Changes in Inorganic Composition of Cocoa Cotyledons During Germination Under Different Nursery Shade Conditions[1]

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ABSTRACT

The dynamics of inorganic constituents of three cocoa (*Theobroma cacao* L.) seed cultivars during germination under eight different nursery shade materials was investigated. Variations were observed in the environments provided by each of these shade materials which appeared to affect the metabolism of these constituents with elephant grass (*Pennisetum purpureum*) shade providing the most comparable conditions to oil palm frond shade. Statistical differences among cocoa seed cultivars in the residual contents of all the inorganic constituents in the abscissed cotyledons were significant, while shade effects were also significant but only for the micronutrient constituents. Cultivar – shade interaction effects were also significant. Relative to the initial composition of the cocoa seeds, levels of Mg, Ca, Zn, Mn and Fe in the abscissed cotyledons increased while levels of P, K and Cu decreased. From the results, it is suggested that the cotyledon is not the only source of inorganic nutrients for the seedling during germination as these nutrients may be supplemented from soil solutes. The results also show that the seedling may have a higher requirement and therefore greater absorption capacity for Mg, Ca, Zn, Mn and Fe. In addition, elephant grass, commonly known as Naphier fodder, is suggested as an adequate replacement for the increasingly cost-prohibitive oil palm fronds in cocoa nursery shading.

COMPENDIO

Se investigó la dinámica de los elementos inorgánicos en tres cultivares de semillas de cacao (*Theobroma cacao* L.) durante su germinación en vivero y a la sombra de ocho diferentes plantas. Se observaron variaciones en el ambiente propiciado por cada una de estas plantas, el cual parece afectar el metabolismo de dichos elementos. La sombra arrojada por el "pasto elefante" (*Pennisetum purpureum*) determina condiciones similares a las existentes bajo la sombra de las hojas de palma aceitera. Fueron significativas las diferencias estadísticas entre los cultivares de semillas de cacao con respecto a los contenidos residuales de todos los elementos inorgánicos, en los cotiledones cortados por abscisión; mientras que los efectos producidos por la sombra también fueron significativos, pero sólo para los micronutrimentos. También fueron importantes los efectos de la interacción entre la sombra y el cultivar. En comparación con la composición inicial de las semillas de cacao, aumentaron los niveles de Mg, Ca, Zn, Mn y Fe en los cotiledones cortados por abscisión; mientras que disminuyeron los niveles de P, K y cobre. Con base en los resultados se indica que el cotiledón no es la única fuente de nutrimentos inorgánicos para la plántula durante la etapa de germinación, ya que ellos pueden también derivarse de los solutos del suelo. Los resultados también muestran que la mayor capacidad para absorber Mg, Ca, Zn, Mn y Fe puede ser atribuida a un requerimiento mayor de los mismos por parte de la plántula. Además, porque resulta altamente costoso usar la palma aceitera para propiciar sombra en los viveros de cacao, se recomienda el empleo del "pasto elefante" como una alternativa adecuada.

INTRODUCTION

Theobroma cacao L. seeds exhibit the epigeous type of germination. Apart from the effect of the prevailing environment, its seedling establishment ability is largely dependent on the physiological activities and the genotype of the cotyledons. Yet relatively little is known about the changes in composition of the cocoa cotyledons during germination. Olofinboba (10) showed that the removal of the cotyledon before the formation of four fully expanded leaves results in growth retardation of the seedling, thus indicating its indispensable role as a food reserve organ. More recently, Ogunmoyela and Esan (11) showed that lipids, carbohydrates and proteins represent the three main classes of organic nutrients substantially lost from the cocoa cotyledon during the germination process and that definite cultivar differences exist in the metabolism of basic nutrients, modified by some weather components, namely, temperature, light intensity and relative humidity.

