design in complete randomization with factorial combination of variants of factors was used. Such modifications concerned the association of staminodes and petals in co-cultivation. The genotype, explant and medium are the used factors. The factorial combination was organized as follows: for each genotype (eight in all), two explants (staminodes and petals) were sowed in bulk on three distinct primary callus growth media (PCG1, PCG3 and PCG4). These three calli induction media were characterized by the same hormonal balance, but some different hormonal concentrations. Thus, medium PCG3 was the least concentrated among three, with a concentration in 2,4-D/TDZ of 4.52 μM/11.35 *n*M. The PCG1 medium was twofold as concentrated as PCG3. Its concentration in 2,4-D/TDZ was 9.04 μM/22.70 *n*M. As regards the induction medium PCG4, it was fourfold as concentrated as PCG3. Its concentration in 2,4-D/TDZ was 18.08 μM/45.40 *n*M. All of the treatments were triplicate.

Measured and calculated climatic parameters

The climatic data were collected by the Meteorological Department of CNRA (Centre National de Recherche Agronomique) located at Bingerville in Côte d'Ivoire. Minimal temperature, maximal temperature, rainfall, sunshine and relative humidity were measured, whereas temperature gaps and mean temperature were calculated. In order to normalize and equalize the variance of the collected data some transformations were applied to them (Table 2). The choice of the best parameters of the climatic variation was based on the correlation, through the cosine^2, between each of climatic parameters and the principal component on which it was very well represented. Within the same principal component, this choice was guided by the sign of the Pearson's linear correlation. Thus, when this sign between two parameters was positive, the best represented climatic parameter was chosen only one. However, when this sign was negative, both parameters were it.

Measured variables for tissue culture

At the end of each culture cycle of three months, five variables were

Table 1. Summary on the origin and the characteristics of each of used genotypes 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 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. BC1F1* came from the cross ICS1 (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.

BC1F1*: Back cross 1 for which the donor parent is SCA6 and the recurrent parent is ICS1.

Table 2. Summary of used climatic parameters, their nature, applied transformations and abbreviation of each of them for the study of their relationship with SE of cocoa tree.

Climatic parameters	Nature of parameter	Undergone transformation*	Abbreviation	Associated period
Minimal temperature	Monthly mean of weekly mean minimal temperatures.	-	TMIN*	TI
Rainfall	Monthly mean of weekly pluviometrical total.	log(x+1)	RAIN	RA
Maximal temperature	Monthly mean of weekly mean maximal temperatures.	log(x)	TMAX	TM
Mean temperature	Monthly mean of weekly mean temperatures.	log(x)	TMOY	TN
Temperature gaps	Monthly mean of weekly mean temperature gaps.	log(x)	ETM	TG
Sunshine	Monthly mean of weekly mean sunshine.	log(x+1)	SUN	S
Relative humidity	Monthly mean of weekly mean relative humidity.	arcsin√ percentage	HRELA	H

Undergone transformation*: log is the abbreviation of decimal logarithm. Arcsin√ percentage is also an abbreviation, but of arc sine of square root of percentage. TMIN*: Minimal temperature presented a normal distribution, consequently it was not transformed.

measured for each genotype: (1) callogenic explants number, (2) embryogenic explants number, (3) embryos number per embryogenic explant, (4) average of embryos per embryogenic explant and (5) percentage of embryogenesis. Square root transformation was applied to the first four variables, while the percentage of embryo-genesis underwent arcsin√ transformation. These transformations allowed the normalization and stabilization of the variance of analysed populations.

Structuring of climatic parameters in stable periods

Structuring of the four climatic parameters in stable periods, were done in three steps. In the first phase, the monthly variations of climatic parameters were analyzed. For this purpose, two statistical tools were used: (1) comparison of monthly average to annual average of each of four climatic parameters and (2) comparison of monthly reliability coefficient at 20% level. Thus, two classes as time intervals were defined in comparison with the annual average: classes of months of which the average values were higher or lower than the annual average. Within each class, sub-groups were identified relatively to their reliability coefficient in abbreviated RC. Thus, the months which recorded high fluctuations (RC > 20%) were separate of those of which the variations were low (RC < 20%). However, this structuring did not give full satisfaction, because of

Table 3. Classification of averages of seven used climatic parameters as a function of two years of study.

