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CACAO: NEW RESEARCH ADVANCES AND APPLICATIONS OF BIOTECHNOLOGY*

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ABSTRACT

Cacao has been cultivated for more than 400 years and this crop still faces numerous problems with diseases, pests and the lack of high yielding clone materials. Cellular genetics and molecular biology could play an important role to complement existing germplasm evaluation and breeding efforts.

Recent progress has been made on the recovery of somatic embryos from non-sexual explants (petals and nucellus tissues). This progress is now opening the door for large scale micropropagation methods for cacao. Superior donor plants could be selected in the field and subjected to a cloning process. In addition, progress has been made to complete germination and plantlet development for cacao somatic embryos. Cacao improvement programs that would rely on transformation methods can now use the new somatic embryogenesis process derived from nucellus or petal tissues and recover intact plants. Reports have also been made on the recovery of shoots derived from axillary buds. This technique can be very useful for multiplying valuable genotypes for clonal orchards or germplasm banks.

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Future work on cacao needs to focus on methods for haploid production, embyrogenic cell suspensions, protoplast cultures and transformation techniques. At the same time, refinement of the micropropagation methods for non-sexual explants should be completed.

1. INTRODUCTION

A cacao pod has ca. 40 seeds which are utilized to prepared chocolate after defating, roasting, and grinding. Cacao butter is always in short supply because the chocolate manufacturers use more fat in the product than the original fat composition of the seed (ca. 50%). The ratio of saturated and unsaturated fatty acids is very important for the chocolate industry since it will determine the shelf stability and the consumer acceptance of the final product (more unsaturated fats will melt at lower temperatures). The fatty acid composition is affected by the environment and higher levels of unsaturated fat is produced from cacao plantations growing at lower temperatures. The modern use of the word "cocoa" refers to the drink made from its seeds and the word "cacao" refers to the tree (Ojeke 1982).

Extensive variability exists for cacao trees in the Upper Amazon region of South America. The Maya civilization was growing cacao plants before the 16th Century (arrival of the Conquistadors). With the decline of the Maya and Aztec civilizations, cacao plantations were established throughout South America (Brazil and Ecuador) and Central America to fulfill the demand from European consumers. According with Cuatreases (1964), the genus *Theobroma* has 22 species and *T. cacao* L. is further divided into two sub-species: (a) *T. cacao* subsp. *cacao* (varieties criollo, Amelonado, Trinitario, and Pentagona) and (b) *T. cacao* subsp.

sphaerocarpum (variety Calabacillo). The variety Criollo is identified by a thin-skinned pod, light-colored seeds and high quality beans. The varieties Trinitario and Amelonado typically have thick-skinned pods with light purple seeds. The variety Calabacillo has small pods, inferior quality purple seeds and it is considered a source of disease resistance (Hunter 1990).

Africa is the leading cacao producing/exporting area (61% of total) followed by South America (28%). This relative Africa position in the cacao market has been fairly similar since 1951. The total cacao production has increased from 0.7 to 1.6 million tons during the period of 1950-80 (Table 1).

Entering the final decade of this century, cacao prices are at another low point owing to current overproduction with relation to demand. Producers in the western hemisphere now face the added problem of increasing cacao production in the Far East. For example, although Malaysia produced only 26,000 metric tons of cocoa beans in 1978-79, production has increased to 255,000 metric tons in 1988 (Hunter 1990). However, Aderman (1989) predicted that cacao prices will rise this year and stabilize above $3500 per metric ton, and Crotty (1986) predicted, based upon a comparison of actual and simulated cocoa prices, that within the next decade, a metric ton of beans could be worth in excess of $4500. If these projections are true, this is the ideal moment to extend or initiate plantings of new trees in anticipation of rising prices.