However, the metabolism of inorganic constituents of the cocoa cotyledon during germination is not yet reported. Studies by Krieg and Bartee (8) with cotton

1 Received for publication 10 October 1988
We thank the Director, Cocoa Research Institute of Nigeria for permission to publish this paper; and Messrs P.A. Akinrinade and O.I. Adegbola for their contributions to the work.

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seeds have shown that substantial losses of inorganic constituents which are influenced by temperature and cultivar differences occur during germination. Krieg and Carroll (9) also observed that the transfer of mineral nutrients K, Ca, Mg and P from the cotton seed cotyledon to the seedling axis varied according to cultivar and temperature, with no apparent correlation with seedling growth

According to Olofinboba (10), cocoa cotyledons are not carbon-dioxide fixing organs. However, their ability to photosynthesize is no longer in doubt from more recent observations *In vivo* vegetative propagation of cocoa requires a photosynthetic site, specifically the leaf (17). However *in vitro*, the photosynthetic requirements can be met by other sources. Using tissue culture techniques Esan (3) reported that the detached cocoa cotyledon which is a vegetative organ, has been made to root both *in vitro* and *in vivo*. More recently, he also showed (4) that rooting was significantly superior under light conditions than in dark and that this observed enhancement of rooting ability by the light effect is a clear indication of the photosynthetic capability of the cocoa cotyledon

In addition to the organic materials produced during photosynthesis, plant cells require a wide variety of inorganic elements for structural purposes (e.g. Ca, P, Mg) as well as for specific enzymic reactions which regulate cell metabolic processes (e.g. K, Mg, Fe, Cu, Zn, Mn, Mo). Plant cotyledons often contain sufficient stored quantities of some of these inorganic elements to eliminate the necessity of external sources. According to Galston (5), if the cotyledons of large-seeded plants like garden bean are removed, requirements of essential inorganics can be met through uptake from soil nutrients by the growing seedling. However, plants have the partial ability to select the rate at which they absorb various ions, hence absorption is usually not in direct proportion to nutrient availability (13). Hitherto, the dynamics of the inorganics of cocoa cotyledons have not been reported. Yet it must be noted that the relative changes in the total composition (organic and inorganic) of the cocoa cotyledons, from pre-germination to the post-abscission stage under specified nursery conditions, may be useful for characterising and thus formulating an empirical index for indentifying cocoa populations or cultivars. Similar indices have been shown to be reliable criteria for estimating and selecting outstanding cultivars in cocoa and other plants cultivated for their seeds, e.g. pod value (15); pod index (2); and seed density (16).

The present study was therefore conducted to examine the changes in inorganic constituents of the cocoa cotyledon during pre-germination and post-abscission stages, with the aim of establishing the dynamics of these constituents during the germination process. The possibility of formulating an empirical index for identifying cocoa populations or cultivars from the information thus obtained is also examined. The influence of nursery shade regimes on the exhaustion of these cotyledon inorganics (P, K, Ca, Mg, Cu, Fe, Mn and Zn) is also discussed.

MATERIALS AND METHODS

Cocoa beans used in this investigation were selected from bulk populations of the three cultivars, F3 Amazon, Amelonado and CRIN hybrid. The beans were extracted from ripe, healthy and freshly harvested pods of each population and the mucilage immediately removed by rubbing with dry sawdust. Shade materials used were carefully selected after a survey of commonly available materials.

Selection was based primarily on cost and durability. Thus, eight different shade materials were used. These were from raphia fronds (RF), oil palm (*Elaeis quineensis*) fronds (O-PF), elephant grass (*P. purpureum*) (EG), frosty polyethylene sheets of 2.33 mm thickness (FP), blue polyethylene sheets of 1.00 mm thickness (BP), green nylon netting of 2.00 mm perforation (GN), rainbow polyethylene sheets of 1.00 mm thickness (RP) and direct sunlight as control (UNSH). Daily readings of some weather components, such as temperature, relative light intensity and relative humidity, under the different shade materials, were taken throughout the experimental period and from which arithmetic averages were obtained. Nursery aspects of this investigation are as discussed by Ogunmoyela and Esan (11).