Climatic parameters	Year	Transformed average*	RC (%)*	Untransformed average*
TMIN	Year 1	21.121 a	0.14	-
	Year 2	19.205 b	0.16	-
RAIN	Year 1	0.865 a	1.04	6.328 mm
	Year 2	0.988 b	0.91	8.727 mm
TMAX	Year 1	1.490 a	0.00	30.903°C
	Year 2	1.481 b	0.00	30.269°C
TMOY	Year 1	1.415 a	0.00	26.002°C
	Year 2	1.392 b	0.00	24,660 °C
ETM	Year 1	0.975 a	0.21	9.441°C
	Year 2	1.025 b	0.20	10.593°C
SUN	Year 1	0.806 a	0.25	5.397 h/day
	Year 2	0.705 b	0.28	4.070 h/day
HRELA	Year 1	1.136 a	0.00	82.25%
	Year 2	1.135 a	0.00	82.18%

TMIN: Minimal temperature; RAIN: rainfall; TMAX: maximal temperature; TMOY: mean temperature; ETM: temperature gaps; SUN: sunshine; HRELA: relative humidity. Transformed average*: Values bearing the same letter in the third column are not significantly different according to Student-Fisher's test at 5% level. Untransformed average*: Presented values were obtained from reciprocal function 10^X for TMAX, TMOY, ETM and $y = 10^X-1$ for both RAIN and SUN. Values of HRELA were calculated from reciprocal function $y = a (\sin x)^2$, where a = 100. Untransformed values of TMIN were not calculated, because they did not undergo any transformation. RC*: Reliability coefficient. TMIN*: legend is as indicated under Table 2.

the plethoric number of generated time intervals. In the second step, some comparisons of the SE averages of these time intervals allowed progressive clustering into stable periods. A period was defined as a time interval composed of one or several months characterized by a reliability coefficient lower than 20%. Considering 20% threshold, all of periods of which the reliability coefficient exceeded it were not proposed to optimize the SE. The structuring of the SE variation commanded that of climatic parameters. In the third time, to confirm the stability and the reliability of identified periods, two complementary analyses were carried out. The first which was from a distance the most important, bore upon the appreciation of the significance of correlation coefficient between climatic parameter and SE for each identified period. The second consisted in appreciating the significance of annual correlation coefficient between each climatic parameter and SE as well as the sign of this one. From first, the comparison between the variation sense of the considered period average and the sign of correlation coefficient was performed. Consequently, the significance of correlation coefficient as well as the concordance between the variation sense of period averages and the sign of correlation coefficient finally conferred the status of reliable and stable climatic period (Issali et al., 2008c).

Statistical analyses

The versions 12.0.1 and 7.5.3 of SPSS and Xlstat softwares, respectively, were used for the statistical analyses as a whole. In order to identify the best parameters of the climatic variation, the comparison of means and the Principal Component Analysis were applied. This comparison of means was performed according to Student-Fisher's test at 5% level. Likewise, to identify the climatic periods, the SE averages as well as those of climatic parameters were compared. Such comparisons were carried out according to LSD Student-Fisher's test at 5% likelihood. Likewise, to optimize the SE from concentration in 2.4D/TDZ in favourable and unfavourable periods, means were separate according to LSD Student-Fisher's test at 5% level. In order to search for the links between the climatic periods and SE, the Pearson's linear correlations at 5%, 1% and 1‰ thresholds were tested. Likewise to look for the annual global links the Pearson's linear correlations were tested too. Moreover, correlations are tested according to Pearson's linear correlation at 5 and 1% as well as 1‰ probability. Furthermore, relationship between concentration in 2,4-D/TDZ and SE inside favourable and unfavourable periods is looked for understanding the influence of these two types of periods. For this purpose, the Student-Fisher LSD test at 5% threshold is used.

RESULTS

Best parameters of climatic variation for SE of cocoa tree

For the annual variations, except for the relative humidity of which data did not significantly vary, those of the six other climatic parameters statistically varied, from year to year (Table 3). Consequently, the relative humidity was eliminated from the study. Continuation of works was only carried out with the six remaining climatic parameters. Likewise, because of the existence of these annual variations, analyses were performed year by year. The dispersion of both measured and calculated climatic parameters around the average was weak, because the reliability coefficients spread out from 0 to 1.04%.

Identification of the best climatic parameters from principal components revealed that maximal temperature and mean temperature have a similar behaviour, because

Table 4. Link between the principal components and the climatic parameters through the cosine^2 on two years of study.

Year	Climatic parameters	Principal components*			
		F1	F2	F3	F4
Year 1	RAIN	0.001	0.001	0.984	0.014
	TMAX	0.000	0.858	0.001	0.140
	TMOY	0.533	0.445	0.000	0.022
	ETM	0.753	0.152	0.002	0.093
	SUN	0.025	0.118	0.028	0.829
	TMIN	0.941	0.053	0.000	0.005
Year 2	RAIN	0.013	0.000	0.985	0.002
	TMAX	0.000	0.913	0.000	0.087
	TMOY	0.620	0.289	0.006	0.085
	ETM	0.930	0.054	0.009	0.007
	SUN	0.083	0.131	0.004	0.783
	TMIN	0.898	0.045	0.009	0.048