The tragedy of cacao growing in the western hemisphere today is that, outside of a few varieties, most of which have not been subjected to rigorous testing, little is currently available for farmers in the way of superior planting material. There is data from field trials in such countries as Puerto Rico, Guatemala, and Trinidad of a number of different clones that show promise, but in the overall scheme of things this is presently of minimal value (Hunter 1990).

Several cacao germplasm collections are available in Africa (Ivory Coast), Brazil (CEPEC, Belem), USA (Miami), Puerto Rico, Trinidad (ICGT), and Costa Rica (CATIE, Turrialba), but little effort is being spent on a systematic evaluation/screening of these gene pools for cacao improvement programs. Very limited information is available on field performance and ecological requirements to recommend planting any specific clone at any particular locality (Hunter 1990).

2. CACAO IMPROVEMENT OPPORTUNITIES

Cacao production still has numerous agronomic limitations, and consequently, there is a great deal of attention for improvement programs to focus on agricultural problems. Total butter production, fatty acid composition, flavor, and theobromine content could also be subject to future cacao improvement programs.

Access to high yielding clones (or hybrids) carrying genes for resistance to major diseases and pests are presently the main emphasis for the majority of cacao improvement programs.

Three fungal diseases cause serious limitations for cacao production in the world. The "black pod" is caused by *Phytophtora palmivora* which also can affect stems and leaves. The "Witches Broom", caused by the fungus *Crinipellis perniciosus* stahel (former Marasmius p.), in indigenous of South America (Ecuador, Colombia, Peru, Venzuela, and Brazil). The "monilia disease" has the fungus *Monilia roreri* as the causing agent and it has caused losses of pods in Colombia, Peru, and Venezuela. Other fungus diseases of minor importance include root diseases caused by fungi of the group *Armillaria*, *Rosellenia*, and *Fomes* (Dublin 1984).

The "Swollen Shoot" is the most serious disease for cacao production in Africa and it is caused by a virus transmitted by mealy bugs. This virus disease has been one of the most limiting factors on cacao production. It was first noticed in Nigeria and later in Ghana, Ivory Coast, and Sierra Leone (Opeke 1982). Other viruses of relative importance are "Cacao Mottle Leaf Virus" and "Cacao Necrosis Virus" (Dublin 1984).

The most serious pest for cacao production in West Africa is the "Cacao Mirids" also called "Capsids" or "Jori-jori". Three species of this insect are found in West Africa: (a) the Brown Mirid (*Sahlbergella singularis*), (b) the Black Mirid (*Distantiella theobroma*), and (c) the Cacao Mosquito (*Helopeltis bergrothi*). The

mirids attack the pods and young shoots and suck the sap through a feeding puncture (Opeke 1982). Other pests of minor importance include the thrips that attack young leaves and borers that produce holes in the stems (Dublin 1984).

Selection of high yielding clones or parental lines for hybrid seed production are being pursued by many cacao improvement programs. Pod index (no. pods per kg of dry cacao) has been used as a yield selection criterion in Trinidad (Kennedy et al. 1987). TSH clones with yield potential of 2 tons/ha/year and pod index of 8-9 pods/kg dry cacao have been reported in Trinidad (Hunter 1990).

3. GENETICS AND BREEDING

Very little is known about the genetic and heritability of useful characteristics such as yield factors, vigor and disease resistance in cacao. The genetic of sexual incompatibility and some morphological characteristics such as axil spot and bean color are a little better understood.

Axil spot is a red anthocyanin coloration at the junction of the petiole with the main axis. The color intensity varies from petioles that are completely dark red to those with a spot of red at the petiole junction. This axil spot character, used for the detection of haploids (Dublin 1973a), is controlled by two complementary genes (Harland and Frecheville, 1927).

The bean color varies from white to purple and includes various shades of purple. According to Wellensieck (1932), the purple color is due to the action of a dominant allele and white results from the action of a recessive allele. Similarly, both the number and size of seeds have high inheritability (Dublin 1984).

