R. P. F. III

FINAL REPORT

1. Institute Code No. Phy XXII(231,299)

2. I. C. A. R. Code No. P_1-86/3-ICI-F-60/0311 & 2110

3. Name and Address of Research Institute/Centre:
   Central Plantation Crops Research Institute, Kasaragod

4. Project Title: Investigations on screening for drought tolerance in coconut and cocoa

5. Name and Designation of Project Leader:
   D.Balasimha, Sr.Scientist (25%)

6. Name(s) and Designation(s) of Project Associates including Project Leader and work to be done:

Sl. No.	Name and Designation	Time spent	Work done
1.	V.Rajagopal, Sr.Scientist	25%	
2.	K.V.Kasturi Bai, "	25%	
3.	EV Daniel, "	50%	
4.	S.Shivashankar, "	40%	
5.	B.Chempakam, "	25%	

7. Location of Research Project with complete address (Division/Section/Sub-Centre)
   CPCRI, Kasaragod and CPCRI Regional Station, Vittal

8. Date of start : 1986

9. Date of termination : 1990-91

10. (a) Objectives (Not more than 150 words)

The yield stability in coconut and cocoa genotypes is important factor in assessing drought tolerance. The genotypes vary in various morphological and physiological characteristics which determin yield under unfavourable conditions. The main objective of this project is to screen germplasm holding of coconut and cocoa for leaf characters, stomatal behaviour, water relations, wax contents; other biochemical factors; yield and yield components in order to identify drought tolerant genotypes. This is to enable to select promising genotypes for use in further breeding programmes.

(b) Practical Utility including background information (Not more than 150 words)

Both coconut and cocoa are predominantly grown under rainfed conditions which experience prolonged drought frequently resulting in considerable yield losses. This is especially pronounced in cocoa which is extremely sensitive to water stress. Several leaf characteristics impart tolerance to drought and this is one of the reasons why some genotypes/varieties show better productivity under moisture limiting conditions. The precise reasons for the responses are not fully understood and the project envisages to find out parameters which help in adapting to stress environments. Identification of genotypes showing drought tolerant characteristics will help in reducing crop losses due to stress and also as parent material for breeding programmes.

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11. Technical Programme

Date of Start: 1986

Materials: Coconut genotypes/hybrids available at Kasaragod and cocoa accessions at Vittal will be used.

Techniques/Instruments: Soil moisture to be monitored at different depths either with neutron probe or gravimetrically. Steady state porometer/photosynthetic system, pressure chamber and osmometer will be employed to measure stomatal characteristics and water relation components. Epicuticular wax will be determined spectrophotometrically.

Biochemical studies include some enzyme/isozyme systems affected by stress. These will be determined by standard procedures.

Yield components and yield will be recorded as per usual agronomic practices. Meteorological data available will be made use of for correlation studies.

FINAL REPORT

Coconut:

Twenty three genotypes of coconut comprising ten talls, six dwarfs and seven hybrids were screened for their tolerance to drought using the sensitive parameters viz. stomatal resistance (rs), leaf water potential (Ψ) and epicuticular wax content (EW). These genotypes had varying responses to the prevailing soil and atmospheric droughts during the summer months. In general, tall genotypes had relatively high stomatal resistance, leaf water potential and ECW content as compared to the dwarfs. Some of the hybrids resembled the female parent in these characters. In response to the development of water stress between November and March some of the tall palms and tall hybrids showed relatively high rs resulting in effective conservation of water in the tissues where as the dwarfs were sensitive to stress with a tendency to lose more water. Parametric relationships with drought tolerance based on the rank sum led to the identification of the genotypes tolerant to drought with the desirable characteristics (Table 1).

The rapid screening method for drought tolerance was developed by determining the changes in leaf water potential and acid phosphatase activity in excised leaf lets exposed to air desication.with increase in duration of stress, the leaf water potential was reduced and the enzyme activity increased to different degrees among the genotypes (Table 2). The genotypes like LO x GB, LO x COD and WCT which maintained higher leaf water potential and stress increase in enzyme activity (7.0% to 22.8% on initial value) are considered drought tolerant.