Principal components*: F1, F2, F3 and F4 are some new parameters not correlated among them. In bold type, the cosine^2 of climatic parameters are the best represented on the principal component.
Principal Component Analysis: For each of two years of study, the first four components explained 100% of total variation. Whatever the year, the component 1 described low temperatures. It explained in the first year and in the second year 37.55 and 42.40%, respectively, of variation. The minimal temperature and the temperature gaps were represented there. These two climatic parameters were unfavourably correlated (r/TMIN-ETM/year 1 = - 0.774; r/TMIN-ETM year 2 = - 0.892). The component 2 expressed the strong heat. It described in the first year 27.14%, as against 23.86% in the second year of residual variation unexplained by the component 1 of each year. In the course of the two years of study, maximal temperature and mean temperature were the most salient on this component 2. They were positively correlated (r/TMAX-TMOY/year 1 = +0.682; r/TMAX-TMOY/year 2 = + 0.612). The component 3 described precipitations. In the first year, it explained 16.91%, as against 16.86% in the second year with respect to remaining variation unexpressed by the component 2 of each year. Only the rainfall was important there. The component 4 valorised the solar radiation. In the first and the second year, it showed 18.40 and 16.88%, respectively, of staying variation unexplained by the component 3. The sunshine was well represented on this one.

they are positively correlated. Therefore, mean temperature was eliminated due to its weak representativity on the Principal Component 2, from year to year. Thus, minimal temperature, temperature gaps, maximal temperature, rainfall and sunshine were chosen as the best climatic parameters on which the study continued (Table 4).

Organization of the best parameters of the climatic variation in stable periods

For minimal temperature, in hybrids, whatever the year, no perceptible variation was recorded. Thus, in the first year and the second year, only one time interval of eleven and twelve months, respectively, was identified (Table 5). In control clones, from year to year, two periods were evidenced. In the first year, period TI_1, including months of September, October, November, December and January, characterized by a weak minimal temperature. In the opposite, period TI_2 constituted of months of February, March, May, June, July and August was marked by a strong minimal temperature. In the second year, period TI_1 composed of months of January, February, March, April, May, June, July and August was distinguishable by a low minimal temperature. However, period TI_2 comprising months of September, October, November and December was marked by a high minimal temperature (Table 5). With respect to temperature gaps, in hybrids, whatever the year, temperature gaps did not significantly vary. Thus, in the first and the second year, only one period of eleven and twelve months, respectively, was observed (Table 5).

In control clones, for two years of study, two time intervals were pointed up in the course of each of them. In the first year, period TG_1, constituted of months of September and August, was characterized by some weak temperature gaps. The second year, period TG_1 of low temperature gaps was composed of September, October, November and December. In the opposite, in the first year period TG_2, consisted of months of October, November, December, January, February, March, May, June and July, was marked by some strong temperature gaps. In the second year, period TG_2 of elevated temperature gaps was composed of months of January, February, March, April, May, June, July and August (Table 5).

Regarding maximal temperature, in hybrids, in the first year two periods were identified, against one the second year. Indeed, in the first year, period TM_1, constituted of months of September, October and July was distinguishable by a weak maximal temperature. Period TM_2 of the same year, characterized by a strong maximal temperature was composed of months of November, December, January, February, March, May, June, August and December. In the second year no significant variation was registered. Sure enough, only one period of twelve months was observed (Table 5). In control clones, whatever the year, two time intervals were revealed. In the first year, period TM_1 of weak maximal temperature was constituted of months of September, October, July and August. This time interval divided into two sub time intervals on a level with SE. The first sub time interval $TM_{1/1}$ comprised months of September and October, whereas the second time interval $TM_{1/2}$ consisted of months of August and July. In the second year, period TM_1, comprising months of September, October, November and December, was marked by low maximal temperature. On the other hand, in the first year, period T_2 of strong maximal temperature was composed of months of November, December, January, February, March, May and June. In the second year, period T_2, including months of January, February, March, April, May, June, July and August, was characterized by a high maximal temperature (Table 5).

As regards rainfall, in hybrids, whatever the year, two

Table 5. Classification of averages of climatic periods, of SE which associated with them and the Pearson's linear correlation coefficient between climatic parameters and SE in each of periods.