Pound (1932) was the first to discover the existence of incompatibility in cacao. The Trinidad trees tested by Pound were classified based on their setting capacity as self incompatible (SI) and self compatible (SC). Pound found that pollen SC trees is effective on any stigma, whereas pollen from SI trees only caused setting on SC trees. Self incompatible trees have been found in several other countries including Java, Colombia and Ghana. In contradiction with the early findings of Pound, successful crosses between two SI trees have been reported by several authors. Muntzing (1947) successfully crosses two SI trees in Ecuador and Posnette (1945a), working with a small population of Amazon trees introduced from the upper Amazon, found all tested trees were SI but cross compatible to some extent.

Cacao provides an unique example of incompatibility, where the diploid tissue of the style does not prevent fertilization. All incompatibility reactions take place in the embryo sac. The incompatibility mechanism in cacao is based on the genetic control of the success or failure of syngamy (Knight and Rogers, 1953, 1955; Cope 1962).

The emergence of SC types from SI individuals of the original cacao population may have occurred somewhere in the lower Amazon. All material collected in and near the origin of cacao is SI. The vast population of trees in West Africa, which provides the bulk of the world cacao production, is uniformly SC and is reputed to have initiated from a small population of cacao collected in the low Amazon region.

Genetic improvement of cultivated cacao is mainly based upon exploitation of the heterosis that occurs in hybrids between upper Amazon types and the Amelonado or Trinitario genotypes. Hybrid vigor was first reported in cultivated cacao many years ago. In Indonesia, the Djati Roenggo hybrids, which were famous for vigor and production during the early 1900s were actually derived from spontaneous hybrids of a Forastero type introduced from Venezuela and the local Java Criollo.

The cacao trees from the upper Amazon have greater vigor than existing varieties and offer unique sources of disease resistance. The improved early vigor, ease of establishment, and precocity are so great that hybrids with Amazon types are being used increasingly in most cacao-growing countries. Since several authors have demonstrated the existence of hybrid vigor in cacao, the genetic improvement of this plant has been essentially based on the utilization of group heterosis, which occurs when the Amazon parent is combined with an Amelonado or Trinitario parent. These Amazonian hybrids have several advantages over local cultivars

(vigor, earliness, disease resistance) but are highly heterogeneous. This heterogenicity of hybrids is a direct consequence of the heterozygosity of the upper Amazon parent obtaining a homozygous, self-fertile Amazon parent should permit elimination of this heterogeneity and lead to homogeneous hybrids of greater vigor. Hence, a great deal of emphasis has recently been placed on the production of haploid plants for cacao improvement.

The first known haploid seedlings of cacao (n = x = 10) were obtained by Dublin (1972). These first haploid cacao trees were obtained following the dissecation of embryos from polyembryonic seeds. The ploidy levels were verified by counting chromosomes of young leaves. Haploid plantlets have also been obtained by germinating flat beans under controlled environmental conditions. Under ordinary conditions, these beans have a low germination rate so that the recovery rat of haploid embryos has been very low (Dublin, 1973a). Haploids have also been obtained by screening seedlings derived from monoembryogenic seeds. Haploid seedlings derived from monoembryogenic seeds developed more rapidly and tolerated colchicine treatment better than haploids derived from polyembryos or flat beans.

Homozygous diploid cacao trees derived from haploids develop well vegetatively, produce flowers, and set normal fruits. These homozygous trees, derived from diploidized of haploids (dihaploids), have been used for crossing with Amelonado parents in the genetic improvement of cacao trees in the Ivory Coast.

Interspecific and Intergeneric Hybridization

Many of the wild species of *Theobroma* or *Herrania*, another member of Sterculiaceae, have desirable characters that would be worth transferring into *T. cacao*. These include thick pods in *T. bicolor*, resistance to black pod and viral diseases in *T. grandiflora* (Martinson 1966), and high butter fat content (50-60%) in *T. grandiflora*.

