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UNIVERSITE DE YAOUNDE I
FACULTE DES SCIENCES
DEPARTEMENT DE BIOLOGIE ET PHYSIOLOGIE VEGETALES
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REPUBLIC OF CAMEROUN
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UNIVERSITY OF YAOUNDE I
FACULTY OF SCIENCE
DEPARTMENT OF PLANT BIOLOGY
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Scaling up oil palm (Elaeis guineensis Jacq.) seedling production through modified dry heat scarification and embryo rescue

THESIS
Submitted in partial fulfilment of requirements for the award of a Doctor of Philosophy Degree (PhD) in Plant Biology

Par : **TABI KINGSLEY MBI**
MSc Plant Physiology

Sous la direction de
YOUMBI Emmanuel
Professor

Année Académique : 2018

UNIVERSITE DE YAOUNDE I
THE UNIVERSITY OF YAOUNDE I

FACULTE DES SCIENCES
FACULTY OF SCIENCE

DEPARTEMENT DE BIOLOGIE ET PHYSIOLOGIE VEGETALES
DEPARTEMENT OF PLANT BIOLOGY

Yaoundé, le 12 FEV 2018

ATTESTATION DE CORRECTION

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Nous soussignés, membres du jury de soutenance de la thèse de **Doctorat / PhD** en **Biologie et Physiologie Végétales**, soutenue le mardi **04 Janvier 2018** par M **TABI KINGSLEY MBI**, Master ès Science, Matricule **07S400**, intitulée **«Scaling up oil palm (*Elaeis guineensis* Jacq.) seedling production through modified dry heat scarification and embryo rescue»**, certifions qu'il a effectué les corrections conformément aux remarques et recommandations du jury.

En foi de quoi, nous lui délivrons cette attestation de correction pour servir et valoir ce que de droit. /-

Président du Jury	**Examinateur**	**Rapporteur**
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Professeur	Professeur	Professeur

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DATE D'ACTUALISATION : 2017

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25	EDONGUE HERVAIS	Chargé de Cours	En poste
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28	MBINACK Clément	Chargé de Cours	En Poste
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19	EYONG John TAKEM	Chargé de Cours	*En poste*
20	FUH Calistus Gentry	Chargé de Cours	*En poste*
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22	GHOGOMU Richard TANWI	Chargé de Cours	*Chef de Dépt IMIP/Maroua*
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27	NGO BELNOUN Rose Noël	Chargé de Cours	En poste
28	NGO BIDJECK Louise Marie	Chargé de Cours	En poste
29	NGUEUTCHOUA Gabriel	Chargé de Cours	CEA MINRESI
30	NJOM Bernard de Lattre	Chargé de Cours	En poste
31	NYECK Bruno	Chargé de Cours	En poste
32	ONANA Vincent	Chargé de Cours	En poste
33	TCHAKOUNTE Jacqueline ép. NUMBEM	Chargé de cours	*CEA MINRESI*
34	TCHOUANKOUE Jean-Pierre	Chargé de Cours	En poste
35	ZO'O ZAME Philémon	Chargé de Cours	*S.G. MINTP*
36	TEMGA Jean Pierre	Chargé de Cours	*En poste*
37	ANABA ONANA Achille Basile	Assistant	*En Poste*
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39	METANG Victor	Assistant	*En poste*
40	NOMO NEGUE Emmanuel	Assistant	En poste
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42	TCHAPTCHET TCHATO De Pesquidoux	Assistant	En poste
43	TEHNA Nathanaël	Assistant	CS/ *MINMIDT*

Répartition chiffrée des enseignants permanents par Département

Département	**Nombre d'enseignants**				
	Pr	**MC**	**CC**	**ASS**	**Total**
BC	5 (1)	10 (4)	14 (9)	11 (5)	**40 (19)**
BPA	10 (0)	10 (3)	18 (7)	6 (1)	**44 (11)**
BPV	3 (0)	6 (1)	13 (3)	5 (5)	**27 (9)**
C.I.	9 (1)	7 (0)	15 (3)	4 (2)	**35 (6)**
C.O.	9 (0)	10 (3)	12 (3)	2 (0)	**33 (6)**
IN	4 (1)	1 (0)	8 (0)	17 (4)	**30 (5)**
MA	3 (0)	4 (0)	19 (1)	7(0)	**33 (1)**
MB	1 (0)	6 (2)	4 (1)	2 (0)	**13 (3)**
PH	8 (0)	14 (1)	15 (3)	4 (1)	**41 (5)**
ST	3 (0)	10 (1)	23 (4)	7 (0)	**43 (5)**
Total	**55 (3)**	**78 (15)**	**141 (34)**	**65 (18)**	***339 (70)***

Soit un total de 339 (70) dont

Professeurs : 55 (3)
Maîtres de Conférences : 78 (15)
Chargés de Cours : 141 (34)
Assistants : 65 (18)
( ) = Nombre de femmes

DEDICATION

THIS WORK IS DEDICATED TO MY SON NNEMMOR MBI TABI KING JR AND MY WIFE MRS TABI MBI NEE LINDA OBI

ACKNOWLEDGEMENTS

Special thanks to all lecturers in the Department of Plant Biology of the University of Yaounde I, not forgetting the late Head of Department Pr Amougou Akoa for fortifying me with a strong theoretical background without which the field and laboratory work of this thesis would not have been realized with ease.

My profound gratitude goes to my supervisor Pr Youmbi Emmanuel for academic orientation, psychological preparedness and development/orientation of methodology throughout the study. I also appreciate him, for provision of reagents and facilitating access into the tissue culture Laboratory of CARBAP. In fact he remains my mentor for life.

I sincerely pay much appreciations to my field supervisor Dr Ngando Ebongue G. F. (MR), Chief of Centre and Chief of Oleaginous Plants Program IRAD and Research Officer at service in CEREPAH for providing this topic. Besides, he also provided technical orientation and personnel. His soft spoken but rigorous follow up gave me a push whenever indolence tried to step in. Moreover, his relentless editing and contributions in manuscripts of publications derived from this thesis cannot be over emphasized.

I immensely thank the administration of CEREPAH for lodging facilities throughout the field work and most especially for a financial grant in the form of reagents worth 2 000 000 FCFA, and oil palm seeds worth 2 100 000 FCFA without which this thesis would not have been possible.

I will forever remain indebted to Dr Godswill Ntsomboh, assistant researcher at IRAD-CEREPAH for his tireless contributions in the shaping of methodological protocols of this thesis, in facilitating acquisition of reagents and in data analysis. Besides indefatigable editing of manuscripts and published articles derived from this thesis, his advice and encouraging words gave me a push each time things appeared to be at a standstill throughout this work, I pray God bless you.

Candid appreciations to Dr Tonfack Libert Brice for his encouragement which makes me anxious to grow in the academic ladder. He contributed enormously in publications originated from this thesis. I am able to write, edit and publish scientific articles today thanks to several workshops on methodological approach in peer review journal we held together.

I abundantly acknowledge prolific encouragement from other researchers in CEREPAH like Dr Anjambang Walters, Dr Mondjeli Constantin, Mr Anaba Bienvenu, Mr Bilong Eloi Gervais and Mr Madi Galdima. Above all I express thanks to personnel of the seed production chain in CEREPAH especially field technicians of the pollen laboratory like Mr Dama Jean

Pièrre, Mr Djoko Gilbert, Mr Ngoa Essogo, those of the physical analysis laboratory like Mr Kwe Eric, Mr Akwanji Robert, Mr Esome, Mrs Nkusima Yvette, as well as those of seed preparation, storage and the germination room like Mrs Kouenkang Rose Nicole, Miss Ekassi Marie Lydie, Mr Sali Brai, Mr Bisohong Messack, and Mr Bakari for their ever readiness to assist me any time. I also thank CEREPAH secretaries like Mrs Mboule Eveline and Ngo Djebe Mispa for making available some vital documents.

I am indeed appreciative to the laboratory of Biotechnology of the Centre Africain de Recherche sur Bananiers et Plantains (CARBAP) Njombé for accepting part of this work to take place within their structure and for provision of hardwares and reagents for tissue culture.

Special tributes to Mr Ngaha Dieudonne and Mr Njembele Celestin for their assistance in preparation of culture media and follow up of different developmental stages in mature zygotic embryo rescue of oil palm. I also thank Mr Alino for cleaning of glasswares in the tissue culture laboratory. I do acknowledged moral support from other students on internship at CARBAP like Mr Likeng Benoit, Mr Ongagn Alphones, Mr Camille, Mr Kelvin, Miss Flora and Miss Yolanda.

I remain indebted to my parents, late Pa Nchenge Steven Tabi and Ma Regina Ayuk Tabi for bringing me in to this world, my elder brothers Mr Nchenge Peter, Mr Tabi Edwin Tabi and Wife Njabe Christiancia, Mr/Mrs Tabinyanga Emmanuel Mbi, my elder sisters Mrs Enow Grace of blessed memory and Mrs Emoh Mourine Mbeng for their love, prayers and care throughout, not forgetting Mr/Mrs Oru Nchenge Francis for their upbringing.

I will forever remain grateful to Sesekou Besong Bakia and wife Mrs Bakia nee Besem Heline of Yaounde for their advice, financial and material support without which this work wouldn't have seen its lamp light; I pray the almighty God blesses them abundantly.

I acknowledge academic encouragement from Mr/Mrs Eyong Alfred Tabot, Mrs Teboh Hanna and Mr/Mrs Egbe Ben Besong. I do appreciate ample moral support and psychological refurbishment from friends like Dr Ngwane Ntongwetape, Mr Mfomor Bakia, Mr Nso Martin Etchi, Mr Tabe kingsely Eyong, Mr Enow James Ayuk, Mr Atem Elias, Mr Abunaw John, Mr Clayton Tabe, OP/Mrs Eyongeta Fieldings, Eng. Besongngem Enowmbi, Dr Besongngem Akotanchi, Mr Asueyoung-Oru Roosevelt and Ntenako youths cultural and development association of Yaounde.

Unique cheers to all members of the Laboratory of Biotechnology and Environment, particularly those of the Unit for Plant Physiology and Improvement, of the University of Yaounde I, for positive criticisms and moral support during scientific animations.