Characterization of identified genotypes:

Investigations on the physiological characterization of drought tolerant/susceptible genotypes revealed the differences not only in the extractability of soil moisture by the roots, but also on the conservation of moisture (i.e, low transpiration rate) in leaf tissues aided by the wax coating (ECW) on the leaf surface. By reducing the transpiration rate through the effective control of stomata, the tolerant genotypes could conserve water in the tissues for various physiological and metabolic processes, whereas the susceptible genotypes tended to lose more water and hence became sesitive to stress conditions. The fact that the most tolerant genotypes like LO x GB and LO x GOD have significantly higher wax content (120 $\mu g\ cm^{-2}$) clearly indicated the significance of cuticular wax in imparting tolerance to drought .

The thin layer chromatographic technique was employed to characteristize the composition of cutiwular wax. In general, the number of components of ECW was more during the period of moisture stress than other periods. The membrane stability was disturbed to different degrees among the genotypes due to moisture stress, the tolerant genotype had less electrolyte leakage than a susceptible one.

The biochemical characterization of coconut genotypes centred around the assay of different moisture sensitive enzymes like acid phosphatase, L-aspartate: 2-oxoglutamate aminotransferase, polyphenol oxidase and isozymes, separated by polyacrylamide gel ectrophoresis, from the osmotically stressed leaves showed two additional fast moving bands in a drought susceptible genotype (COD x WCT) while the same was absent in a relatively tolerant one ie., WCT. Based on the threshold leaf water potential to trigger the enzyme activity the APH was found to be highly sensitive to moisture stress. Further studies on the purification and characterization of APH isozymes isolated from normal and stressed leaves revealed that during stress development the Aph isozymes II probably undergoes changes in its conformation as evidenced by the shift on its optimum temperature (Fig). This enzyme also showed differences in its rate of hydrolysis of various naturally occurring substrates.

Responses of the genotypes to simulated stress by air desiccation and by 30% PEG revealed that the relative water content on the leaves of all the genotypes was reduced more due to air desiccation than the osmatic stress. Except in the dwarfs which exhibited higher proline content in PEG stressed leaves, the other genotypes did not show any difference (Table 3). There was inverse relationship between RWC and proline accumulation. The data revealed that proline content is not associated with drought tolerance in coconut.

Photosynthetic characteristic of the identified drought tolerant and susceptible genotypes have been studied during the prestress and stress period. As compared to the prestress condition there was nearly 35 to 70% reduction in the net photosynthetic rate (PN) during the stress period. Instantaneous water use efficiency (WUE) was higher both in LO x GB and LO x COD, the two identified drought tolerant and released gentoypes (Table 4). Day time fluctuations of these parameters showed a mid day reduction in WUE indicating the mid day closure of stomata by checking the transpiration rate. Photosynthesis and related parameters such as WUE, Carboxylation efficiency and the efficiency of the stomata in the gas exchange were found to vary according to the maturity of the leaf. Irrespective of the genotypes all these parameters were found to be maximum in the 6th leaf.

Lipid peroxidation which is an important parameter in drought tolerant studies had shown lower levels in the tolerant genotypes than the susceptible ones. The former group had higher SOD and catalase activities and lower peroxidase and PPO activities.

Relationship between physiological characters and nut yield revealed significant correlation between the two. Some of the genotypes which were identified as drought tolerant such as LO x GB, LO x COD and WCT x COD ~~identified as drought tolerant~~ were found to be good yielders. This highlights the significance of using the physiological characters in screening for drought tolerant varieties with the ultimate object of aiding the breeding strategy in coconut.