Posnette (1945b) was the first to suggest transfer of desirable characters from wild species of *Theobroma* into the cultivated varieties and was the first to work on interspecific hybridization between *T. cacao* and related species.

The results of several interspecific crosses between *T. cacao* and related species of the genus *Theobroma* and *Herrania* have produced very small numbers of fruits. The percentage of flower set is low (Williams 1975), and only a small amount of fruit is obtained (Jacob and Opeke 1971). Interspecific crosses in *T. cacao* generally produce only a few hybrid seeds that are capable of germination. The growth of hybrid seedlings from *T. cacao* x

T. grandiflora can be improved by grafting the hybrid plant on rootstock of either T. cacao or T. grandiflora (Martinson 1966).

4. TISSUE CULTURE

To date there have been a limited number of studies on tissue culture of *Theobroma cacao*. In general, all attempts to initiate callus of cacao were successful as callus was rapidly obtained from various organs or explants on a wide range of culture media. On the contrary, all attempts to regenerate plantlets from cacao callus have failed.

Archibald (1954) was the first to investigate tissue culture of cacao. He obtained callus from explants of bark or stem on culture media of Gautheret or White without andy growth regulators.

The second attempt to culture cacao in vitro was reported by Ibanez (1964). Ibanez reported on the action of different sugars (sucrose, dextrose, maltose, lactose, and sorbose) on the respiration rate of cotyledon-free mature cacao embryos under sterile conditions. The best callus growth occurred on MS media supplemented with 11.0 μM IAA, 0.47 μM KIN, and twice the normal concentration of MS vitamins. A large range of media supplemented with extracts derived from leaves, pod walls, and young seeds were tested in attempts to regenerate plantlets from callus. With the exception of periodic root initiation, no organogenesis was obtained.

Orchard et al. (1979) examined in vitro culture of apical buds of cacao for vegetative propagation. Some growth was observed on both agar and liquid medium but the degree of response varied with the stage of bud development and with hormone treatment. Breakage of dormancy as manifested by bud swelling followed by stipule opening was prompted by both KIN and GA. No intact plants were recovered from cultured dormant buds.

Both Esan (1975) and Pence et al. (1979) were able to obtain somatic embryos in vitro from cultured cotyledon and hypocotyl tissues of very young cacao seed embryos. Esan (1975), in attempts to develop a method for production of cacao plantlets in vitro, used numerous explants including ovules from fruit 6-8 weeks old, immature embryos from 90-day-old fruit, and the embryo axis (axes of cacao bean) from mature unripe pods and anthers. Addition of IAA to the basal culture medium prompted direct somatic embryogenesis rather than an increase of root growth. The ensuing adventive embryos that were spherical or bell-shaped developed through a budding process. Most of these somatic embryos were derived from the hypocotyl portion of the seedling embryo, while some were derived from the adaxial portion of the cotyledon. The initiation of these adventive embryos was not preceded by callus formation.

Pence et al. (1979) initiated tissue cultures of cacao in order to establish the necessary conditions for cacao regeneration in vitro. Various explants including leaves, pericarp, ovules,

immature embryos, cotyledons from mature embryos, and the axis of mature embryos were cultured on different culture media to determine morphogenetic potential. Callus was obtained with all explants used and on practically all media tested. Immature sexual embryos cultivated in dark or in light on basal medium supplemented with NAA and CW produced adventive embryos, which proliferated by budding from the cotyledon of the immature sexual embryo (Pence et al. 1981). When transferred from a solid medium to a liquid medium, these adventive embryos developed roots and primary leaves (Pence et al. 1981). Further development and complete normal plantlets were not obtained. In some treatments, up to 80% of the cotyledons of the sexual embryos initiated asexual embryos.