Year	Genotype	Climatic parameter	Period*	Average of Climatic parameter*	RC (%)*	Average of Associated SE*	RC (%)*	Correlation with SE*	p*
Year 1	Hybrid	Minimal temperature	TI	20.797	0.15	1.411	10.69	+0.10	0.795
	Clone		TI_1	19.275 a	0.16	5.698 a	8.97	-0.005	0.950
			TI_2	22.491 b	0.15	2.152 b	8.39	+0.270***	<0.0001
Year 2	Hybrid		TI	19.011	0.16	1.047	11.05	+0.030	0.405
	Clone		TI_1	17.219 a	0.16	12.397	2.53	+0.017	0.722
			TI_2	22.856 b	0.22	10.030	4.20	-0.079	0.271
Year 1	Hybrid	Temperature gaps	TG	9.780	0.613	1.411	10.69	-0.098**	0.009
	Clone		TG_1	4.764 a	1.77	8.994 a	8.97	-0.283***	<0.0001
			TG_2	10.568 b	0.59	2.945 b	8.39	+0.114*	0.046
Year 2	Hybrid		TG	10.568	0.49	1.047	11.05	-0.024	0.505
	Clone		TG_1	6.966 a	0.47	10.030 a	4.20	+0.033	0.643
			TG_2	12.972 b	0.27	12.397 b	2.53	+0.104**	0.009
Year 1	Hybrid	Maximal temperature	TM_1	29.717 a	0.07	2.883 a	13.55	-0.074	0.059
			TM_2	31.261 b	0.07	0.941 b	15.57	-0.117**	0.002
	Clone		TM_1:$TM_{1/1}$	29.174 a	0.07	0.379 a	50.32	+0.286	0.031
			$TM_{1/2}$			10.311c	7.38	-0.163**	0.002
			TM_2	31.696 b	0.07	3.309 b	8.91	+0.198**	0.001
Year 2	Hybrid		TM	30.269	0.07	1.047	11.05	+0.022	0.533
	Clone		TM_1	20.417 a	0.15	10.030 a	4.20	-0.056	0.440
			TM_2	30.200 b	0.07	12.397 b	2.53	+0.050	0.213
Year 1	Hybrid	Rainfall	RA_1	4.458 a	4.07	1.960 a	9.79	+0.069	0.067
			RA_2	20.429 b	5.11	0.011 b	301.94	+0.116**	0.005
	Clone		RA_1	1.213 a	14.49	7.907 a	6.54	-0.075	0.154
			RA_2	13.689 b	3.86	1.764 b	12.65	-0.087	0.158
Year 2	Hybrid		RA_1	6.362 a	3.11	0.830 a	13.72	+0.052	0.146
			RA_2	38.902 b	3.56	2.338 b	17.33	+0.142*	0.046
	Clone		RA_1	5.353 a	4.61	9.809 a	3.10	+0.017	0.674
			RA_2	14.171 b	3.56	14.304 b	2.93	+ 0.035	0.426
Year 1	Hybrid	Sunshine	S	5.281	0.75	1.414	10.69	-0.043	0.251
	Clone		S_1	2.396 a	2.82	8.994 a	8.97	-0.191***	0.000
			S_2	5.966 b	0.95	2.945 b	8.39	+0.113*	0.049
Year 2	Hybrid		S	4.093	0.99	1.047	11.05	+0.030	0.410
	Clone		S	4.012	1.14	11.642	2.17	-0.018	0.660

Period*: Time intervals of each of four climatic parameters previously identified as the best. Average of climatic parameter*: Values bearing the same letter are not statistically different according to LSD Student-Fisher's test at 5% probability. RC (%)*: Reliability coefficient in percentage. Average of associated SE*: Value of somatic embryogenesis corresponding to the period of identified climatic parameter. SE: Used values come from average of embryos per embryogenic explant (MEXEMB). Correlation with SE*: Values of correlation coefficient of each of periods of climatic parameters. P*: Calculated probability to compare at 5 and 1% as well as 1‰ thresholds after the Pearson's linear correlation.

periods were identified. In the first year, period RA_1, composed of months of September, October, November, December, January, February, March, July and August, was characterized by a weak rainfall. In the second year, period RA_1 of low rainfall was constituted of months of January, February, March, April, May, June, July, August, September and December. In contrast, in the first year, period RA_2, including months of May and June, was distinguishable by a strong rainfall. In the second year, period RA_2, comprising months of October and November, recorded a high rainfall (Table 5). In control clones, whatever the year, two periods were observed too. In the first year, period RA_1 of weak rainfall was constituted of months of January, March, July and August. In the course of the second year, period of low rainfall RA_1 included months of January, February, July,

Table 6. Annual global link between five climatic parameters climatic parameters and SE in hybrids through the Pearson's linear correlation at 5, 1% and 1‰ levels.

Pearson's linear correlation											
		Year 1					Year 2				
Geno.*	Var.*	TI*	TG*	TM*	RAIN*	S*	TI	TG	TM	RAIN	S
Hybrid	SE*	0.010	-0.098**	-0.117**	+0.069	-0.043	+0.030	-0.024	+0.022	+0.052	+0.030
Clone	SE	+0.241***	-0.283***	-0.163**	-0.075	-0.191***	-0.085*	+0.104**	+0.050	+0.017	-0.018

Geno*: Genotype. TI*: Annual minimal temperature. TG*: Annual temperature gaps. TM*: Annual maximal temperature. RAIN*: Annual rainfall. S*: Annual sunshine. Var.*: Variable for tissue culture. SE*: Average of embryos per embryogenic explant (MEXEMB).

August, October, November and December. In contrast, in the first year period RA_2 of strong rainfall was composed of months of September, October, November, December, February, May and June. In the second year, however, period RA_2 of high rainfall comprised months of March, April, May, June and September (Table 5). As to sunshine, in hybrids, from year to year, no significant variation was recorded. Thus, only one period, respectively, of eleven and twelve months was observed (Table 5).

In both control clones, the first year provided two periods, while the second year no variation was registered. With respect to this first year, period S_1, constituted of months of August and September, was marked by a low sunshine. Period S_2 of high sunshine was composed of months of October, November, December, January, February, March, May, June and July (Table 5).