Samples of the fresh, mucilage-free cocoa beans, as well as those of the abscissed cotyledons from each of three replications of the three cultivar populations under the different shade regimes, were oven-dried to constant weight at 65 °C and stored in stoppered glass sample bottles

Analyses of the fresh cocoa beans from the three cultivar populations were replicated three times while those of abscissed cotyledons from cultivar shade interaction were replicated only twice due to limited sample size. Arithmetic averages were statistically analysed using the analysis of variance technique (1)

One gram sample in each case was carefully weighed and after removal of organic material by dry-ashing using the procedure described by Osborne and Voogt (12) contents of Ca, K, Mg, Cu, Fe, Mn and Zn were determined with the Hitachi Atomic Absorption Spectrophotometer at the appropriate wavelengths of each of these elements P was determined using the colorimetric molybdophosphate-ascorbic acid method on the Technicon auto-analyzer with the same ash extracts.

RESULTS AND DISCUSSION

Table 1 lists the average readings obtained over the experimental period for the weather components (temperature, relative light intensity and relative humidity), under the different shade materials.

Wide variations are observed in the temperature conditions under the different shades. FP shade provided the highest average temperature reading of 40.8 °C compared to 24.5 °C and 24.7 °C under O-PF and EG shades respectively. The light intensity was also found to be lowest under O-PF shade (25.61 %) and EG shade (30.76 %) compared to the unshaded (100 %). Oil palm fronds have long been established as an adequate shading material for cocoa in the nursery (7). However, since the cost of oil palm fronds is becoming highly prohibitive to small-scale farmers, primarily due to the difficulty in obtaining them, the use of elephant grass, a cheap, readily available weed material in most cocoa-growing areas, appears an adequate substitute for oil palm fronds in nursery shading. Usually about 4-8 m high and with bamboo-like stems often growing to 2.5 cm in diameter, its ready propagation from roots, seeds or stem cuttings (14) ensures availability in significant quantities all year round and with little labour costs compared to oil palm fronds. Table 2 outlines the inorganic components of fresh cocoa seeds analysed.

Table 1. Average readings of weather components obtained under different shade material.

	Nursery shade materials							
Weather component	Oilpalm-F (O–PF)	Rainbow-P (RP)	G-Netting (GN)	Raphia-F (RF)	Blue-P (BP)	E-Grass (EG)	Frosty-P (FP)	Unshaded (UNSH)
Temperature* (°C)	24.50	30.30	34.40	28.60	29.80	24.70	40.80	35.80
Rel. light intensity (%)**	25.61	55.38	61.53	55.38	46.15	30.76	69.23	100.00
Rel. humidity (%)***	60.20	65.40	50.80	55.80	65.70	60.30	65.50	50.50

* Temperature obtained using a mercury-in-glass thermometer
** Light intensity readings determined using the AMI SAV –A– PLANT II portable lightmeter.
*** Records obtained using Fisher's Hair-Hygrometer

The data show very low levels of Ca (0.042-0.099 %) relative to other macro-nutrients (0.16-0.17 % Mg; 0.39-0.41 % K and 0.44-0.45 % P). This is in agreement with the results of Lockhard and Burridge (6) who reported mean values of 0.08 % Ca, compared to 2.43 % N, 0.46 % P, 0.85 % K and 0.16 % Mg, from analyses of Ghana cocoa beans. Intercultivar differences for Ca are significant ($P < 0.05$) but not for other nutrients. Values of Mn and Cu in the cocoa seeds also compare with mean values of 28 ppm Mn and 19 ppm Cu reported by Lockhard

Table 2. Inorganic composition of cocoa beans of different cultivars. (Means of three replicate values are presented).