Jalal and Collin (1977, 1979) suggested using callus of cacao to investigate the biosynthesis of polyphenols and flavor compounds in cacao. The polyphenols of the cacao bean have long been regarded as important components of flavor in the roasted fermented cotyledons of cacao. Although some polyphenols were found both in cacao callus and in tissue of the intact plant, most of the polyphenols discovered in the callus were not detected in the plant.

Studies were made to evaluate the in vitro synthesis of cocoa butter by cultured somatic embryos (Janick et al. 1982). Somatic embryos grown in nutrient medium with increased sucrose concentrations (3-9-15-21-27 and 33%) produced lipids with similar composition of those present in seed embryos.

Subsequent studies on somatic embryogenesis from cultured immature zygotic embryos were reported by Abu-Ampomah et al. (1988) and Duhem et al. (1989). Direct and indirect somatic embryos were identified and histological sections confirmed the single cell origin of these embryos (Abu-Ampomah et al. 1988). The recovery of cacao plantlets was achieved by removal of the cotyledons and increasing the gas exchange inside the culture vessels (Duhem et al. 1989).

The first attempts to develop micropropagation methods for cacao were reported by Litz (1986). Axillary buds were induced to proliferate but it was not possible to sustain a rapid proliferation and subsequent growth. In addition, attempts were made to develop a somatic embryogenesis method from non-sexual tissues. Leaf callus of Amelenado trees was induced to differentiate somatic embryos from the globular to late heart stage. It was not possible to stimulate development of cacao somatic embryos beyond this stage. Expansion of the cotyledons was accomplished by gradual necrosis (Litz 1986).

Two successful attempts on culture of cacao axillary buds were recently reported. Flynn et al. (1990) described the recovery of some cacao plants in the greenhouse after rooting and hardened off the propagules. Axillary buds were obtained from either orthotropic or plagiotropic shoots of UF-667 or EZX-100 genotypes. The beneficial effect of increased CO_2 (20,000 ppm) in the presence

of 150-200 μmol/s/m_2 of light for promoting in vitro growth of axillary shoots of cacao was reported by Figueira et al. (1991). Cotyledonary nodes and single-node cuttings from mature plants and shoots were used to induce axillary shoot development.

5. NEW ADVANCES

Until today, a process is not available that would permit the scaling up of individual superior plants from germplasm collection to establish commercial plantations. Considering the existing agronomic limitations for cacao production, DNAP has embarked on a long-term program to develop a method(s) for micropropagation of cacao.

Eleven different explant sources that represent the mother plant (not sexual in origin) were studied in diallelic culture medium design for embryo regeneration. Two types of explants provided some encouraging results: young petals and nucellus.

Petal explants were obtained from cacao clones growing under greenhouse conditions. The best flower bud size was 3-5 mm in length. After sterilization, petals were cultured on callus induction medium for 4 weeks and then subcultured to a regeneration medium. Somatic embryos were isolated after 2-4 months on the regeneration medium. Using more than 9,000 petal explants, a

regeneration rate of 4.3% was observed for primary embryos. Some of these embryos were allowed to produce secondary embryos which were then transferred to maturation, germination, and plantlet development/hardening phases (Table 2).

Nucellus explants were excised from young cacao fruits with 7-9 cm in length. After the nucellus was isolated from the seed coat, the portion containing the zygotic embryo was cut and discarded. Twenty nucellus explants were cultured on 100 x 10 mm Petri dishes charged with the primary medium. Primary embryos were visible after 4 weeks of inoculation. The cultures were examined on a weekly basis to check for possible escapes of zygotic embryos due to abnormal positioning. If present, the zygotic embryos would develop quickly giving rise to very large embryos after 2-3 weeks of culture. The embryo regeneration process from nucellus tissues can take place through a direct or indirect pathway. The indirect pathway is characterized by the proliferation of an embryogenic tissue which leads to a large number of embryos. A certain number of nucellus embryos were recultured to produce large numbers of secondary embryos. The regeneration frequency of somatic embryos from the culture of more than 29,000 nucellus explants has been ca. 2.0% (Table 2). The somatic embryos recovered were transferred to Maturation, Germination, and Plantlet Development/Hardening phases to complete the culture process.