Table 1: Ranking of genotypes based on water relation components

Genotype		Ψ (-MPa)	ECW (μg.cm^{-2})	Rank
<u>Talls</u>				
	WCT	1.48	109.0	11
	SS Apricot	1.14	102.1	12
	Andaman Ordinary	1.24	91.9	14
	Laccadive Micro	1.41	96.2	15
	Andaman Giant	1.13	99.2	5
	FMS	1.10	116.7	2
	Fiji	1.23	104.7	4
	Philippines Ordinary	1.32	113.4	6
	Cochin China	1.27	110.4	8
	Java Giant	1.41	116.2	3
<u>Dwarfs</u>				
	SS Green	1.32	98.4	17
	MGD	1.36	94.0	18
	MOD	1.52	77.0	21
	MYD	1.49	79.0	20
	GB	1.46	90.3	19
	COD	1.01	87.6	13
<u>Hybrids</u>				
	WCT x COD	1.19	116.7	10
	COD x WCT	1.25	110.5	16
	WCT x WCT	1.17	117.4	1
	COD x COD	1.36	109.2	7
	WCT x GB	1.16	109.6	9
	LO x GB	1.32	132.6	11
	LO x COD	1.26	120.7	5

Table 2. Changes in the activities of APh and GOT in the coconut genotype during stress development

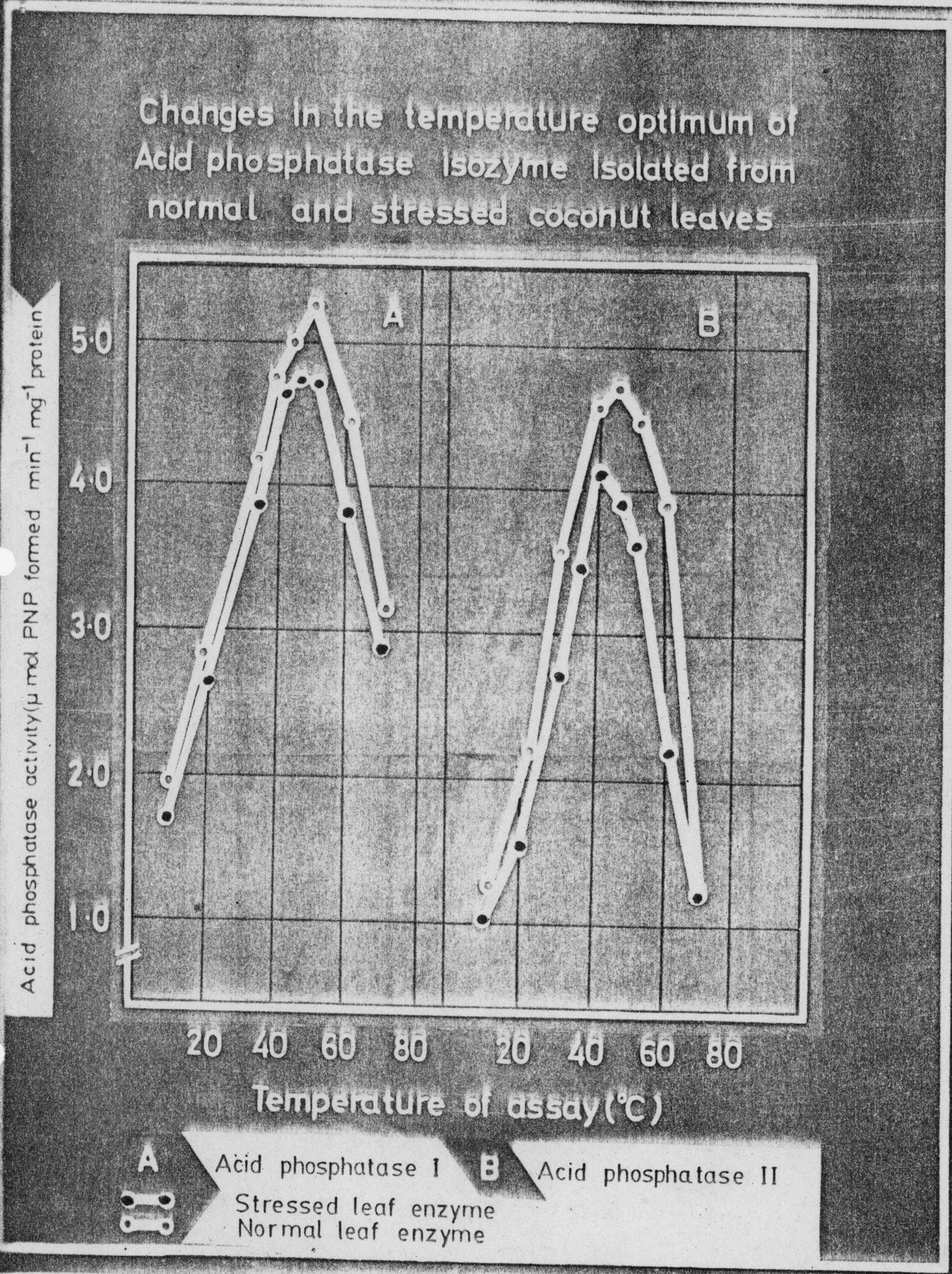
Genotypes	Activity at '0' hr	% increase at the end of		
		3hr	6hr	9hr
(a) Acid phosphatase activity (µ moles PNP liberated g^{-1} DWt				
WCT	253.32	0	0	7.1
LO x GB	278.39	0	0	18.1
LO x COD	269.84	0.8	1.5	22.8
COD	289.12	31.3	56.1	130.4
WCT x COD	209.45	0	11.5	143.8
COD x WCT	224.63	16.1	20.6	86.2
(b) Glutamate oxalic acid transaminase (µg pyruvate mg^{-1} protein)				
WCT	3.056	5.4	5.4	18.1
LO x GB	3.620	0	3.1	12.4
LO x COD	3.813	1.4	2.9	7.6
COD	4.102	16.6	19.1	104.2
WCT x COD	3.755	10.8	17.2	115.3
COD x WCT	3.522	4.5	8.8	86.2
(c) Soluble protein (mg. g. dry wt^{-1})				
WCT	2.42	2.1	2.8	13.2
LO x GB	2.68	7.4	11.1	11.2
LO x COD	2.71	0.7	7.9	16.6
COD	2.38	11.7	11.7	42.5
WCT x COD	2.69	7.1	14.2	32.6
COD x WCT	2.76	7.2	22.6	53.2