	Inorganic nutrient							
Cultivar	Mg (%)	Ca (%)	P (%)	K (%)	Mn (ppm)	Zn (ppm)	Cu (ppm)	Fe (ppm)
F3 Amazon	0.17	0.042	0.45	0.39	21	98	40	119
Crin hybrid	0.16	0.099	0.44	0.41	12	115	37	121
Amelonado	0.16	0.080	0.45	0.41	19	122	39	185

and Burridge (6) while Zn and Fe values of 54 ppm and 28 ppm respectively reported by the same workers are much lower than those obtained here. Table 3 lists the mean values of two replications for the inorganic nutrient composition of the abscissed cotyledons of the different cocoa cultivars under the different shade conditions.

When the mean square estimates for each of these inorganics (Table 4) are examined, cultivar differences are found to be highly significant ($P < 0.001$). However, while no significant differences are observed for the shade effect on macro-nutrients, the shade effect is highly significant for micronutrients. Cultivar shade interaction effects are also significant ($P < 0.01$) for both macronutrients ($P < 0.01$) and micronutrients ($P < 0.001$).

Table 3. Mean values (two replications) of inorganic nutrients in the abscissed cotyledons of different cocoa cultivars under different shade conditions.

		Olipalm-F (O-PF)	Rainbow-P (RP)	G-Netting (GN)	Raphia-F (RF)	Blue-P (BP)	E-Grass (EG)	Frosty-P (FP)	Unshaded UNSH	S.E. (MEAN)
F3 Amazon	Mg	0.50	0.22	0.21	0.22	0.32	0.38	0.22	0.33	0.03
	Ca	0.23	0.68	0.13	0.14	0.36	0.15	0.28	0.17	0.04
	P	0.39	0.39	0.42	0.40	0.38	0.42	0.44	0.32	0.01
	K	0.32	0.28	0.26	0.22	0.34	0.39	0.20	0.32	0.02
	Mn	93	66	68	99	94	91	88	94	3.02
	Zn	158	200	175	190	163	182	228	161	5.72
	Cu	28	14	12	28	14	23	28	12	1.82
	Fe	98	95	101	74	94	102	109	101	2.48
Crin hybrid	Mg	0.36	0.22	0.20	0.21	0.28	0.23	0.22	0.36	0.02
	Ca	0.15	0.65	0.12	0.12	0.24	0.10	0.87	0.46	0.07
	P	0.48	0.44	0.44	0.42	0.44	0.50	0.42	0.32	0.01
	K	0.33	0.21	0.28	0.25	0.32	0.39	0.42	0.40	0.02
	Mn	99	60	50	59	89	89	89	97	4.67
	Zn	165	191	163	181	174	174	197	169	2.93
	Cu	19	8	19	24	25	24	22	19	1.66
	Fe	95	106	107	105	93	108	97	107	1.48
Amelonado	Mg	0.34	0.22	0.21	0.22	0.40	0.25	0.22	0.29	0.02
	Ca	0.10	0.45	0.12	0.12	0.31	0.13	0.54	0.08	0.04
	P	0.38	0.37	0.36	0.41	0.35	0.39	0.45	0.31	0.01
	K	0.30	0.20	0.30	0.34	0.20	0.34	0.20	0.33	0.01
	Mn	91	58	55	72	92	94	74	89	3.78
	Zn	173	174	165	178	165	188	206	156	3.76
	Cu	32	7	6	13	15	26	9	9	2.29
	Fe	103	76	119	111	90	119	123	93	4.04

When the initial inorganic composition of the cocoa seed is compared to the compostion of the abscissed cotyledon, differences are found to exist in the contents of specific inorganic nurients. While Mg, Ca, Zn, Fe and Mn increased in the abscissed cotyledons relative to the cocoa seeds, P, K and Cu decreased. It appears from these observations that cocoa seed cotyledons normally contain sufficient stored quantities of P, K and Cu which are depleted during the germination process, and for which the need for absorption from soil solutes probably does not arise. On the other hand, the increase in the nutrients Mg, Ca, Zn, Fe and Mn appears to suggest that these are absorbed from the soil solutes, presumably as a result of the insufficiency of cotyledon reserves in meeting the requirements of the germinating seedling for these nutrients. According to Salisbury and Ross (13), plants have a partial ability to select the rate at which they absorb various ions from soil solutes. It is thus possible that these nutrients are absorbed more rapidly from the soil solutes than they are utilized in the metabolic activities of the germinating seedling compared to those nutrients for which decreases are recorded.