Relationship between stable climatic periods and SE

On the whole, sole maximal temperature did not record conformity between its time intervals and those of SE. Indeed, time interval TM_1 of control clones during the first year was divided into two sub time interval designated $TM_{1/1}$ and $TM_{1/2}$. Consequently, periods of maximal temperature (in number two) differed from those of SE (in number three; Table 5).

For minimal temperature and SE, in hybrids, whatever the year, no statistically different variation was registered. Nevertheless, some SE averages of each of observed annual periods were calculated (Table 5). No significant link was detected between SE and minimal temperature (Table 6). In control clones, from year to year, period TI_1 of weak minimal temperature corresponded to a high SE, while period TI_2 of strong minimal temperature was associated with a low SE. Sole in the period TI_2 of first year where the variation sense between SE and minimal temperature corresponded to a link positive and very highly significant (Table 5). Moreover, annual global link was favourable and very highly significant in the first year, whereas in the second year, it was significant, but unfavourably (Table 6). Regarding temperature gaps, in hybrids, from year to year, no statistical difference was noted on a level with their SE. The means of SE of time intervals of eleven and twelve months constituting each of years were calculated (Table 5). In the first year, although the correlation between both is unfavourable but significant, the lack of distinct period does not allow an analysis of this one (Table 5). Furthermore, sole in the first year the annual global link between temperature gaps and SE was very significant, but unfavourably correlated (Table 6).

In both control clones, whatever the year, two periods were determined. In the first year variation sense of periods of temperature gaps contrasted with that of SE, whereas in the second year this one was comparable. Indeed, in the first year, period TG_1 of low temperature gaps was suited to a high SE, while period TG_2 of high temperature gaps was associated with a low SE. In the second year, period TG_1 of weak temperature gaps was linked with a weak SE, in the same way, period TG_2 of strong temperature was suited to a strong SE (Table 5). Within the identified periods, variation sense of periods TG_1 and TG_2, respectively, for the first and second years corresponded to sign of associated correlation coefficients between this climatic parameter and SE. These ones were very highly significant in the first year, but very significant in the second year (Table 5). Furthermore, annual global relationship was very highly unfavourable, in the first year, whereas the second year it was very favourably correlated (Table 6). Concerning maximal temperature and SE in hybrids, only in the first year a significant variation was registered. In the course of this first year, variation sense of time intervals of maximal temperature contrasted with those of SE. Indeed, period TM_1 of weak maximal temperature corresponded to strong SE, while period TM_2 of strong maximal temperature was suited to a weak SE (Table 5). In the first year, in time interval TM_2, the opposite variation sense between maximal temperature and SE was proved to be very negatively correlated (Table 5). Moreover, only in the first year the annual global correlation between maximal temperature and SE was very unfavourable too (Table 6).

In both control clones, a shift between time intervals of maximal temperature (in number two) and those of SE (in number three) was recorded in the first year. However, in the second year, two periods of equal length were identified. Sure enough, in the first year, period TM_1 of weak maximal temperature was divided into two on a level with time intervals of SE. Thus, the sub time interval $TM_{1/1}$,

characterized by a weak SE; whereas sub time interval $TM_{1/2}$ was marked by a strong SE. On the other hand, period TM_2 of strong maximal temperature revealed a mean SE. In the second year, period TM_1 of low maximal temperature was linked with a low SE, likewise period TM_2 of strong maximal temperature corresponded to an elevated SE (Table 5). In the first year, sub period $TM_{1/2}$ and period TM_2 for which variation sense of maximal temperature was opposite to that of SE, expressed, respectively, some unfavourable and favourable links. These links were very significant (Table 5). Furthermore, as in hybrids, the entire first year provided a very unfavourable link between maximal temperature and SE (Table 6).

As regards rainfall and SE, in hybrids, two time intervals were defined. The first year, the variation sense of rainfall periods did not correspond with that of SE, while the second year, such a correspondence was perfect. Indeed, the first year period RA_1 of weak rainfall corresponded with a high SE, whereas period RA_2 of strong rainfall was suited to a low SE. However, the second year period RA_1 of low rainfall was linked with low SE, likewise period RA_2 of high rainfall corresponded a high SE (Table 5). However, both periods RA_2 of first and second year recorded some variation senses in relation to SE opposite the first year, but similar in the second year. In these two periods, the link although it is positive, was respectively very strong the first year, but least the second year (Table 5). Furthermore, in hybrids, from year to year, no significant link was recorded between rainfall and SE (Table 6). In both control clones, two periods were identified as in hybrids. In the first year, variation sense of rainfall periods did not coincide with that of SE, whereas in the second year such a coincidence was effective. Sure enough, in the first year period RA_1 of weak rainfall was linked with a strong SE, whereas the period RA_2 of strong rainfall was suited to weak SE. In contrast, in the second year, period RA_1 of low rainfall was in accordance with a low SE, likewise period RA_2 of high rainfall corresponded with high SE (Table 5). No significant link was established between rainfall and SE (Table 5). Here also, whatever the year, no significant relation was revealed between rainfall and SE (Table 6).