Table 3. Proline content ($\mu g\ g^{-1}$ dry wt) in the leaf tissues of coconut genotypes.

Genotype	Initial 'O' h	After 24 hrs		
		Control	Osmotic stress	Air desiccation
WCT	4.0	8.4	12.0	11.6
LO	6.5	8.2	15.8	15.1
GB	6.6	15.3	31.8	19.6
COD	5.6	13.2	36.1	12.0
LO x GB	3.6	5.3	15.1	16.9
LO x COD	4.0	4.5	15.3	13.8
WCT x COD	4.5	8.5	17.2	17.8
COD x WCT	4.1	4.1	16.4	21.7

Table 4: Water use efficiency (μ mole/m $mole^{-1}$) in the identified genotypes.

Genotype	Pre-stress	Stress	% reduction
GB	0.88	0.36	59
COD	1.07	0.42	60
WCT	0.79	0.36	55
WCT x COD	0.88	0.42	53
COD x WCT	0.88	0.60	32
LO x GB	1.20	0.82	32
LO x COD	1.02	0.72	30



Changes in the temperature optimum of Acid phosphatase isozyme isolated from normal and stressed coconut leaves
A
B
5·0
4·0
3·0
2·0
1·0
Acid phosphatase activity (μ mol PNP formed min-1 mg-1 protein
20 40 60 80
20 40 60 80
Temperature of assay (°C)
A Acid phosphatase I
B Acid phosphatase II
Stressed leaf enzyme
Normal leaf enzyme

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12. Final Report: 1986 —1991 Date of Start· 1986

Cocoa

Stomatal behaviour: Fourteen cocoa accessions of Nigerian collection were screened for drought tolerance using parameters like stomatal resistance, water potential components and biochemical constituents during prestress, stress, recovery stages. Based on the parametric relationships with drought tolerance the rank sums of these accessions lead to a selection of five of them NC 23, NC 29, NC 31, NC 39 and NC 42. These drought tolerant accessions had effective stomatal regulation resulting in decreased transpiration under water stress, which seems to be an important strategy for adaptation in cocoa. Although a decrease in osmotic potential and NR activity and an increase in soluble sugar were noticed in response to stress, there was no relationship with respect to drought tolerance. The stomatal diffusive resistance was negatively correlated with transpiration during pre-stress (-0.82), stress (-0.81); Leaf temperature was positively correlated with transpiration during prestress (0.69) and recovery (0.56); soluble sugars positively correlated with osmotic potential under stress (0.61). The cocoa accessions with higher drought tolerance characteristics and higher yield potential under drought stress should be useful basal material for selective breeding.