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LIST OF ABBREVIATIONS

ABA: Abscisic acid

AC: Activated charcoal

AS: Acid scarification

CARBAP: Centre Africain sur la Recherché en bananier plantain

CEREPAH: Centre specialise pour la recherché en palmier a l'huile

Cat 1-10: The ten categories of the commercial oil palm seeds produced by CEREPAH

CH_2N_2: Hydrogen cyanamide

CM: Culture medium

CVG: Coefficient of velocity of germination

DHT: Dry heat treatment

DW: Dry weight

FF: Fresh fruits

FFB: Fresh fruit bunch

FW: Fresh weight

GA: Gibberellic acid

GP: Germination capacity

GPC: Growth promoting chemicals

H_2O_2: Hydrogen peroxide

HWS: Hot water scarification

IAA: Indole -3-acetic acid

IBA: Indole-3- butyric acid

IRAD: Institut de recherché agricole pour le development

MC: Moisture content

MD: Morphological dormancy

MLIT: Mean length of incubation time

MPD: Morpho-physiological dormancy

MS: Murashige and Skoog medium

MZE: Mature zygotic embryo

NAA: Naphthalene acetic acid

PD: Physiological dormancy

PGR: Plant growth regulators

PY: Physical dormancy

TTZ: 2, 3, 5 triphenyltetrazolium chloride

LIST OF APPENDICE

ABSTRACT

Germination of oil palm seeds is naturally very slow, low and unsynchronized due to severe dormancy. Considering that in practice the propagation of oil palm is accomplished mainly by seeds, slow, low and worse still, failure to germinate obviously has serious consequences at the agronomic and socioeconomic scales. The present consumption rate of palm oil in the food and the manufacturing industries coupled with its recent use in production of biodiesel, call for an increase in productivity for fear that demand does not outweigh supply at the global scale. To mitigate this deficit, the government of Cameroon in accord with local councils has been allocating extensive hectares of land to international and national companies in the South West, Littoral and Centre Regions to extablish oil palm plantations. The latter need improved seeds adapted to the local agroecological zone. Unfortunately, CEREPAH, 'the major producer and distributor of improved oil palm seeds in Cameroon witnessed a constant decrease in annual germination capacity (GP) of ≈5 % per annum from 2009-2013. Hence demand for improved oil palm seeds became greater than CEREPAH can supply. In response, CEREPAH is envisaging new approaches to be used alongside the conventional dry heat scarification (DHT) to maximize supply of improved oil palm seeds. It is within this context that this study was conceived.

The general objective was to to identify approaches that could ameliorate annual germination rate of improved oil palm seeds at the IRAD-CEREPAH, La Dibamba, in groundwork to satisfy the present and future huge demand. Four specific objectives were set to attain this goal, amongst which where: to determine the causes of low annual germination capacity (GP); to optimize the traditional DHT by incorporating some growth promoting chemicals (GPC); to evaluate acid and hot water scarifications as alternative methods of breaking dormancy and finally to assess production of oil palm plantlets via mature zygotic embryo rescue.

To determine the cause of unsynchronized GP, some biometric parameters were studied. Position dependent effect of seeds and the influence of harvesting date on germination were carried out to determine the causes of general low GP. In the second approach, the effect of incorporating different concentrations of some growth promoting chemicals (GPC) to the traditional DHT was appraised. Regeneration potential of *vitro* plantlets via MZE rescue of the ten CEREPAH commercial oil palm cultivars was tested on 10 modified Murashige and Skoog culture media (CM1-10).

As concerns the causes of variation in GP, the results showed the existence of significant variation in biometric parameters like length, weight, number of kernel/seeds, shell

thickness between the 10 tenera cultivars commercialised by CERPAH. Similarly, it was found that the basal seeds (Orange coloured fruits), significantly higher than apex seeds (deep red coloured fruits) in terms of number, scored a lower germination compared to apical seeds. The results also showed that the harvesting date of FFB destined for seed production significantly influences germination capacity positively.

As concerns optimization of DHT, it was observed that, Gibberellic acid (GA_3) and hydrogen peroxide (H_2O_2) incorporation to DHT improved germination capacity (GP) than DHT alone. Significant differences were noted between the GPC and the control for parameters like GP, mean length of incubation time (MLIT) and coefficient of velocity of germination (CVG). Generally incorporation of GA_3 and H_2O_2 significantly improved GP while CN_2H_2 decreased GP even lower than the control. H_2O_2 recorded the least MLIT (30-36 days) while CN_2H_2 registered the longest (90-120 days) for all seed cultivars tested. H_2O_2 also scored the highest CVG compared to other treatments. No germination was observed when the seeds were subjected to acid and hot water scarifications without prior scarification by DHT.

Embryo rescue revealed a positive response to *in vitro* germination in all the ten seed cultvars. Cv1, Cv5 and Cv9 scored the highest germination capacity (90 %) while the least of 14 % was recorded in Cv8. Most suitable culture medium (CM5) registered 72 % while the least germination (48 %) was observed in CM1 and CM8. Robust *vitro* plantlets with complete differentiation into a shoot and root bud axes occurred in CM2, CM5, CM7 and CM10. Among the three auxin types compared, rooting of *vitro* plants was highly significant when treated with IBA at 1.5 mg/L.

Key words: *Elaeis guineensis* Jacq., Dormancy rupture, Germination, Hydrogen peroxide, Gibberellic acid, Embryo rescue

RESUME

La germination des graines de palmier à huile est naturellement très lente, et désynchronisé avec un faible taux en raison de la dormance sévère. Tout ceci a évidemment des conséquences graves sur les plans agronomiques et socio-économiques. Le taux actuel de consommation d'huile de palme dans les industries alimentaires et autres y compris son utilisation récente pour la production de biodiesel, appelle à une augmentation de la production par crainte que la demande ne l'emporte sur l'offre à l'échelle mondiale. Raison pour laquelle, recement le governement Camerounais en accord avec les autorités locales à accordé aux producteurs nationaux et internationaux les étendus des hectares dans le Sud Ouest, Littoral et la Region du Centre pour etablir les plantations. Ces dernierre ont besoin des semences adaptées à des conditions agroecologiques locales. Malheureusement, de 2009 à 2013, le CEREPAH, premier producteur et distributeur des semences ameliorées de palmier à huile, a connu une baisse du taux de germination d'environ ≈5 % par an, rendant ainsi la demande supérieure à ce que le CEREPAH peut fournir. En réponse, le CEREPAH exploite différentes méthodes parallèlement à la scarification par chaleur sèche afin de maximiser l'offre de semences améliorées. C'est dans ce contexte que cette étude a été lancée pour tenter d'expliquer le faible taux de germination, ainsi que chercher des moyens d'améliorer ce taux.

L'objectif général de cette étude été d'identifier les techniques qui permettent d'optimiser le taux de germination des semences ameliorees de palmier à huile afin de satisfaire la demande. Plus spécifiquement, il été question de deteminer les cause du faible taux de germination, optimiser la technique conventionelle en y associant des produits chimique et enfin le sauvetage d'embryons comme une methode alternative pour la production des semences de palmier à huile.

En ce qui concerne la determination des causes du faible taux de germination (TG) et la variation de TG parmis les dix cultivars commercialisee par le CEREPAH, trois aspect ont été étudiée; les parametres physique des graines, le TG en fonction de la position des fruits sur le régime et l'influence de la date de récolte sur TG.

Pour améliorer la method conventionelle de scarification a chaleur seche, l'effet d'association de certaines produits chimiques et hormone de croissance tels que GA_3, H_2O_2 et CN_2H_2 à différentes concentrations a été évalué. La capacité de germination, la durée moyenne du temps d'incubation (MLIT) et le coefficient de vitesse de la germination (CVG) ont été appréciées. La scarification avec acide et avec de l'eau chaude ont été utilisés comme des alternatives à la scarification par chaleur seche. La troisième approche été axée sur la régénération directe de plants de palmiers à huile *in vitro* en procédant au sauvetage d'

embryons zygotique matures (MZE) sur 10 milieux de culture Murashige et Skoog modifié (CM1-10).

En ce qui concerne les causes des faible TG, les resultats ont montrés que le nombre de graines basales (fruits de couleur orange) était significativement plus élevé que celui des graines de l'apex (fruits de couleur rouge foncée). Pour chaque régime, les graines basales avaient un TG plus faible par rapport à ceux de l'apex. Les résultats ont aussi montré que la récolte tardive des régimes (≥7 mois) augmente le TG par rapport a ceux récolté ≤ 6 mois après la pollinisation.

Concernant, l'association des stimulateurs de croissanc à la scarification par chaleur seche, GA_3 et H_2O_2 ont améliorés le taux de germination par rapport au témion. En général, l'incorporation de GA_3 et H_2O_2 a considérablement amélioré la TG tandis que CN_2H_2 la diminué jusqu'à un taux plus faible que le témoin. Pamis les traitements, l'association avec H_2O_2 a enregistré la plus forte CVG.

Pour la deuxième approche, aucune germination n'a été observée sur les graines soumises à une scarification acide et l'eau chaude sans scarification à chaleur seche au préalable.

Pour la troisième approche, tous les dix cultivars de semences ont montré une réponse positive à la germination. Néanmoins, des variations importantes au niveau de l'organogenèse ont été observées entre les cultivars et les milieux de culture. Cv1, Cv5 et Cv9 ont enregistrées les meilleurs taux de germination (90 %) tandis que Cv8 a enregistrée le plus faible taux (14 %). Le meilleur milieu de culture (CM5) a enregistré 72 % de germination tandis que le moins bon (48 %) a été observé dans les milieux CM1 et CM8. Les plantules les plus robustes avec une différenciation complète en bourgeons et racines ont été produites dans CM2, CM5, CM7 et CM10. Parmi les trois types d'auxins comparés, la rhizogenèse des *vitro* plants a été très significatif en présence d'IBA à la concentration des 1.5 mg/L.

Mots Clés : *Elaeis guineensis* Jacq, Levée de dormance, Eaux oxygénée, Acide gibbérellique, Sauvetage d'embryon

CHAPTER I. GENERALITIES

I.1. Introduction

The oil palm (*Elaeis guineensis* Jacq.) is a perennial monocot of the *Arecaceae* family with diploid chromosome number (2n) = 32 (Castilho et *al*., 2000). It is the most important and highest oil yielding crop in the world with world average oil yield of 3.5 MT palm oil/ha/year, almost 7 times higher than that of soy (0.5 t /ha/year) whose oil is also highly demanded in the world market (Corley & Tinker, 2003). Thanks to its year-round harvestability and multi-decade productive lifetime, the oil palm cropping system has unique advantages over annual oil crops such as soy beans, rape seed or sun flower. Thus the oil palm has been reflected in its rapid rise to become the largest global oil crop (Murphy, 2009). The crop is grown on a large scale in Africa, Equatorial America, South East Asia and South of the Pacific and in smaller scales in other parts of the world (Koh & Wilcove, 2008). Oils obtained from its fruits are crude palm oil (CPO) extracted from the mesocarp and palm kernel oil (PKO) extracted from the endosperm. Palm oil holds huge economic importance in the food, non-food derivatives, oleo chemical and biofuel industries (Mya et *al*., 2010).

During the past decade there has been a growing interest in bioenergy, driven by concerns about global climate change, growing energy demand, and depleting fossil fuel reserves. For biodiesel production, a high yield crop is needed, and the large amount of oil produced by the oil palm fruit makes this species highly suitable (Thawaro & Te-chato, 2010). The predicted rise in biofuel demand makes it important to understand the potential consequences of expanding biofuel cultivation (Murphy, 2009). Worldwide demand for vegetable oils is expected to increase by the end of this decade, with biofuels accounting for one-third of the increase (Anonymous, 2011a). This is probably owing to the continued high demand of palm oil as a source of edible oil and a biofuel feedstock (Corley, 2009; Koh, 2007). The present consumption rate of palm oil both in the food and the oleochemical industries coupled with its recent use in production of biodiesel, calls for an increase in production for fear that demand does not outweigh supply at the global scale. Increasing the sizes of plantations alone is not a solution enough to equate current and future demand and supply. Rather, intensifying oil palm cultivation by planting of improved seedlings among other factors may appear to be a sustainable solution to increase oil palm productivity while reducing habitats and biodiversity distruction of tropical forest. However, the rise in prices of oil crop derivatives, which are closely linked to demand (Rudel et *al*., 2009), indicate that the market for palm oil is still expanding. Recently, six new foreign investors have been allocated hundreds of hectares of

land (50 000-600 000 ha) in the South West, Littoral and Centre Regions of Cameroon, for the establishment of oil palm plantations (Hoyle and Levang, 2012); harnessing the fact that global demand for palm oil and its derivatives is on a rise.