Table 5 shows the coefficients of relationship between the inorganics of fresh cocoa seeds and the abscissed cotyledons. Highly significant ($P < 0.01$) but inverse relationships are obtained between seed

Table 4. Mean square estimates of inorganic nutrients from analysis of variance.

Source of variation	df	Inorganic nutrient							
		Mg (%)	Ca (%)	P (%)	K (%)	Mn (ppm)	Zn (ppm)	Cu (ppm)	Fe (ppm)
Cultivars	2	*** 1 53	*** 1.62	*** 3.24	*** 1.82	*** 132 497 6	*** 670 249.3	*** 6 801.3	*** 204 678 6
Shades	7	n.s. 0 15	n.s. 0.12	n s 0.34	n s. 0.19	*** 13 904.0	*** 474 097 7	*** 577.4	*** 19 899.9
Cultivar x shade interaction	14	** 0 26	** 0 28	** 0.54	** 0 30	*** 11 138 3	*** 141 244 5	*** 640 7	*** 18 022 3
Error	23	0 07	0 07	0 15	0.08	510.0	2 387 3	25 7	771.6

** P < 0.01.
*** P < 0 001.
n.s. = non significant.

contents and abscissed cotyledon contents of Ca/Mg, P/K, K/Mn, Zn/Zn and Fe/Cu respectively, while the relationships for the relative contents of Mg/Mn, Zn/Fe and Cu/Mn, were positively significant ($P < 0.01$).

It appears from the foregoing that both the micro- and macronutrients are supplementarily absorbed, though selectively and at different rates from soil solutes, although the requirement for Mg, Ca, Zn and Mn appears much higher at this stage and thus necessitates higher absorption of these ions from the soil solutes. This variability in nutrient requirements for seedling growth is not uncommon in plant nutrition since certain inorganics are required in larger or smaller quantities at different stages of plant growth.

Although significant intercultivar differences ($P < 0.001$) are found between the levels of the inorganics in the abscissed cotyledons (Table 4), the dynamics of these constituents during the germination process appear to follow the same trend for all the cultivars. These differences do not therefore give any clear indication of the possibility of using variations in inorganic composition between the cultivars as a basis for characterising the cultivars. Thus, variations in proximate constituents of the abscissed cotyledons (especially lipids, protein and nitrogen-free extract values) provide a more useful guide for the identification of bulk populations of cocoa cultivars.

Table 5. Coefficients of relationship (r) between micronutrient levels in fresh cocoa seed nibs and the abscissed cotyledons.

		Abscissed Cocoa Cotyledons							
		Mg	Ca	P	K	Mn	Zn	Cu	Fe
Cocoa seed nib	Mg	0.98	–0.18	–0 21	–0.42	0 99	0.98	0.48	–0.97
	Ca	–0 99	0.38	0 51	0.69	–0.91	–0.88	–0 17	0.83
	P	0.67	–0.94	–0.95	–0.99	0.42	0 35	–0 52	–0.26
	K	–0.97	0.18	0.20	0.42	0.99	–0.98	–0 48	0.97
	Mn	0.81	–0.85	–0.86	–0.95	0.60	0 54	–0 32	–0.46
	Zn	0.72	–0.10	–0.08	0.14	–0.98	–0.99	–0 71	–0.99
	Cu	0.88	–0.78	–0 79	–0.91	0.99	0.64	–0.21	–0.56
	Fe	–0 33	–0.74	–0.73	–0 55	–0.60	–0.65	–0.99	0.72

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