As to sunshine and SE, in hybrids, from year to year, no variation significantly different was observed. Yet, the time intervals of eleven and twelve months, respectively, identified in the first and in the second year provided some means of SE (Table 5). In control clones, in the first year, the period S_1 of low sunshine was suited to a high SE, whereas the period S_2 of high sunshine corresponded to low SE (Table 5). Within the identified periods, in control clones only period S1 of the first year, variation sense between SE and sunshine corresponded with negative sign of correlation coefficient. This correlation coefficient was very highly significant (Table 5). Furthermore, annual global link between SE and sunshine was very highly, but unfavourably significant (Table 6).

Relationship between concentration in 2,4-D/TDZ and SE within the favourable and unfavourable periods

Concerning the favourable periods, whatever year and clone the variations of concentration in 2,4-D/TDZ induced unstructurable and independent variations in distinct classes of average of embryos per embryogenic explant. Variability of measured values around average stretched out from 4.30 to 19.45% (Table 7).

For unfavourable periods, in the first year, only the variations of concentration in 2,4-D/TDZ in period TI_1 of control clones triggered structurable and observable variations in distinct groups of average of embryos per embryogenic explant. Thus, the induction media at weak hormonal concentration, PCG3 and PCG1, respectively, favoured SE, while the induction medium at high hormonal concentration PCG4 reduced SE. Amplitude of gaps of measured values spread out from 9.77 to infinity %. Regarding the second year, the variations of concentration in 2,4-D/TDZ in periods TI, RA_1, RA_2 of hybrids as well as TI_2, TG_1, TM_1, RA_1 and S of control clones induced organizable and perceptible variations in separate classes of SE. During this second year, from genotypes group to genotypes group and in concerned unfavourable periods, the variations of hormonal concentrations provided some comparable effects. Indeed, the calli induction media at low hormonal concentration, PCG3 and PCG1, respectively, induced a low SE, where as the one at high hormonal concentration PCG4 highlighted a high SE. Percentage of gaps between average and observed values stretched out from 4.57 to 60.32% (Table 8).

DISCUSSION

The relationship between five climatic parameters and SE was looked for through the identification of favourable and unfavourable periods. Likewise, from these ones the attempt to optimize the SE in unfavourable periods was undertaken through the analysis of the variation of their SE. The year strongly influenced the variations of minimal temperature, rainfall, maximal temperature, mean temperature, temperature gaps and sunshine. Such an influence reflects not only the differences of received solar energy by the land area, but also the atmospheric general circulation, the oceanic general circulation as well as those of the relief. However, in spite of the existence of the annual variations, these ones remain compatible with the evolution of life on earth (Encyclopaedia Encarta, 2006). Consequently, the data of two years were analysed year by year.

The minimal temperature, the temperature gaps, the maximal temperature, the rainfall and the sunshine were the most variable (Table 3). These are the best parameters of the climatic variation of our study. This might be due to recorded reasonable gaps, from day to day. They were

Table 7: Classification of embryogenesis averages as a function of effect of concentration in 2.4 D/TDZ of PCG induction media in unfavourable periods for two years of study in cocoa tree.