Beside land, opening an oil palm plantation requires improved oil palm seeds. According to Corley and Tinker (2003), much research has been carried out on production of improved seeds but the major challenge affecting the oil palm seed production is shortage due to a complex and expensive technique involved in overcoming deep dormancy. Rees (1963) reported that in the wild, the seeds may remain viable and begin germination even after 25 years in the soil. The author found that oil palm germination rate is uplifted if shade is suppressed to let seeds receive sun light, an indication that light or high temperature probably act as stimulants. Early works on this topic indicated that if sown normally, the seeds can take up to nine months to give just 50 % germination (Ferwerda, 1956). Faced with this challenge, improved oil palm seed production centres employ techniques that permit ≥ 70 % germination of oil palm seeds within 4 months rather than the natural lengthy germination time in which ≥ 9 months are required to obtain just 50 % germination.

In Cameroon, improved oil palm seeds are produced in two research institutes i.e. PAMOL Plantations Limited found in Ndian Division in the South West Region and the Specialized Center for Oil Palm Research (IRAD-CEREPAH) of La Dibamba found in the Littoral Region. Of these two, CEREPAH is a reputable producer and distributor of improved seeds in Cameroon and abroad. Out of the several techniques used in seed technology to overcome deep dormancy observed in oil palm, CEREPAH practices the dry heat scarification technique (DHT) developed by Rees (1959) to improve and speed up seed germination for a sustainable supply of improved seeds to the planters. CEREPAH's criterion to harvest fresh fruit bunches (FFB) to be used for seed production is the abscission of 2 fresh fruits (FF) likely to take place 5-6 months after pollination. Apart from the fact that this criterion varies with environmental conditions and age of plants, it is generally used to assess ripeness of FFB meant for extraction of CPO (Mohd et *al*., 2012). Using the present technique, CEREPAH anticipates at least 80 % germination for each seed lot subjected under the germination circuit, but this has not always been the case. In 2013 alone, CEREPAH registered 1186539 (40 %) none germinated seeds. This is a great loss from the socioeconomic and agronomic stand points, considering that a germinated oil palm seed of CEREPAH sold averagely at 237.5 FCFA (Anonymous, 2015). By multiplying the 1 186 539 ungerminated seeds in 2013 by 225 FCFA / seed, the sum of 281 803 013 FCFA was lost by CEREPAH in 2013 alone, all things being equal. On the other hand, if the seeds germinated and were grown in the nursery for 8

months, CEREPAH would have made 1 186 539 000 FCFA if sold at average 1000 FCFA / seedling. Similarly, if these seeds germinated and were planted by whoever, an increase in world planted area of 8297.4 ha would have been made if we consider the conventional planting density of 143 oil palms / ha.

Reports from 2009-2013, signpost annual germination capacity (GP) far below the 80 % conventional threshold, hence a difficult task for CEREPAH to match demand and supply, especially at a time when many nationals and foreign farmers in the CEMAC zone are gaining interest in the oil palm (Hoyle and Levang, 2012). The recent geometric increase in consumption of crude palm and kernel oil at the global scale has fuelled an emerging seed market (Hoyle & Levang, 2012). Seed production unit is the first link in the palm oil supply chain. Seed production unit is followed by the nursery to produce seedling, the plantation to produce fresh fruit bunches (FFB), the mill to produce crude palm oil and palm kernel, the kernel crushers to produce crude palm kernel oil, the refinery to produce refined palm oil and finally the palm biodiesel plant to produce palm biodiesel (Halimah et *al*., 2014). Low or slow production of improved oil palm seeds or worse still failure to germinate, therefore strains the activities of the entire oil palm chain value. In an effort to scale up availability of improved oil palm planting materials, CEREPAH is currently exploring other seed production techniques to be used alongside the dry heat scarification. It was within this context that this thesis was initiated.

General objective

The general objective of this study was to identify approaches that could ameliorate annual germination rate of improved oil palm seeds at the specialised centre for Oil palm research, IRAD La Dibamba as groundwork to satisfy the present and future huge demand.

In an effort to develop approaches that would enhance germination capacity and shorten germination time, four specific objectives were formulayed as follows:

- to determine the causes of low germination capacity and germination variation among cultivars;
- to optimize the conventional DHT by incorporating some growth promoting chemicals;
- to verify the potentials of acid and hot water scarifications as alternatives to the conventional scarification by DHT and
- to assess the production of oil palm plantlets via mature zygotic embyo rescue.

I.2. Review of literature

I.2.1. The oil palm

I.2.1.1. Origin and distribution of the oil palm

The oil palm is a tropical plant (Fig. 1) with two main species; the African oil palm (*Elaeis guineensis*) and the Latin America oil palm (*Elaeis oleifera*). The African oil palm was first illustrated by Nicholas Jacquin in 1763 in the Gulf of Guinea, hence its scientific name *Elaeis guineensis* jacq. (Hartley, 1988). Analysis of the species natural genetic diversity suggests that wild populations could be separated into three groups located at the extreme West of Africa, equatorial Africa and on Madagascar Island. The highest allelic diversity was found among Nigerian palm populations, indicating the possible centre of origin (Bakoumé et *al*., 2015). The tropical rain forest region of West Africa is therefore the natural home of *E. guineensis* Jacq. with the main belt running through the southern latitudes of Cameroon, Côte d'Ivoire, Ghana, Liberia, Nigeria, Sierra Leone, Togo and into the equatorial region of Guinea, Angola and the Congo (Fig. 1). The other species (*E. oleifera*) is native to South America, with the main areas of distribution being Surinam and the Amazon basin passing through Venezuela, Columbia, Costa Rica, and Panama up to Nicaragua (Meunier & Boudin, 1975). Cook (1942) suggested that, the oil palm originated in Brazil, mainly because the great majority of species in the palm subfamily Cocoideae, including the other species of *Elaeis* like *E. oleifera*, *E. odora* are native to South America. Wessel–Boea (1965) considered that the American and African species are so closely related that separation into different genera is not justified. This argument is supported by the production of viable seeds in controlled pollination trials between the two species (Corley et *al*., 1971). In the hybrids, *E. oleifera* showed dominance over *E. guineensis* for several traits, suggesting that *E. oleifera* is more primitive and *E. guineensis* represents a derived species (Hardon, 1969). With ever increasing interest in the oil palm and its related products, research has enabled the plant to grow out of its original ecosystem. Today the crop has been distributed to over 43 countries in the globe (Fig.1).

Meunier & Boudin (1975) reported that several morphological features can be used to distinguish *E. oleifera* from *E. guineensis* but that the main distinctive feature between the two is the fact that unlike *E. guineensis*, *E. oleifera* is a much shorter, often procumbent trunk, a trait from which species is also known as a walking palm. After procumbence, the basal part of the plant dies whilst adventitious roots sprouting from the part in contact with soil allow the plant to restart growth. The high proportion of parthenocarpic fruits that may constitute up to 90 % of the total is another striking characteristic of the *E. oleifera* fruit bunches as compared

to the African species. Immature fruits are green turning orange at maturity, which resembles *virescens* African oil palms. *E. oleifera* leaves are different from those of *E. guineensis* with respect to the positioning of leaflets on the front (Corley and Tinker, 2003). *E. oleifera* pollination depends on insects. However, the profile of volatiles emitted by the inflorescences at anthesis is different and species do not synthesize methyl chavicol (Gomes, 2011). Annual rate of vertical growth is another criteria used by some authors to further differentiate the two species. Corley and Tinker (2003) stated that in relation to the environment and genetic makeup, African oil palm accessions have height increment of 45–75 cm a year while annual height increment of *E. oleifera* may be only 5–10 cm. Interspecific F1 hybrids between *E. guineensis* and *E. oleifera* have a mid-parent growth phenotype of 15–25 cm annual height increment, indicating a useful gene introgression source. Intraspecific variation enabled Malaysian breeding programs to develop PORIM series of dwarfish palms with a yield potential of 7 tons and annual height increment of 40 cm (Rajanaidu et *al*., 2000). Hybrids from crosses of Bamenda and Ekona *E. guineensis* accessions developed by ASD Costa Rica are slower growing (45–50 cm / year) and are also known for overall high cold and drought tolerance (Barcelos et *al*., 2015).

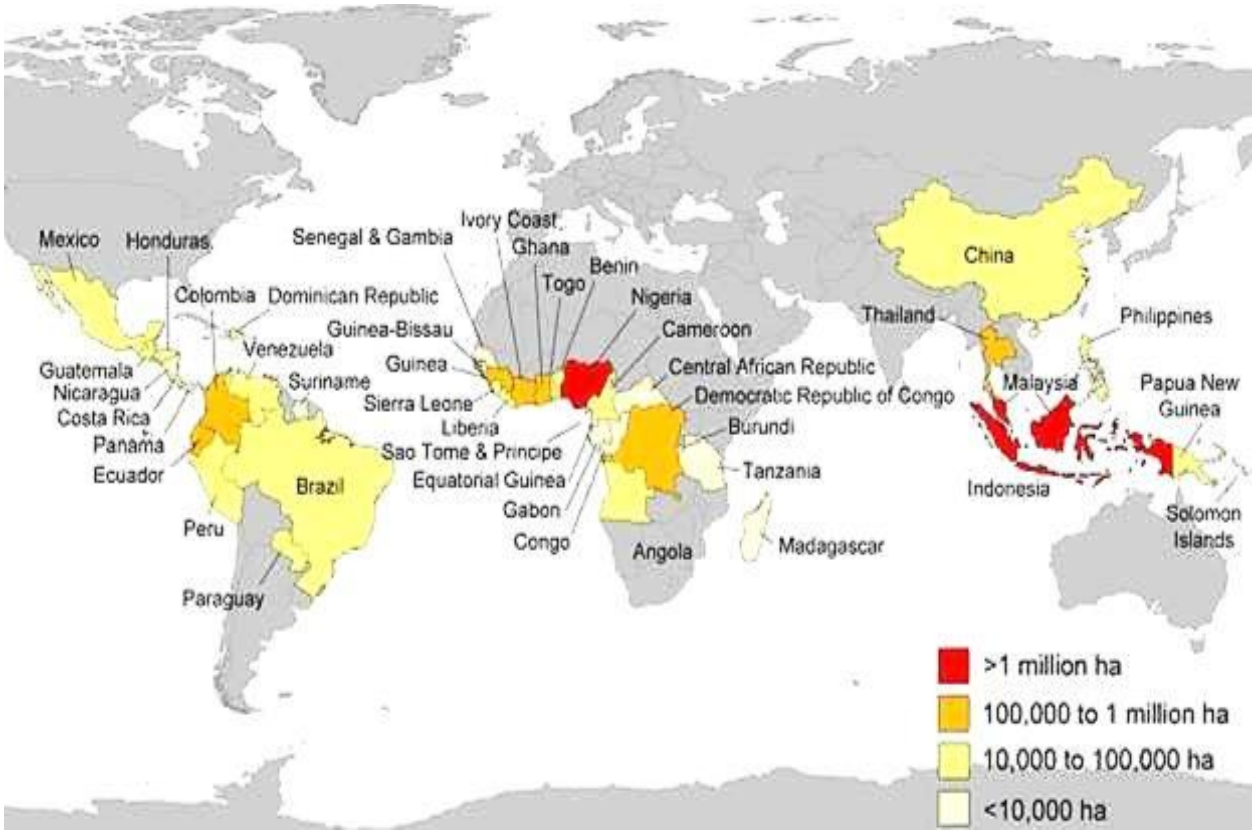

Fig. 1. Land allocation in 43 palm oil producing countries in the world (Koh & Wilcove, 2008)

I.2.1.2. Taxonomy

The Arecaceae family includes palm trees of economic importance both as a source of agricultural produce, as well as ornamental components in landscaping projects (Henderson et *al*., 1995). The palm family is one of the largest families of monocots with an estimated, 190 genera and 2 364 species (Govaerts & Dransfield, 2005).