Effect of stress on seedlings: Cocoa seedlings of drought tolerant and susceptible accessions were subjected to water stress (from 17.6 to 6.7% soil moisture). Drought caused decrease in water potential to a greater extent in susceptible as compared to tolerant accessions. This was accompanied by increased activities of phosphatase and amylase. Starch, sugar, phenol and protein did not show any significant differences. The changes in epicuticular wax(EW) lipids and electrolytic leaching in seedlings revealed that tolerant ones had higher EW and better membrane stability.

Effect of weather variables: An increase in evaporative demand owing to lowered humidity changes stomatal resistance. r_s was increased with decreased RH which was magnified by soil moisture stress. The correlation coefficients among various environmental factors and plant responses indicate that ambient temperature did not influence stomatal resistance but showed high correlation with leaf temperature (0.90). Stomatal regulation by internal (stress) and external factors (RH) lead to decrease in transpiration which was a major adaptation for water conservation.

Screening technique: After evaluating the mechanism of drought tolerance in cocoa, it was found that as a result of reduced transpirational water loss in cocoa under drought leaf turgidity was maintained thereby giving higher water potential. A rapid screening method for drought tolerance in cocoa has been described which eliminates difficulty of using field plants. This method utilizes measurement of water potential in excised leaves. The decrease in water potential was more pronounced in susceptible as compared to tolerant accessions under laboratory stress.

Screening germplasm holding: In all a total of 65 cocoa accessions have been screened for drought tolerance. Six of them have been identified as drought tolerant based on the leaf characteristics and beahviour in laboratory screening and subsequently under field testing. These are NC 23, NC 29, NC 31, NC 39, NC 42 and Amel x Na 33.

Photosynthetic studies: Photosynthesis (P_N) was measured in accession types in relation to microclimatic variables. P_N, transpiration and stomatal conductance showed significant seasonal variations. During periods of low VPD, P_N was highest. Diurnal patterns indicated transient midday water deficits reducing P_N. The drought tolerant accessions maintained higher WP and there were no significant differences in P_N. The depressions of P_N was principally due to lowered stomatal conductance which was positively correlated. There was no

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significant differences in intercellular CO_2 showing that it did not limit net assimilation rate.

<u>Lipid, EW and membrane ~~leachings~~ stability:</u> Water stress decreased the total lipid content of leaf to an extent of 30-37%, the decrease being more pronounced in susceptible accessions. Glycolipids and phospholipids decreased while neutral lipids increased as a result of water stress. There were no qualitative changes in neutral and glycolipids while two components disappeared in phospholipid fraction with a concomitant increase in another fraction due to stress. The unsaturated fatty acid fraction generally increased in response to stress. The electrolytic leaching was lower in tolerant accessions with higher sterol/phospholipid ratio, probably indicating lesser membrane damage due to stress.

As a result of ~~PEG~~ air-drying stress on excised leaves the lipid peroxidation was lower with higher SOD stability in tolerant accessions. This also contributes to the better membrane stability.

The EW constituted about 0.4 - 0.8% of total leaf dry weight and varied with season, light intensity and water stress. Water stress and higher light intensity increased wax deposition. Tolerant accessions showed 22-27% higher EW as compared to susceptible one which helped in reducing water loss. There were 7 wax components which did not show appreciable differences among accession types.

14. **Publications**

a) Research papers

1. Balasimha,D., Rajagopal,V. Daniel, E.V., Nair,R.V. & Bhagavan,S. 1988. Comparative drought tolerance of cocoa accessions. Trop. Agric. 65: 271-74.

2. Balasimha,D., & Daniel,E.V. 1988. A screening method for drought tolerance in cocoa. Current Science 57: 385.

3. Balasimha,D. & Rajagopal,V. 1988. Stomatal responses of cocoa to changes in climatic factors. Indian J. agric. Sci. 58:213-216.