Considering a normal flow of the somatic embryogenesis process practiced for petal and nucellus tissues, a total time of 40 weeks

are required to transfer plantlets to soil. The protocols currently under development offer great hope for a future application of micropropagation for cacao. This is the first time that embryos and plantlets have been recovered from non-zygotic tissues of cacao which is critical to reproduce the phenotype of the donor plants. The process still need refinements since low efficiency rates are being observed for maturation and germination, and plantlet development. Germination and plantlet development has been a problem in previous studies based on somatic embryos derived from seed embryos. There are conditions and methods that will need to be optimized to allow a large scale utilization of this process for establishing cacao plantations.

REFERENCES

ADU-AMPOMAH, Y., NOVAK, F.J., AFZA, R., DUREN, M. van, and PEREA-DALLOS, M. 1988. Initiation and growth of somatic embryos of cocoa (*Theobroma cacao* L.) Cafe Cacao The 32(3):187-200.

ARCHIBALD, J.F. 1954. Culture "in vitro" of cambial tissue of cocoa. Nature London 173:351-352.

COPE, F.W. 1962. The mechanism of pollen incompatibility in *Theobroma cacao* L. Heredity 17:157-182.

CUATRECASAS, J. 1964. Cacao and its allies. A taxonomic revision of the genus Theobroma. Contrib. US Nat. Mus. 35(6):379-614.

DUBLIN, P. 1984. Cacao. In: Handbook of Plant Cell Culture - Crop Species. pp. 541-563. P.V. Ammirato et al. (eds.). Macmillan Publ. Co., New York.

DUBLIN, P. 1973a. On the use of a genetic marker in the search for haploids in the cacao tree (*Theobroma cacao* L.). Cafe Cacao The 17:205-210.

DUBLIN, P. 1973b. Hybridisation intérspecifique et matromorphie chez *Theobroma cacao*. Rap. Ann. EFCC Côte d'Ivoire 2:73-76.

DUBLIN, P. 1972. Polyembryony and haploidy in *Theobroma cacao*. Cafe Cacao Thé 16:295-311.

DUHEM, K., MERCIER, N. and BOXUS, P. 1989. Données nouvelles sur l'induction et le development d'embryons somatiques chez *Thoebroma cacao* L. Café Cacao Thé 33(1):9-14.

ESAN, E.B. 1975. Tissue culture studies on cacao (*Theobroma cacao* L.). In: Proceedings, Fifth International Conference on Cacao Research (Ibadan, Nigeria) pp. 116-124.

FIGUEIRA, A., WHIPKEY, A. and JANICK, J. 1991. Increase CO_2 and light promote in vitro shoot growth and development of *Theobroma cacao*. J. Amer. Soc. Hort. Sci. 116(3):585-589.

FLYNN, W.P. GLICENSTEIN, L.J. and FRITZ, P.J. 1990. *Theobroma cacao*: an axillary bud in vitro propagation procedure. Plant Cell, Tissue and Organ Culture 20:111-117.

HARLAND, S.C. and FRECHEVILLE, G.E. 1927. Natural crossing and the genetic of axis spot in cacao. Genetica 9:279-288.

HUNTER, J.R. 1990. The status of cacao (*Theobroma cacao, sterculiaceae*) in the Western Hemisphere. Economic Botany 44(4):425-439.

IBANEZ, M.L. 1964. The cultivation of cacao embryos in sterile culture. Trop. Agric. Trinidad 41:325-328.

JACOB, V.K. and OPEKE, L.K. 1971. Interspecific hybridization in *Theobroma cacao*. In: Proceedings, Third International Conference on Cacao Research (Accra Ghana, 1969) pp. 552-555.