- Domain**:** *Eukaryota*
- Kingdom: *Plantae*
- Subkingdom: *Viridaeplantae*
- Phylum: *Tracheophyta*
- Sub phylum: *Euphyllophytina*
- Infra phylum**:** *Radiatopses*
- Class: *Liliopsida*
- Sub class: *Arecidae*
- Super order: *Arecanae*
- Order: *Arecales*
- Family: *Arecaceae*
- Sub family: *Arecoideae*
- Tribe: *Cocoeae*
- Genus**:** *Elaeis*
- Specific epithet**:** *guineensis* Jacq.
  Botanical name**:** *Elaeis guineensis*

I.2.1.3. Biology of the oil palm tree

E. guineensis is a monocotyledonous plant that thrives best in ecological zones with the following abiotic characteristics; annual rainfall of 2000 mm or more evenly distributed throughout the year with no marked dry season (at least 100 mm / month), temperature of 22-33 °C, sunshine of at least 5 hours a day in all months of the year and rising to 7 hours per day in some months, latosol characterized by low silica sesquioxide ratios of the kaolinitic clay fraction, medium to low cation exchange capacities, a relatively high degree of aggregate stability and a relatively low content of primary minerals except quartz, are the best (Hartley, 1988). At the level of the plantations, 130–143 palms are planted/hectare in a rectangular spacing pattern with an appropriate distance between two palms being 9 m. However, high density planting is species depend. Reproduction starts 2–3 years after planting, and can be

exploited up to 25–30 years before replacing (Soh et *al.*, 1989; Wahid et *al*., 2005). However, the lifespan of the oil palm as demonstrated by specimens planted in the Bogor Botanical Garden, Indonesia, is at least 120 years (Corley& Tinker, 2003; Mohd et *al*., 2004).

I.2.1.3.1. Botanical description

A mature oil palm tree is made up of only one stem that ends with many fronds and unlike some wild types, it is one of the largest palm species that does not produce suckers from the base. The plant presents a single vegetative growing point, situated in a depression at the stem apex as in other palms. The meristem is continuously active, producing a new leaf primordium about every two weeks in mature palms (Hartley, 1988; Corley & Tinker, 2003).

I.2.1.3.2. Leaf or frond

The leaf is pinnate, with the pinnae 'leaflets' arranged in two planes on both sides of the rachis, numbering 200-300 per leaf, about 90–100 cm long and 1.5-2.0 cm wide, with entire margins. Leaflets cover the distal 2/3 of the frond, and the lower 1/3 possess spines whose lengths increase acropetally. The leaf can measure up to 7.5 m long in mature palm trees. Leaf bases are persistent for years, and prominent leaf scars are arranged spirally on the trunk of mature palms where bases have fallen. Corley (1973b) suggested that the lifespan of leaves depends on the intensity of the light reaching them through the canopy, hence at high planting densities, leaf duration reduces considerably while at normal densities of 140–150 palms / hectare without pruning, senescence of leaves will probably start at about leaf 48–50.

I.2.1.3.3. Trunk

The trunk of the oil palm is made up of a discrete vascular bundle buried in the parenchymatous tissues. No secondary growth occurs; stem thickening and extension are due to activities of primary meristem and the trunk reaches its maximum girth just below the apex. Generally, trunk growth is affected by environmental factors, but a maximum increase in height of between 33–75 cm occurs / year. Internodes length varies from 14–23 mm in different progenies in Ivory Coast while in Malaysia, internodes length varies from 15 mm in 4.5 years old oil palms to 25 mm in 10.5 years palms (Gray, 1969). Old leaf bases persist on the trunk until the palm is between 11–15 years old before they begin to fall, usually from the middle of the palm trunk and then extend upward and downward, the trunk of an old palm will totally be free of old leaf bases except just below the crown. The reason for this behaviour is not known but a treatment to accelerate leaf base abscission might be useful, since the old

bases may act as a focus for upper stem rot infection (Turner, 1969) and they also turn to retain fruits during harvesting.

I.2.1.3.4. Root system

As the seed grows, its radicle is replaced by adventiceous primary roots emanating from the radical–hypocotyls junction and then from the lower internodes of the stem which are formed in to a massive basal cone or bole. The latter retains the meristematic capacity, producing roots well above ground level. In some rare cases roots may develop up to 1 m above the ground, even though these roots often dry before reaching the soil. At maturity thousands of primary roots spread rapidly from the bole (Fig. 2) and new primaries are continuously replacing dead ones (Jourdan et *al*., 2000). The anatomy of oil palm roots is described by Tomlinson (1961).

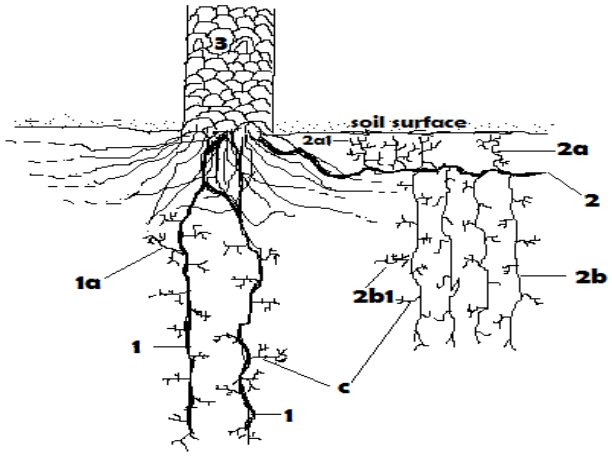

Fig. 2. The root system of an adult oil palm

1. Primary root with vertical downward growth, 1a.secondary root with general horizontal growth, 2. Primary root with horizontal growth, 2a.secondary roots with upward vertical growth, 2b. Secondary roots with downward vertical growth, 2a1. Superficial tertiary roots, 2b1.quartenary roots 3. Deep lying tertiary roots. C. Deep lying tertiary roots, 3. Trunk (Jourdan et *al*., 2000 *modified*)

I.2.1.3.5. Inflorescence

Palms have a number of interesting features in their reproductive development, such as variable modes of sex determination and single-or mixed-sex flower clusters, which may define specific clades (Hélène et *al*., 2005). Oil palms are monoecious, producing separate male and female inflorescences on the same palm in varying cycles. The inflorescences are born from leaf axils, with either male or female and never both inflorescences per leaf axil. On an average, each year an individual palm bears 10 ± 2.5 male inflorescences and 7±2 female

inflorescences, giving a male to female ratio of 1.4:1 (Rajesh et *al*., 2001). The inflorescence of both sexes is a compound spadix with 100-200 branches, initially enclosed in a spathe or bract that splits two weeks prior to anthesis (Corley & Tinker, 2003).

I.2.1.3.5.1. Inflorescence abortion

Abortion refers to the failure of an inflorescence to develop to anthesis. Generally, the aborted inflorescences are not visible externally, but a careful removal of the older leaves at leaf base reveals small, often rotten inflorescences in the leaf axils (Hartley, 1988). Inflorescence abortion is the second main factor determining final bunch number. The physiological mechanisms surrounding inflorescence abortion have not yet been satisfactorily explained but a good number of approaches have been carried out in Malaysia, Nigeria etc to determine the actual age at which abortion occurs (Broekmans 1957). However the severity of the dry season, high density of planting and rate of fruiting activity are factors among others that affect abortion rate (Desmarest, 1967; Gray, 1969), while Corley (1973b) found that severe defoliation caused abortion of female inflorescences but not of the male.

I.2.1.3.5.2. Sex differentiation

The oil palm produces functionally unisexual male and female inflorescences in an alternating cycle on the same plant, resulting in an allogamous mode of reproduction. The sex ratio of an oil palm stand is influenced by both genetic and environmental factors. In particular, the enhancement of male inflorescence production in response to water stress has been well documented (Héléne et *al.,* 2005). According to Hartley (1988), each flower primordium is a potential producer of both male and female organs but in very rare cases both androecium and the gynoecium develop fully to give a hermaphrodite flower.

I.2.1.3.5.3. Male inflorescence

It is born on a peduncle longer than that of the female inflorescence and contains long finger–like cylindrical spikelet (Fig. 3) and unlike the female, it does not bear spines. The male spikelets bear 600–1500 thick and fibrous bracts, each subtending and enclosing a single flower. Spikelets measure up between 10–20 cm in length and 0.8–1.5 cm in breadth. Flowers start opening from the base of the spikelet and all flowers on the spikelet usually open within 2 days though during rainy weather the opening may prolong to 4 days. Most pollen is shed within the first 2–3 days following the start of anthesis and production ceases within 5 days. Viability of late produced pollen is low (Turner, 1969).

Fig. 3. Male inflorescence of oil palm at anthesis

I.2.1.3.5.4. Female inflorescence

It measures a length greater than or equal to 30 cm before anthesis. Each female spikelet bears about 10–20 floral groups of flower clusters, arranged spirally around the rachis (Hartley, 1988). Tomlinson & Moore (1968) regarded the flower cluster as a series of condensed sympodial branches of successively higher order, each bearing one bracteole and terminating in a flower by a bract which is drawn up into a spine at the cavity and subtended end of the spikelet. According to Corley & Tinker (2003), the total number of flowers in an inflorescence is approximately 900; out of which 150-250 open per day during anthesis (Fig. 4). Turner and Gilbert (1974) reported that each flower in the female inflorescence is tricarpelous even though only one kernel generally develops.

Fig.4. Female inflorescence of oil palm at anthesis

I.2.1.3.5.6. Hermaphrodite or mixed inflorescence

There is a great variety of hermaphrodite or mixed inflorescence formed in oil palm, usually male, female, and mixed spikelet appear on the same bunch but in widely differing

proportions and position. In the first kind of inflorescence, female flowers are located at the base while the male flowers are found at the summit. In the second kind, it may occur that, the gynoecium instead develops in flower on the male spikelets and a so–called androemoephous inflorescence result. This is particularly common in young palms (Williams & Thomas, 1970).