4. Shivashankar,S.1988. Polyphenol oxidase isozymes in coconut genotypes under water stress. Plant Physiol. Biochem. 15: 87-91.

5. Rajagopal,V., Shivashankar,S., Kasturi Bai,K.V. & Voleti,S.R., 1988. Leaf water potential as an index of drought tolerance in coconut. Ibid, 80-86.

6. Kasturi Bai, K.V., Voleti,S.R. & Rajagopal,V. 1988. Water relations of coconut palms as influenced by environmental variables Agric. Forest. Meteorol. 43: 193-1991.

7. Bhat,P.G., Daniel,E.V. & Balasimha,D.1990. Epicuticular Wax, lipids and membrane stability of cocoa trees in relation to drought tolerance. Indian J. exp. Biol. 28: 1171-73.

8. Balasimha,D., Daniel,E.V. & Bhat,P.G. 1991. Influence of environmental factors on photosynthesis in cocoa trees. Agric. Forest Meteorol. (in press).

9. Balasimha,D. 1988. Leaf characteristics of cocoa in relation to drought tolerance. 75th Indian Science Congress, Pune, 7-12 Jan. 1988.

10. Balasimha,D. 1988. Water relations of cocoa accessions and their adaptability to drought. International Congress of Plant Physiology New Delhi, 15-20 Feb.1988.

11. Rajagopal,V., Kasturi Bai,K.V., Voleti,S.R., & Shiavashankar,S. 1988. Water stress in coconut palms. Ibid.

12. Shiavashankar,S.1990. Thermal stability of nitrate reductase in relation to drought tolerance in coconut. PLACROSYM IX, Bangalore,5-7 Dec.1990.

13. Rajagopal,V., Kasturi Bai,K.V., Pillai,R.V. & Vijayakumar,K. 1990. Relationship between physiological characters and nut yield in coconut genotypes under rainfed conditions. Ibid.

14. Balasimha,D.1987. Cocoa. In: Tree Crop Physiology (Eds. Sethuraj,M.R. & Raghavendra,A.S.) Elsevier, Amsterdam. pp 263-285.

15. Ramadasan,A. & Rajagopal,V. Coconut. Ibid. pp.169-192.

*16.

b) Popular articles

16. Balasimha,D.1989. Cocoa intercropping under areca palms. The Hindu, 6 Dec.,1989, p.20.

*16. Voleti, S.R., Kasturi Bai, K.V., Rajagopal, V and Shivashankar, S. 1990. Relative water content and proline accumulation in Coconut genotypes under moisture stress. Journal of Plantation Crops. 18(2): 88-95

13. Approximate expenditure incurred in the Project: (Give reasons for variation, if any, from original estimated cost)

14. Publications and material (one copy each to be supplied with this proforma)

a) Research papers — See annexure

b) Popular articles — -do-

c) Reports — --

d) Seminars and workshops (Relevant to the Project) in which the Scientists have participated:

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e) Material developed (such as new varieties of crops or breeds of farm animals, implements, products, etc.)

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15. Details (Nos. etc.) of Field/Laboratory Note books and final material and their location

Note books kept in respective Physiology Sections

16. Comments/suggestions of Project Leader regarding possible future line of work that may be taken up arising of this project:

The results obtained in the project have given a basic understanding of mechanism of drought tolerance in both coconut and cocoa. The accessions/genotypes which display specific favourable attributes can be used as a source for breeding to bring desirable characters into a single ideotype with a good expectation of increasing drought tolerance. Future work should attempt to establish criteria for drought tolerance and their applicability to seedling progenies. The future studies on F_1 hybrid seedlings may help to know the extent of inheritance of some of these characters.

17. Signatures with name of Project Leader and Associates:

D. Balasimha

V. Rajagopal

K.V. Kasturi Bai

E.V. Daniel

S. Shivashankar

B. Chempakam

18. Signature (with comments, if any) of Head of Division/Section/Station: The project has given a good lead for breeding programme on drought tolerance.

V. Rajagopal

19. Signature (with comments, if any) of Director:

Director

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