Year	Genotype	Unfav. period*	Induction Medium	Transf. Average*	RC (%)*	Untransf. average*	Year	Genotype	Unfav. period	Induction Medium	Transf. Average*	RC (%)*	Untransf. average*
Year 1	Hybrid	TI	PCG3	0.932 a	23.50	0.869	Year 2	Hybrid	TI	PCG3	0.592 a	32.94	0.350
			PCG4	1.235 a	17.57	1.525				PCG1	0.769 a	25.36	0.591
			PCG1	1.401 a	15.85	1.963				PCG4	1.681 b	11.36	2.826
	Clone	TI_1	PCG4	0.701a	45.08	0.491		Clone	TI_1	PCG1	3.488 a	4.30	12.166
			PCG1	1.612 b	19.42	2.599				PCG3	3.434 a	4.66	11.792
			PCG3	2.040 b	14.90	4.162				PCG4	3.656 a	4.51	13.366
		TI_2	PCG4	2.106 a	14.58	4.435		Clone	TI_2	PCG3	2.804 a	7.74	7.862
			PCG1	2.264 a	13.38	5.126				PCG1	2.900 a	7.10	8.410
			PCG3	2.784 a	10.88	7.751				PCG4	3.777 b	5.53	14.266
		TG_2	PCG4	0.000 a	∞	0.000		Clone	TG_1	PCG3	2.804 a	7.74	59.891
			PCG3	0.313 a	101.28	0.098				PCG1	2.900 a	7.10	50.459
			PCG1	0.618 a	48.38	0.382				PCG4	3.777 b	5.53	30.620
	Hybrid	TM_1	PCG3	1.327 a	37.30	1.761		Clone	TM_1	PCG3	2.804 a	7.74	7.862
			PCG1	1.428 a	34.38	2.039				PCG1	2.900 a	7.10	8.410
			PCG4	2.284 a	20.62	5.217				PCG4	3.777 b	5.53	14.266
		TM_2	PCG4	0.760 a	30.39	0.578		Clone	TM_2	PCG3	3.434 a	54.94	11.792
			PCG3	0.772 a	29.79	0.596				PCG1	3.488 a	52.32	12.166
			PCG1	1.389 a	16.85	1.929				PCG4	3.656 a	60.32	13.366
	Clone	$TM_{1/1}$	PCG4	0.000 a	∞	0.000		Hybrid	RA_1	PCG3	0.482 a	44.61	0.232
			PCG1	0.529 ab	66.92	0.280				PCG1	0.707 a	30.41	0.500
			PCG3	1.219 b	26.91	1.486				PCG4	1.528 b	13.87	2.335
	Hybrid	RA_1	PCG3	1.090 a	23.76	1.188			RA_2	PCG1	1.068 a	43.45	1.141
			PCG4	1.440 a	17.71	2.074				PCG3	1.093 a	41.45	1.195
			PCG1	1.672 a	15.61	2.796				PCG4	2.336 b	18.62	5.457
		RA_2	PCG1	0.000 a	∞	0.000		Clone	RA_1	PCG1	2.954 a	5.55	8.726
			PCG3	0.150 a	82.67	0.023				PCG3	2.966 a	5.83	8.797
			PCG4	0.154 a	81.82	0.024				PCG4	3.500 b	4.97	12.250
	Clone	RA_1	PCG4	2.480 a	13.51	6.150			RA_2	PCG1	3.776 a	4.71	14.258
			PCG1	2.620 a	12.56	6.864				PCG3	3.614 a	5.26	13.061
			PCG3	3.305 a	9.77	10.923				PCG4	3.965 a	4.89	15.721
		RA_2	PCG4	0.743 a	37.82	0.552		Clone	S	PCG3	3.246 a	4.00	10.537
			PCG1	1.473 ab	18.94	2.170				PCG1	3.313 a	3.71	10.976
			PCG3	1.752 b	15.81	3.070				PCG4	3.696 b	3.57	13.660

Table 7. Contd.

		S_2	PCG4	2.853 a	19.45	8.140							
			PCG1	2.978 a	17.33	8.868							
			PCG3	3.158 a	16.91	9.973							

Legend: Unfav. period*: Unfavourable period. Transf. average*: Transformed average. Values followed of the same letter in the column are not significantly different after the LSD Student-Fisher test at 5 % level. RC (%)*: Reliability coefficient in percentage. Untransf. average*: Untransformed average. Sign ∞ in the column of reliability coefficient results in infinite value of this one.

organized in stable climatic periods (Table 5).

The organization of each climatic parameter in stable periods was based on the SE variations to which we associated those of concerned climatic parameter (Table 4). Control clones used in our study were more embryogenic than hybrids. A comparable result was found by Issali et al. (2008b). Consequently, they provided more of periods than hybrids (Table 5). Furthermore, among five climatic parameters structured in periods, sole maximal temperature in the first year in hybrids underwent a duplication of its SE. Sure enough, in the course of this year, a weak variation of maximal temperature, marked by two periods, induced an amplification of SE which resulted in three time intervals. A similar result was obtained relating to the relationship between three phenological parameters and SE in *T. cacao* by Issali et al. (2008c, accepted for publication). However, this result did not happen again neither the second year nor in other climatic parameters. Thus, we can be deduced that it is about isolated phenomenon.

In hybrids, no period was found favourable to SE. However, periods TM_1 and RA_1 that recorded high SE, on the other hand provided some correlation coefficients not significant. However, a time interval stretched out from August to October, including February was found to be favourable to SE in hybrids concerning the relationship between phenology and SE by Issali et al. (2008c). The difference of composition of time intervals could be due to the difference in action mode of climate and phenology. Sure enough, phenology variations are only the expression of climatic variations. But a shift in the time exists between these two variations (Issali, 2008a). Regarding control clones, four periods designated TG_1, $TM_{1/2}$ and S_1 in the first year and TG_2 in the second year were able to increase SE. These periods are respectively those of temperature gaps, maximal temperature and sunshine. However, the influence of temperature gaps seems predominant. Because, upon the four identified periods as a whole, eight months out of nine belongs to period TG_2 of temperature gaps. In all, the months of January, February, March, April, May, June, July, August and September increased SE. In contrast, analysing relationship between phenology and SE, the time interval spread out from February to December was considered to be favourable to SE by Issali et al. (2008b). Although both time intervals is different, the months of February, March, April, May, June, July, August and September are common to them. Their similarity at four months of difference close seems to express a certain identity in action mode of climate and phenology on SE.