I.2.1.3.7. Pollination

The Oil palm is an outcrossing species due to asynchronous maturation of male and female inflorescences on the same plant and on a hermaphrodite inflorescence (Pootakham et *al*., 2015). The outcrossing process is both anemorphillous and entomoephilous, although the latter is prevalent and most effective. Investigations have shown that declining population of pollinating insects is often the cause of the low yields in terms of fruit set and bunch weight, observed in many oil palm plantations (Syed, 1982). In Africa, weevils of *Elaeidobius* spp. account for 99 % pollination, with *E. kamerunicus* and *E. Plagiatus,* cited as the most efficient oil palm pollinators (Yalamoussa et *al*., 2011). In Latin America on the other hand, *Mystrops costaricensis* has been reported to be the commonest and most efficient oil palm pollinators (Syed, 1982). *E. kamerunicus* is considered, the most efficient oil palm pollinator, because of its ability to multiply rapidly under different agro-climatic conditions and its introduction in a plantation generally brings about an increase in fruit set and bunch weight (Syed, 1982). Weevils are considered as the chief pollinating agents, they visit in large numbers (2000–3000 per inflorescence) during the shedding stage to consume starch rich pollen grains. These pollinators are attracted by the strong fennel–like fragrance aroma released by the male inflorescence at anthesis (Ponnama et *al.*, 2004). A significant problem for gender dimorphic plants is that pollinators often avoid female flowers mainly because they do not smell like males. This might explain why female flowers with no functional male organs often suffer inadequate visitation and pollen limitation for seed set (Tia-lynn et *al*., 2005). Though the oil palm also shows gender dimorphism, the female inflorescence also emits the same fragrance aroma of the male hence adequate weevils' visitation to receptive female is ensured. However, duration of weevils stay in the female flower is short (3.3 ± 1.28 seconds per flower) compared to that of male (> 60 seconds per flower) due to lack of reward (Ponnama et *al*., 2004).

Anthesis in oil palm flowers occurs acropetally in both male and female inflorescences. All the female flowers open in 3 days and the male flowers in 4 days. Generally, male flowers open between 08**:**00 am and 09**:**00 am while female flowers open from 09**:**00–10**:**00 am and visitations of weevils to the female flower starts at about 07**:** 00 am and continues till 3**:** 00 pm with maximum activity between 11**:** 00 am -12**:** 00 noon (Tandon et *al.*, 2001).

I.2.1.3.6. Fruit anatomy

The fruit of the oil palm is a sessile drupe, and fruitlets on the same FFB, show variation in shape ranging from nearly spherical to ovoid or elongated and bulging somewhat at the top. In length, fruits vary from about 2–5 cm and 3–30 g in weight (Hartley, 1988). Like all drupes, it is made up of a pericarp of 3 layers; exocarp; mesocarp or pulp; and the endocarp or shell. Generally the endocarp surrounds one but at times up to 4 kernels. The kernel is made up of, a testa, a solid endosperm and an embryo (Purseglove, 1972).

The embryo is straight, small, 3.0 mm long and is embedded in the 9 mm endosperm, opposite to one end of the germ pore. Its distal end lies below the germ pore but is separated from it by an operculum. A slight constriction divides the embryo into two parts; the haustorium, sometimes called the cotyledon and the tigellum. The haustorium is lighter in colour than the rest of the embryo and is marked with shallow longitudinal furrows. The haustorium stores reserves such as carbohydrates in the form of starch grains, lipid bodies and protein bodies (De Mason, 1985). After germination, when the distal part of the embryo has emerged from the germ pore, the haustorium grows and absorbs the endosperm, forming a spongy mass, which eventually fills the entire kernel (Tomlinson, 1990).

Fruit types have been used to classify oil palms into 3 varieties namely Dura, Pisifera and Tenera (Fig. 5). This classification is based on a major bi-allelic co-dominant gene called *Sh* (Table I), that determines the presence or absence of a shell and the degree of endocarp lignifications (Beirnaert & Vanderweyen, 1941). Based on the colour or pigmentation of fruits exocarp before and after ripening, oil palm can be placed into 3 main groups for each cultivar of *E. guineensis*: nigrescens; their fruits appear black in colour when unripe and red in colour when ripe. Virescens; unripe fruit appear green while ripe fruits are orange in colour and albescens; carotenoids are absent in its pulp. Nigrescens is the commonest of the three (Hartley, 1988).

Table I. Varieties of oil palm, their characteristics and interest in research

Variety	Genotype	Mesocarp/fruit ratio (%)	Endocarp (shell)	Kernel /size	Interest in breeding program
Pisifera	Sh^-/Sh^-	Thick (90-99)	Absent	Absent	Male parent (pollen source)
Dura	Sh^+/Sh^+	Thin (35-75)	Thick (2-8mm)	Large	Female parent for hybridization program
Tenera	Sh^+/Sh^-	Thick (55-96)	Thin (0.5-2mm)	Small	Hybrid of cross between dura and pisifera, for commercial reasons

Fig. 5. Fruit anatomy of the 3 varieties of oil palm

I.2.1.3.7. FFB ripeness prediction

Maturation phase of an oil palm fruit begins from 110 days and completes about 150 days after pollination while ripening overlaps with maturation from 140th day and ends 160 days after pollination (Tranbarger et *al*., 2011). The question on whether a FFB is ripe or not vary with authors. The suitable time for harvesting or true ripeness is the stage at which total oil content produced is at optimum quality (Mohd, 2012). Various criteria and techniques to gauge the ripeness standards of oil palm FFB have been developed amongst which are percentage detached fruits from the bunch (Turner & Gillibanks, 1974; Ng & Southworth, 1983), floatation technique (Azis, 1990), application of microstrip sensor to determine the moisture content which will indicate or approximate oil content of fresh mesocarp oil palm (Kaida & Zulkifly, 1992), oil palm colorimeter (Tan et *al*., 2004). Alfatni et *al*. (2008) used red, green and blue digital numbers to distinguish ripeness categories into unripe, ripe, under ripe and over ripped.

I.2.2. Socioeconomic importance of the oil palm

I.2.2.1. The place of oil palm among other oil seeds in the world market

Oil palm (*Elaeis guineensis*, Jacq.) is by far the most productive oil crop and alone is capable to fulfil the large and growing world demand for vegetable oils that is estimated to reach 240 million tons by 2050 (Coley, 2009). Per hectare of cropland, oil palm plantations give 3–8 times more oil than any other temperate or tropical oil crop (Fig. 6). Jacquemard (1995) reported that within the last 30 years, the oil palm has become the main oleaginous plant compared with other major oil crops like rapeseed, soy and sunflower, and the world's production of palm oil witnessed a tremendous evolution from 1 535 million tons in early 60s to 13751 million tons in 1995 giving a steadily increasing annual average of 343.9 million tons. By 2007, land area under

oil palm cultivation had tripled from 1961, while yield and price has also increased substantially (Fig. 7). Nowadays, palm oil occupies the first position among all vegetable oils consumed with over 85 % of the global production coming from Indonesia and Malaysia with Cameroon at the 13th position (Anonymous, 2016).

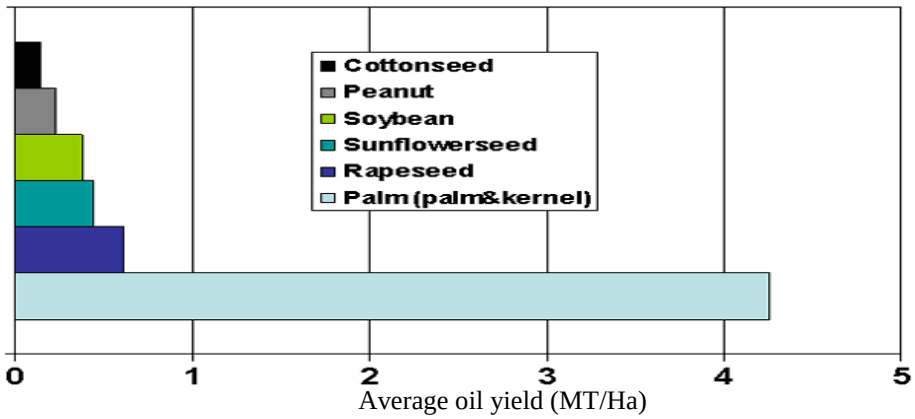

Fig. 6. Comparison of world oilseeds production from 2001-2006 (Anonymous, 2007)

Diffusion of high yielding and disease resistant cultivars produced by different research institutes and the reduction in cost of production account for the remarkable progress since the 60s (Soh et *al.*, 1989; Jacquemard, 1995). Some renowned research institutes that have contributed enormously in the development of the oil palm industry at the global scale are; INRAB, IRAD, NIFOR, IDEFOR, ASD, CENIPALMA, EMBRAPA, PORIM, CIRAD (ex-IRHO) and IOPRI (Hartley, 1988; Jacquemard, 1995; Corley & Tinker, 2003).

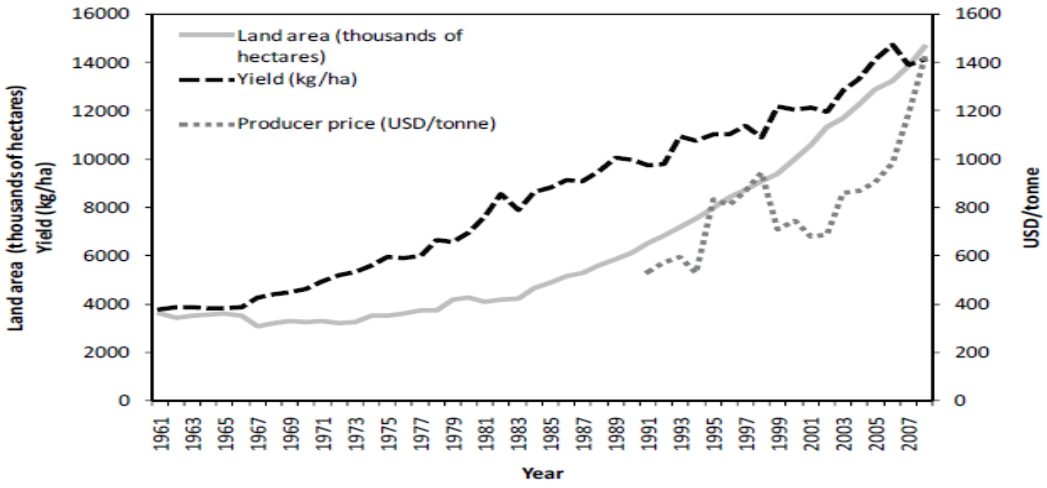

Fig. 7. Global oil palm land area, yield per unit area, and palm oil price (Anonymous, 2011b)

I.2.2.2. Palm oil / palm kernel oils consumption and health

The oil palm is one of the rare oil crops that produce two types of economically important oils i.e. crude palm oil (CPO) extracted from the mesocarp, and palm kernel oil (PKO) extracted from the endosperm. These two oils have very different fatty acid compositions of saturated and unsaturated fatty acids. CPO is 50 % saturated fat and 50 % unsaturated fat. Amongst monounsaturated fatty acids are; 44 % palmitic acid, 5 % stearic acid and 39 % oleic acid. Besides, linoleic acid (10 %) represents the only polyunsaturated acid. Myristic acid and lauric acid are negligible. On the other hand, approximately 82 % of PKO is saturated fat with the main contributors being 48 % lauric acid, 16 % myristic acid, and 8 % palmitic acid. Just 18 % of PKO is unsaturated fat with 15 % oleic acid 'monounsaturates' and 3 % linoleic acid (Anonymous, 2004). Studies on cardiovascular disease usually attribute an increased risk to elevated levels of serum cholesterol, which are thought to be derived from a dietary intake of saturated fats and cholesterol (Mukherjee & Mitra, 2009). Saturated fat means every carbon is bound to as many hydrogens as possible, thus the molecule is absent of double bonds. There is scientific evidence that not all saturated fats are equally cholesterol elevating. Stearic acid for example appears to have a neutral effect on total cholesterol and low-density lipoprotein (LDL), otherwise known as the 'bad' cholesterol (Bonanome & Grundy, 1988). Hayes et *al*. (1991) showed that Lauric acid and myristic acid are examples of saturated fats that increase total blood cholesterol and LDL while palmitic acid increases HDL cholesterol.