JALAL, M.A.F. and COLLIN, H.A. 1977. Polyphenols of mature plant, seedling and tissue cultures of *Theobroma cacao*. Phytochemistry 16:1377-1380.

JALAL, M.A.F. and COLLIN. H.A. 1979. Secondary metabolism in tissue culture of *Theobroma cacao* L. New Phytol. 83:343-349.

JANICK, J., WRIGHT, D.C. and HASEGEWA, P.M. 1982. In vitro production of cacao seed lipids. J. Amer. Soc. Hort. Sci. 107(5):919-922.

KENNEDY, A.J., LOCKWOOD, G., MOSSU, G., SIMMONDS, N.W., and TAU, G.Y. 1987. Cocoa breeding: past, present, and future. Cocoa Growers Bull. 38:5-22.

KNIGHT, R. and ROGERS, H.H. 1953. Sterility in *Theobroma cacao* L. Nature London 172:164.

KNIGHT, R. and ROGERS, H.H. 1955. Incompatibility in *Theobroma cacao*. Heredity 9:69-77.

LITZ, R.E. 1986. Tissue culture studies with *Theobroma cacao*, pp. 111-120. In: Cacao biotechnology symposium. P.S. Dimick (ed.), Dept. Food Science, Penn State Univ., USA.

MARTINSON, V.A. 1966. Hybridization of cacao and *Theobroma grandifora*. Heredity 57:134-136.

MUNTZING, A. 1947. Some observations on the pollination and fruit setting in Ecuador cacao. Hereditas 33:397-404.

OPEKE, L.K. 1982. Cacao. In: Tropical Tree Crops, pp. 67-123. Opeke (ed.), John Wiley and Sons, Chichester.

ORCHARD, J.E., COLLIN, H.A., and HARDWICK, K. 1979. Culture of shoot apices of *Theobroma cacao*. Physiol. Plant. 47:207-210.

PENCE, V.C., HASEGAWA, P.M., and JANICK, J. 1980. Initiation and development of asexual embryos of *Theobroma cacao* in vitro. Z. Pflanzenphysiol. 98:1-4.

POSNETTE, A.F. 1945. Incompatibility in Amazon Cacao. Trop. Agric. Trinidad 22:184-187.

POUND, F.J. 1932. Criteria and methods of selection in cacao. Annu. Rep. Cacao Res. Trinidad 1:10-24.

WELLENSIECK, S.J. 1932. Observations in floral biology of cacao. Arch. Koffie Cult. Med. Indie 6:87-101.

WILLIAMS, J.A. 1975. Interspecific crosses between *Theobroma* and *Herrania* species. In: Proceedings, Fifth International Conference on Cocoa Research (Ibadan, Nigeria) pp. 1-10

TABLE 1. World production of cacao. Data in 1,000 Mt and relative position in % in parenthesis.

Region	1949/51		1959/61		1969/71		1979/81		Average Annual Growth Rate (1950-80)
Africa	489	(66)	786	(70)	1,100	(73)	999	(61)	2.3
Central America	65	(9)	87	(8)	82	(5)	94	(6)	1.2
South America	182	(25)	225	(20)	282	(19)	461	(28)	3.2
Asia	4		7	(1)	11	(1)	48	(3)	8.7
Oceania	4		12	(1)	31	(2)	34	(2)	7.5
Total	744		1,117		1,506		1,636		2.6

TABLE 2. Somatic embryos and plantlet recovery from petals, and nucellus tissues. Data from explants derived from more than 22 different genotypes.

Culture Phase	Petal tissues		Nucellus tissues	
	Number	% Recovery	Number	% Recovery
Primary explants	9,756	-	29,793	-
Primary embryos	424	4.3	633	2.1
Secondary embryos	2,955	697.0	28,907	4,567
Maturation	1,000	33.8	5,143	17.8
Germination	151	15.1	418	8.1
Plantlet development	48	31.8	88	21.1
Plantlet in soil	15	31.2	23	26.1