With respect to relationship between concentration in 2,4-D/TDZ and SE in favourable periods, all of concentrations in 2,4-D/TDZ have some similar effects (Table 6). Such a similarity seems to indicate that the cells get the embryogenic competence when the concentration of substances proceeding from metabolism of 2,4-D and TDZ attained a same level, whatever their initial concentration. Consequently, it could exist a concentration threshold in metabolites of 2,4-D and TDZ responsible for optimizing of SE. Destiny of concerned cells would depend on this threshold. Thus, when this concentration threshold is attained, these cells would acquire the embryogenic competence and consequently would tend towards the embryogenic morphogenesis. This threshold would be mainly influenced by certain climatic parameters such as temperature gaps, maximal temperature and sunshine. In *T. cacao*, primary SE originates from multicellular pathway (Maximova et al., 2002). We can admit that the threshold concentration in metabolites from 2,4-D/TDZ, for every cell involved in formation of a somatic embryo is reached at the same time. Thus, the climate influences SE, but the assessment of the degree of such an influence does not concern this paper. Furthermore, in unfavourable periods, the concentration in 2,4-D/TDZ increasing SE seems to depend on year (Table 8). Indeed, in the first year, the concentration in 2,4-D/TDZ the most weak was that of PCG3, that was the most embryogenic moreover. In the opposite, in the second year, whatever was considered unfavourable period, that was the concentration in 2,4-D/TDZ of PCG4 which was

Table 8. Classification of embryogenesis averages as a function of effect of concentration in 2,4-D/TDZ of PCG induction media in unfavourable periods for two years of study in cocoa tree.

Year	Genotype	Unfav. period*	Induction medium	Transf. average*	RC (%)*	Untransf. average*	Year	Genotype	Unfav. period	Induction medium	Transf. average*	RC (%)*	Untransf. average*
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			PCG1	1.401 a	15.85	1.963				PCG4	1.681 b	11.36	2.826
	Clone	TI_1	PCG4	0.701a	45.08	0.491		Clone	TI_1	PCG1	3.488 a	4.30	12.166
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			PCG3	2.040 b	14.90	4.162				PCG4	3.656 a	4.51	13.366
		TI_2	PCG4	2.106 a	14.58	4.435		Clone	TI_2	PCG3	2.804 a	7.74	7.862
			PCG1	2.264 a	13.38	5.126				PCG1	2.900 a	7.10	8.410
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		TG_2	PCG4	0.000 a	∞	0.000		Clone	TG_1	PCG3	2.804 a	7.74	59.891
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			PCG1	0.618 a	48.38	0.382				PCG4	3.777 b	5.53	30.620
	Hybrid	TM_1	PCG3	1.327 a	37.30	1.761		Clone	TM_1	PCG3	2.804 a	7.74	7.862
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			PCG4	2.284 a	20.62	5.217				PCG4	3.777 b	5.53	14.266
		TM_2	PCG4	0.760 a	30.39	0.578		Clone	TM_2	PCG3	3.434 a	54.94	11.792
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	Clone	$TM_{1/1}$	PCG4	0.000 a	∞	0.000		Hybrid	RA_1	PCG3	0.482 a	44.61	0.232
			PCG1	0.529 ab	66.92	0.280				PCG1	0.707 a	30.41	0.500
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			PCG1	1.672 a	15.61	2.796				PCG4	2.336 b	18.62	5.457
		RA_2	PCG1	0.000 a	∞	0.000		Clone	RA_1	PCG1	2.954 a	5.55	8.726
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	Clone	RA_1	PCG4	2.480 a	13.51	6.150			RA_2	PCG1	3.776 a	4.71	14.258
			PCG1	2.620 a	12.56	6.864				PCG3	3.614 a	5.26	13.061
			PCG3	3.305 a	9.77	10.923				PCG4	3.965 a	4.89	15.721
		RA_2	PCG4	0.743 a	37.82	0.552		Clone	S	PCG3	3.246 a	4.00	10.537
			PCG1	1.473 ab	18.94	2.170				PCG1	3.313 a	3.71	10.976
			PCG3	1.752 b	15.81	3.070				PCG4	3.696 b	3.57	13.660
		S_2	PCG4	2.853 a	19.45	8.140							
			PCG1	2.978 a	17.33	8.868							
			PCG3	3.158 a	16.91	9.973							

Unfav. period*: Unfavourable period. Transf. average*: Transformed average. Values followed of the same letter in the column are not significantly different after the LSD Student-Fisher test at 5 % level. RC (%)*: Reliability coefficient in percentage. Untransf. average*: Untransformed average. Sign ∞ in the column of reliability coefficient results in infinite value of this one.

the most embryogenic. A similar result was obtained by Issali et al. (2008c, accepted for publication) analysing the relationship between phenology and SE. Link among three factors which are year, concentration in 2,4-D/TDZ and SE could express an interaction among them in relation to measured variable for tissue culture (MEXEMB). However, the relationship thus established is only statistical, that is to say numeric. It did not allow the quantification of the level of contribution of these climatic parameters to variations either callogenesis or SE or both.

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