Palm oil consumption and its effects on serum lipid levels and cardiovascular disease in humans is still a subject of debate. Advocacy groups with varying agenda continue to fuel the controversy. The main argument for those advocating against the use of palm oil as edible oil is the fact that, it contains palmitic acid, which is a saturated fatty acid and by extrapolation should give rise to elevated total cholesterol and low-density lipoprotein cholesterol levels (Osaretin et *al*., 2015). On the other hand those advocating for palm oil consumption hold that, Palm oil consumed as a dietary fat as a part of a healthy balanced diet does not have incremental risk for cardiovascular disease. According to the latter, many scientific studies, both in animals (Onyeali et *al*., 2010) and humans (Zhang et *al*., 2003) that clearly show that palm oil consumption does not give rise to elevated serum cholesterol levels and that palm oil is not atherogenic. Apart from palmitic acid, palm oil consists of oleic and linoleic acids which are monounsaturated and polyunsaturated respectively. Palm oil also consists of vitamins A and E, which are powerful antioxidants. Palm oil has been scientifically shown to protect the heart and blood vessels from plaques and ischemic injuries.

I.2. 3. The Oil palm and the environment

Conversion of natural ecosystems to agricultural landscapes has had a severe negative impact on global biodiversity (Sodhi et *al*., 2010), with losses of species already occurring and further regional and global extinctions predicted to occur. Oil palm cultivation is among the main culprits responsible for loss of tropical biodiversity for two reasons. The first reason is its huge increase in planted area in recent years (Anonymous, 2011b). Secondly, oil palm production centres are located in the tropics which are known to be the richest biodiversity regions and habitats on the planet (Sodhi et *al*., 2004). Khalizani & Khalid (2011) reported that the oil palm has the highest potential in the production of biodiesel than any other oil seed (Fig. 8). The ever increasing demand for palm oil in food products and as a biofuel is likely to accelerate environmental changes in the future and at the same time, placing more pressure on remaining natural habitats (Koh & Ghazoul, 2008). Following the initial excitement about biofuel potential as a clean source of energy, increasing concerns have emerged about possible unintended environmental and social costs of biofuels in developing countries (Sheil et *al.*, 2009).

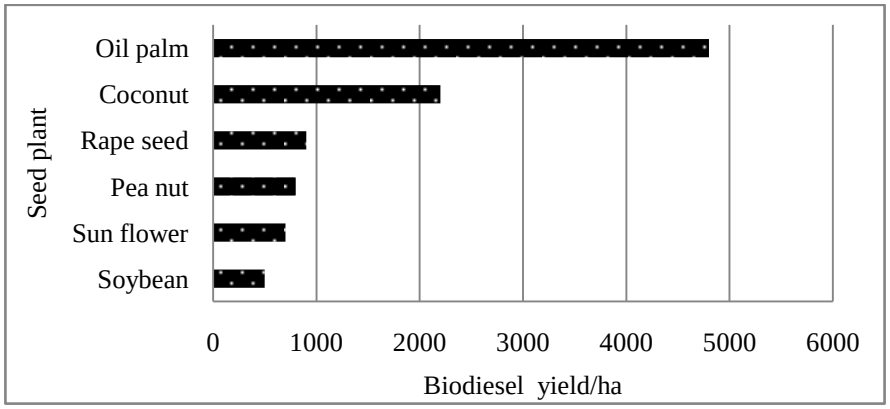

Fig. 8. Comparison of biodiesel yield / ha in different oleaginous plant

The impact of converting natural communities to monocultural landscape like an oil palm plantation can be seen from many angles like species richness, abundance, community composition, and ecosystem functions related to species richness and community composition. Sodhi et *al*. (2004) hold that expanding oil palm planted area in world biodiversity hotspots like Indonesia and Malaysia (Myers et *al*., 2000), and the Congo basin is likely to have a large negative impact on biodiversity at the global scale (Fig. 1). There is now overwhelming evidence that conversion of natural or semi-natural habitats to oil palm has severe negative impacts on biodiversity due to the simplification of the habitat (Foster et *al*., 2011). This includes the obvious loss of the different tree community that forms the basic structure of a

forest which is important in maintaining herbivore diversity (Novotny et *al*., 2006), a reduction in above ground structural complexity and a reduced canopy height. Partly due to this loss of canopy cover, microclimatic conditions are harsher for species in plantations, with temperatures being on average hotter and humidity levels lower. Fluctuations in both temperature and humidity are also greater over 24 hours in plantations compared to forest habitats (Turner & Foster, 2006). Direct disturbance effects, like cutting and spraying of understorey vegetation and a higher proportion of invasive species probably contributes also to species' declines and extinctions (Koh et *al*., 2009).

The question of oil palm expansion being a main culprit to biodiversity lost remains controversial. For instance, out of the 258.9 million ha of the global land under cultivation of the ten major oil crops, the oil palm covers just 14.24 million ha making merely 5.5 %. This percentage is twice lower compared to the global area under cultivation of sunflower, rape and cotton seeds and eight times lower than area covered by soybean (Fig. 4) (Oil world, 2013 cit. Anonymous, 2014). Considering that up to 32 % of world vegetable oil is produced just over approximately 5.5 % world surface area, oil palm producers are of the opinion that conservationist are just giving the oil palm a bad name. Based on these analyses, does oil palm monoculture actually threaten the forest (Oil world, 2013 cit. Anonymous, 2014)? The answer may probably be 'no', when world area under plantation of oil palm is compared to other oil seeds (Fig. 9) and can be 'yes' based on the fact that oil palm cultivation takes place in the tropics which represents world's ecological hot spot.

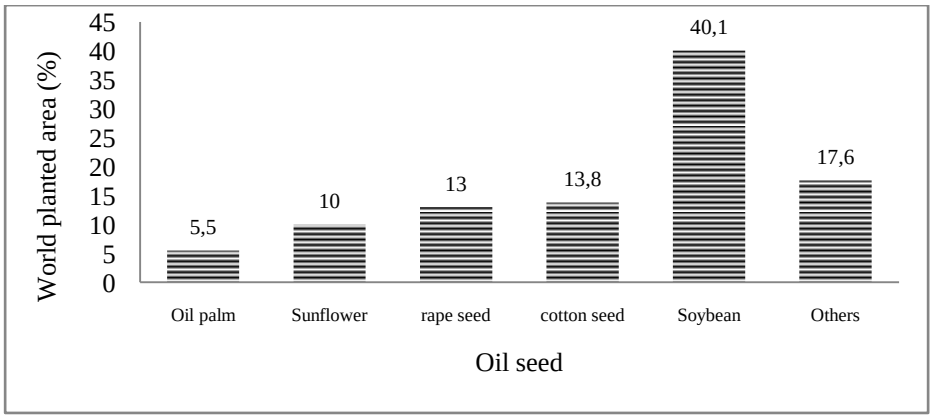

Fig. 9. Oil seeds and world planted area in 2012 (Anonymous, 2014)

Following concerns about potential negative social and environmental consequences due to oil palm monoculture, some voluntary standards have emerged since the beginning of the millennium. Amongst the most prominent are the Roundtable on sustainable Palm oil

(RSPO) established in 2004 and the Roundtable on sustainable biofuels (RSB) in 2007. The major objective behind these platforms is to ensure sustainable cultivation of oil palm while taking in to account protection of the environment.

I.2.4. Seed development, dormancy and germination

I.2.4.1. Seed development

In angiosperms, seed development is initiated with the double fertilization event involving two sperm cells and two female gametes. The female gametes consist of a haploid egg cell and a diploid central cell. Fertilisation of the egg cell with one sperm cell forms the zygote that later differentiates into the embryo (2n) while the fusion of the other sperm cell with the diploid central cell gives the endosperm (3n), hence the term double fertilisation. According to Bewley & Black (1994), even though the diploid embryo and triploid endosperm both originate from the double fertilization event, the developmental fates of the two differ greatly from one another. While the embryo progresses through a fixed pattern of cellular divisions to form a series of specialized organs, the endosperm, which initially grows coenocytically, either becomes a major storage organ as in monocotyledonous species or degenerates as in dicotyledonous species as the seed matures. The monocotyledonous endosperm serves as a crucial provider of nutrition during germination, while the role of the transient dicotyledonous endosperm has remained a puzzle despite more than a century of research (Sabelli & Larkins, 2009).

I.2.4.2. Origin, development and function of seed components

At complete development, the seed of angiosperms is made up of three genetically different constituents; the embryo, the endosperm and the testa (Lopes & Larkins, 1993). The sporophytic generation of higher plants is initiated with double fertilisation event that result in the formation of a single-celled zygote and a progenitor cell of the endosperm. Seed development comprises two major phases: embryo development and seed maturation. Throughout the process of embryogenesis which is a morphogenesis phase, the zygote undergoes a complex series of morphological and cellular changes resulting in the formation of a developmentally arrested mature embryo (Raz et *al*., 2001), comprised of an embryonic axis with a shoot and a root poles with cotyledon(s), often containing high level of stored macromolecules like proteins and lipids (Mayer et *al*., 1991). Hereafter, the seed, containing a

full sized embryo, undergoes maturation during which food reserves accumulate as dormancy and desiccation tolerance develop (Goldberg et *al*., 1994).

I.2.4.2.1. Embryo

The multicellular embryo sac embedded within the ovule has a polar organisation along its micropylar-chalazal axis. The egg cell and synergids are located closest to the micropylar pole of the ovule, whereas the antipodal cells are situated at the opposite end of the embryo sac, closest to the chalazal pole (Mansfield et *al*., 1990). During pollination, the pollen tube penetrates the ovule through the micropyle and delivers a sperm nucleus that fuses with the haploid nucleus of the egg cell to produce the diploid embryo. The embryo contained within the seed is a plant in miniature. Frequently embryo growth is minimal during the early stages of fruit and seed development and only intensifies near the end of the period of seed development although the presence of the embryo may be essential for seed and fruit development to begin (Bewley & Black, 1994).

Embryogenesis in higher plants can be divided conceptually in to three overlapping phases. The first phase is that of morphogenesis, during which the polar axis of the plant body is defined with the specification of the shoot and root apices, and the embryonic tissue and organ systems are formed. The second phase is that of embryo maturation, characterized by the accumulation of storage reserves in the endosperm or cotyledon (Bewley & Black, 1994). The final step before dispersal in the development of most seeds is maturation drying, in which water is lost from the seed while still within the fruit (Karssen et *al.*, 1983), reducing the moisture content thereby increasing the dependence of the seed on external water availability for germination. This step does not exist in recalcitrant seeds (Marilyn & Harada, 1993).

I.2.4.2.2. Endosperm

The endosperm originates when the second spermatic nucleus fuses with two polar bodies, making it triploid; two of the nuclei are maternally derived and one is derived paternally (sakai, 2010). The term “double Fertilization” indicates that the primary endosperm nucleus is considered to be derived from a fertilization event, even though the triple fusion nucleus does not go through a process that results in another organism, as expected from true fertilization (Lopes & Larkins, 1993). The fate of the endosperm, however, varies depending on species i.e. different plants have developed patterns of seed formation that lead to different levels of endosperm persistence during development (Murray, 1988). Based on its presence or absence, seeds have been classified into albuminous, for seeds that contain much of their stored

food in the endosperm and exalbuminous seeds, for seeds whose cotyledons absorb most or all the endosperm, before seed reach maturity (Sakai, 2010). The latter seeds have a relatively large embryo while the former seeds have a small embryo at maturity. Seeds of many species are extremely hard in their mature dry state because the walls of the endosperm cells are heavily thickened with galactomannan or mannan polymers (Bewley & Reid, 1985).

I.2.4.2.3. Testa

The seed coat originates from the ovular tissues precisely the integument and as such, bears the genetical characters of the mother plant. The inner integument gives rise to the tegment and the outer integument gives rise to the testa. The other ovule parts such as the funicle, raphe, chalaza and micropyle can mostly still be recognised in a mature seed and are indicated by the same names. The scar of the funicle is called the hilum, while the micropyle often forms a small depression. After fertilisation, the integuments continue their development by anticlinal path leading to growth in length and periclinal trend causing growth in thickness (Boesewinkel & Bouman, 1991). The role of testa is assumed mainly by controlling germination through dormancy imposition and by limiting the detrimental activities of physical and biological agents during seed storage (Debeaujon et *al*., 2000). However, testa rupture is not enough for radicle protrusion, weakening of endosperm is also required to allow radicle emergence, this process is associated with hydrolysis by cell wall-loosening proteins, such as expansin (Chen & Bradford, 2000), and endo-β-mannanase (Nonogaki et *al*., 2000). Testa rupture and endosperm rupture do not occur simultaneously, testa rupture occurs first followed by endosperm rupture and finally radicle protrusion (Liu et *al*., 2005).

I.2.4.2.4. Endocarp

Seeds from some plant families like *Arecaceae* and *Anacadiaceae* form an outer stony shell called the endocarp which encloses the endosperm. Anatomical studies have revealed that the endocarp of seeds with physical dormancy is made up of three water impermeable palisade layers namely brachysclereid, osteosclereid and macrosclereids from outside to inside bound by an outer layer called the crystalliferous layer (Wannan & Quinne, 1990). The scar and the carpellary micropyle are two areas on the endocarp that differ anatomically from the remainder of the endocarp. The scar corresponds to the origin of the funiculus, by which the ovule is attached to the ovary, while the carpellary micropyle is generally narrower in thickness than the rest of the shell, represents the site of rupture from which the radicle protrudes during

germination. Monosclereid, brachysclereid and osteosclereid of carpellary micropyle is said to be weak because the former are shorter compared to the rest of the endocarp (Li et *al*., 1999).

I.2.4.3. Seed germination

Defining germination can be difficult because the only way to assess germination is by the lack of it (Hilhorst, 1995). Studying germination is complex from a biochemical and molecular point of view because no seed population under the same conditions has ever been reported to successfully complete the process synchronously, hence the difficulty to explain why some should germinate early, others may delay from days to weeks and yet some would not (Still et *al.*, 1997). Bewley (1997) attributed the non-synchronous germination behaviour of a seed lot to the fact that, the threshold stimulus of factors that trigger germination varies greatly among seeds even in an individual population. It has been reported that, embryo, endosperm, testa and endocarp all contribute to the delay of germination either individually or in synergy in different seeds (Jensen & Eriksen, 2001; Karam & Al-Salem, 2001). Seed priming defined as a pre-sowing strategy to influence seed germination and seedling development by modulating pre-germination metabolic activity prior to emergence of the radical is therefore necessary to optimize germination aptitude (Bradford, 1986).

I.2.4.3.1. Developmental phases of seed germination

The process of germination traditionally starts with physical water uptake known as imbibition, and terminates with root protrusion (Bewley & Black, 1994). Bewley (1997b) reported that uptake of water by a mature dry seed is triphasic (Fig. 10)**,** corresponding to the three main events that occur during seed germination. The three events include; activation, reserve mobilisation, radical protrusion.

I.2.4.3.1.1. Phase I or activation or imbibition

This phase is actually referred to as imbibition and it involves a rapid influx of water into the cells of dry seeds. According to Bewley (1997b) imbibition occurs in all the phases of germination but at varying levels (Fig. 10). In the activation phase, imbibition causes temporal structural perturbations, particularly to membranes, which lead to an immediate and rapid leakage of solutes and low molecular weight metabolites into the surrounding solution. This is symptomatic of a transition of the membrane phospholipid components from the gel phase achieved during maturation drying to the normal, hydrated liquid-crystalline state (Crowe & Crowe, 1992). The structures and enzymes necessary for this initial resumption of metabolic

activity are generally assumed to be present within the dry seed, haven survived the desiccation phase. Reintroduction of water during imbibition is sufficient for metabolic activities to resume, with turnover or replacement of components occurring over several hours as full metabolic status is achieved (Nicolas & Aldasoro, 1979).

I.2.4.3.1.2. Phase II or reserve mobilization

In Phase II there is a drastic drop in water influx experienced in phase I, hence it is also known as a lag phase of imbibitions (Fig. 10). At this plateau phase, the lipid and protein reserves accumulated during seed maturation are mobilized to fuel the rapid metabolism, and these storage reserves are mobilized first in the micropylar endosperm (Mansfield & Briarty, 1996). This implies that reserve mobilization during seed germination is essentially the reverse of reserve deposition during seed development. Meanwhile biosynthesis of new proteins essential for the support of normal cellular metabolism precedes germination (Bewley & Marcus, 1990). Major activities in phase II include speeded up of physiological activities, involving synthesis of nucleic acids and proteins, increase in enzyme and respiratory activities, initial reserve breakdown, endosperm constraint is weakened, and testa splits in this phase. This step is called "germination *sensu stricto*" (Côme & Corbineau, 1989).

I.2.4.3.1.3. Phase III or radicle growth

This phase is characterised by a further rapid increase in water uptake (Fig. 10). This is followed by embryonic axis elongation and protrusion from the covering structures, which signals the end of germination and the beginning of post-germination or seedling growth. It is important to note that dormant seeds can only undergo Phases I and II; they cannot enter Phase III until dormancy is broken before they complete germination (Hill, 1999). Dead seeds can absorb water like viable seeds, but they cannot complete germination; therefore, it will never enter Phase III. Before Phase III, cell elongation is believed to be enough for the radicle protrusion since in most cases cell division takes place after radicle emergence (Barroco et *al*., 2005). Osmotic potential, capacity of radicle cells to expand by cell wall loosening and weakening of tissues surrounding radicle cells are three possible methods that control radicle protrusion (Bewley, 1997).

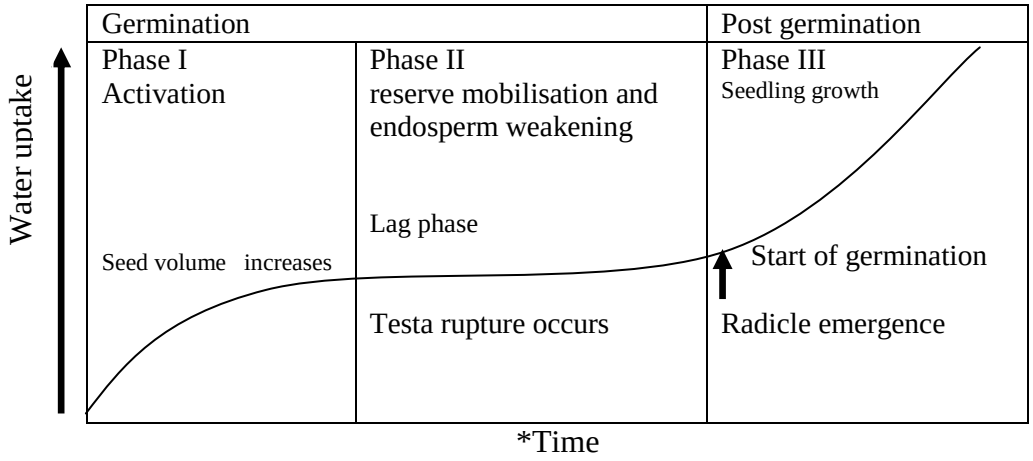

Fig.10. Variations in imbibition during the three phases of seed germination

*Time for events in different phases to be completed ranges from hours to months depending on species and environmental conditions

I.2.4.3.2. Mobilisation of reserves during seed germination

Mobilization of reserves refers to the process by which stored food materials in the endosperm are enzymatically broken down to simpler components and translocated to the embryo, as energy source for growth. The reserves mobilized depend on whether the seed is a monocots, dicots, cereals, oil seeds etc. In monocots, endosperm becomes rich in soluble carbohydrates and peptides which are then translocated to the scutellum. In the latter, lipids and glucose are transformed to sucrose and together with amides is translocated to embryonic axis for energy and new proteins that promote growth (Chandra & Tribikram, 2000).

I.2.4.3.2.1. Carbohydrate mobilization

The amylose and amylopectin in the native starch grain are first hydrolysed by α-amylase, which randomly breaks the (1- 4) glycosidic links between the glucose (Glc) residues. Starch degradation is aided by β-amylase, which cleaves off successive disaccharide maltose units (Glc-Glc) from the non-reducing end of the large oligomers released by prior amylolytic attacks. These enzymes produce Glc and maltose from amylose in addition to highly branched short chains of Glc, called limit dextrins, from amylopectin. A debranching enzyme called limit dextrinase, releases the short chains by hydrolysing the (1-6) branch points, which are further cleaved by the amylases. The plant hormone gibberellic acid is released from the scutellum and diffuses to the living cells of the aleurone layer where it promotes the synthesis of key hydrolytic enzymes like α-amylase and maltase (Yamaguchi, 2008).

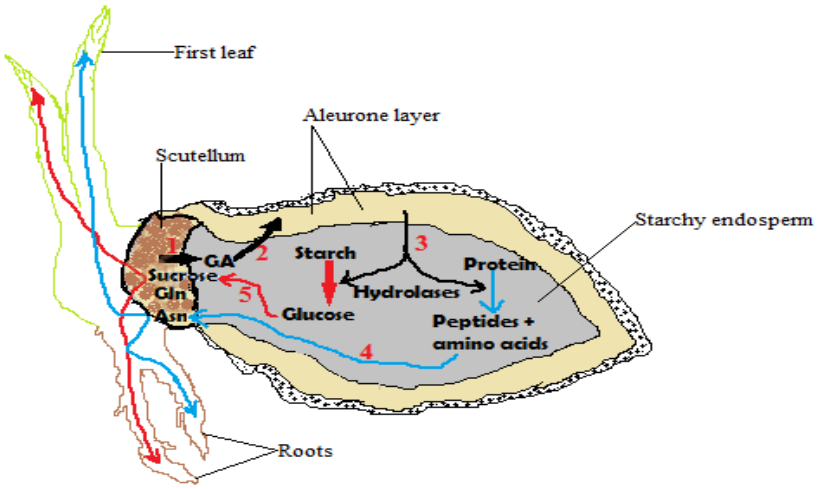

Fig. 11. Major events of carbohydrate and protein metabolism following germination

1 Embryo (scutellum) releases GA, 2. GA diffuses into the Aleurone layer 3. Aleurone layer releases hydrolytic enzymes into the endosperm, 4. Amino acids diffuse in embryo 5. Glucose diffuses into embryo (Scutellum) where sucrose is synthesized and sent to growing regions (Bewley, 2001 *modified*)

I.2.4.3.2.2. Fatty acid metabolism during germination in oil seeds

Storage oil, in the form of triacylglycerol (TAG), is synthesized during the growth of embryos of oil seeds and stored in oil bodies. TAG is then degraded to provide carbon and energy during germination and early seedling growth via the successive operation of β-oxidation, the glyoxylate cycle, partial tricarboxylic acid cycle, and gluconeogenesis (Eastmond & Graham, 2001). During germination and seedling development, TAG is broken down into fatty acids through the action of lipases. Fatty acids are then transported into specialized organelles called peroxisomes, which contain enzymes that catalyze β-oxidation to produce acetyl-CoA (Fig.12). Fatty acid β-oxidation provides carbon for sucrose synthesis in the cytosol (gluconeogenesis) and also substrates for energy production in mitochondria (Eckardt, 2005). Peroxisomal acteyl-CoA is routed into gluconeogenesis through the glyoxylate cycle (Eastmond & Graham, 2001). The five key enzymes of the glyoxylate cycle are considered to be citrate synthase (CSY), aconitase (ACO), isocitrate lyase, malate synthase, and NAD malate dehydrogenase (MDH). Three of these enzymes are located in the peroxisome, and the other two, ACO and MDH, function in the cytosol. Peroxisomal MDH is thought to operate principally in the production of malate from oxaloacetate to regenerate NAD from NADH produced during β-oxidation (Cornah & Smith, 2002). Pracharoenwattana et *al.* (2005) showed that peroxisomal CSY is required for seed germination and the mobilization of TAG and subsequent seedling growth.

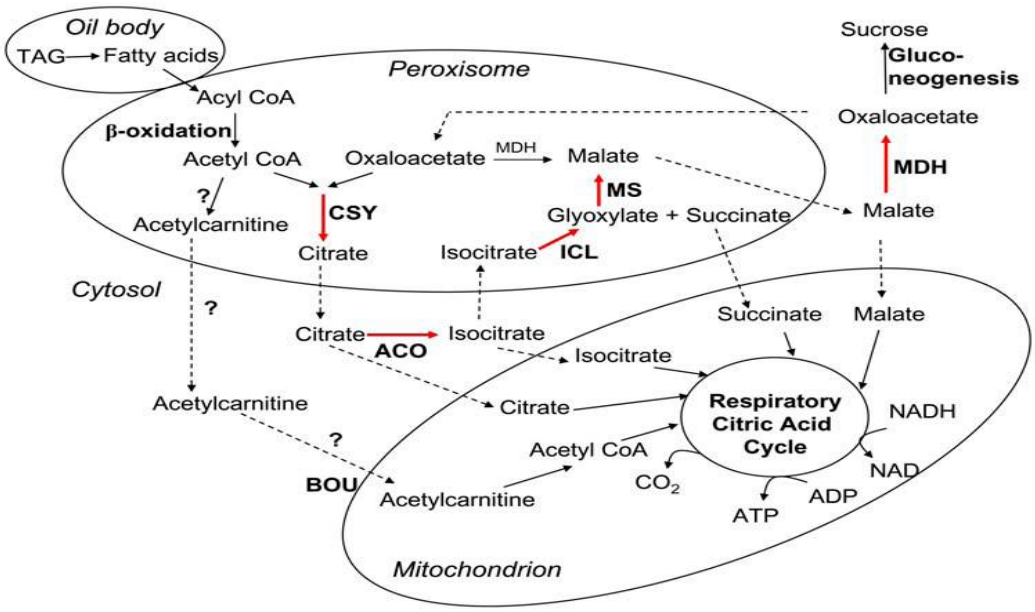

Fig. 12. Major reactions of Peroxisomes and routes during germination in oil seeds

*Glyoxylate cycle reactions are marked with red arrows. (Eckardt, 2005)

I.2.4.3.3. Parameters used in measuring germination response

Practically, it is difficult to establish a clear cut between the commencement and the end of each partial process because molecular and cellular events overlap in a multicellular and complex tissue and also due to the fact that the reactivation of the process is gradual (Labouriau, 1983a).

I.2.4.3.3.1. Measurement of germination capacity

It refers to the percentage of seeds in which the germination process reaches the end, in the experimental conditions, by means of the intra-seminal growth that result in the protrusion or emergence of one live embryo. It is generally expressed in percentage given as;

$$GP = \frac{\text{number of germinated seeds}}{\text{number of tested seeds}} x\ 100$$ (Labouriau, 1983a)

I.2.4.3.3.2. Measurement of germination time

The time for the first germination (t_o) that expresses the time of germination of the fastest seeds has been used by some authors since 1865 (Czabator, 1962; Goloff & Bazzaz 1975; Quintanilla et *al.*, 2000). According to Labouriau (1983a), when used alone, it is an excessive simplification, since this method disregards the behavior of the majority of seeds of

one sample. The use of the two extreme times, time for the first germination (t_o) and time for the last germination (t_g) was proposed in 1878 (Edwards, 1932), perhaps to correct the partiality expressed when only t_o is used, although this method is also limited, concentrating its attention only on the slower and faster seeds (Labouriau, 1983a). A more efficient method to solve this inconvenience is to use measurements of central tendency. Among them is the median, used in the context of germination since 1913 (Czabator 1962; Drake, 1993; Ranal, 1999) to study the time for 50 % of germination denoted as $t_{1/2}$ or T_{50}, defined as the number of days lapsed for half seed sample to germinate. Coolbear et *al.* (1984) proposed the following method to calculate T_{50};

$$T_{50} = t_i + \left[\{((\mathrm{N}+1)/2 - n_i)/(n_j - n_i)\} \times (t_j - t_i)\right]$$

Where;

t_i = the time before reaching 50%,

n_i = the number of seeds emerged at time t_i,

N= the final number of emerged seeds,

t_j = the time next after t_i, and

n_j = the number of seeds emerged at t_j.

However, Czabator (1962) is of the opinion that the mean and median time (T_{50}) can be used to express central tendency of the data if the frequency distribution of germination is symmetric, otherwise where the latter is asymmetric then germination time measurement should be given as mean length of incubation time (MLIT). According to the author, MLIT is a measurement of the average length of time required for maximum germination of a seed lot, and is expressed in terms of the same units of time used in making germination counts (hours or days). It was denoted as $MLIT = (G_1T_1 + G_2T_2 + ... + G_nT_n) / (G_1 + G_2 + ... + G_n)$, where G: germination count on any counting period; T: time. The variance of germination time is given as $S_t^2 = \frac{\sum_{i=1}^{k} n_i(t_i - \bar{t})^2}{(\sum_{i=1}^{k} n_i - 1)}$

Where $\bar{t}$ = mean germination time; t_i= time between the start of the experiment and the i^{th} observation (day or hour); n_i: number of seeds germinated in the time i, and k: last time of germination. The standard deviation is calculated by $S_t = \sqrt{S_t^2}$. The standard error of the mean germination time is another important measurement of variability and is adequate to measure the accuracy of the calculation of the mean germination time. Thus, $S_{\bar{t}} = \frac{S_t}{\sqrt{\sum_{i=1}^{k} n_i}}$

Where: S_t= standard deviation of the germination time;n_i: number of seeds germinated in the time *i*, and *k*: last time of germination.

I.2.4.3.3.3. Measurement of germination rate

Among the germination measurements, rate has received special attention of the researchers (Ranal & Santana, 2006). According to Labouriau (1983a), the first idea of germination rate was expressed by Kotowski (1926). The author presented the coefficient of velocity for 17 cultivated species, and rather used the expression coefficient of velocity (CVG) to rate of germination. He proposed the following formula to measure

$$CVG = 100 \quad \frac{(A_1 + A_2 + ... + A_x)}{(A_1T_1 + A_2T_2 + ... A_xT_x)}$$

Where:

A_1, A_2,... A_x is number of seedlings counted on the first day, second day, and so on until the last day (c), and

T_1, T_2,..., T_x represents the number of days between sowing and the first collection, between the sowing and the second collection, and so on until the last collection.

According to Al Mudaris (1998) the CVG gives an indication of the rapidity of germination and increases when the number of germinated seeds increases and the time required for germination decreases.

$$\text{Rate} = \frac{\text{number of normal seedlings}}{\text{Days to first count}} + ... + \frac{\text{number of normal seedlings}}{\text{Days to first count}}$$

Several indices have also been used to express germination rate, amongst which are; Germination rate index (GRI) expressed as = $[(\frac{G1}{1}) + (\frac{G2}{2}) + (\frac{Gx}{x})]$ where, G = germination on each alternate day after placement 1, 2, x = corresponding day of germination (Esechie, 1994). Corrected germination rate index (CGRI) = $(\frac{\text{GRI}}{\text{FGP}})\times 100$ where, FGP = final germination percentage (Hsu et *al*., 1985).

I.2.4.3.3.4. Measurement of germination synchrony

Generally, seed germination is asynchronized. Nonetheless, it is possible to quantify this characteristic by means of a measurement named synchronization index ($\bar{E}$), measurement of the degree of spreading of germination through time (*U*), or informational entropy (*H*), expressed by Labouriau and Valadares (1976) as $(\bar{E}) = -\sum_{i=1}^{k} f_i \log_2 f_i . f_i$ is given as $f_i =$

$\frac{n_i}{\sum_{i=1}^{k} n_i}$Where: f_i : relative frequency of germination; n_i: number of seeds germinated on the day *i*, and *k*: last day of observation. According to Primack (1980), the synchrony of germination refers to the degree of germination overlapping.The author added the synchrony of one seed (Z) with another included in the same replication of one treatment is given as

$$Z = \frac{\sum Cn_{i,}2}{N}\text{ , with } Cn_{i,}2 = n_{i,}\frac{(n_{i,}-1)}{2} \text{ and} N = Sn_{i,}\frac{(Sn_{i,}-1)}{2}$$

Where:

$Cn_{i,}2$ represents a combination of the seeds germinated in the time *i*, two together, and *ni*: number of seeds germinated in the time *i*. Then, $Z = 1$ when the germination of all seeds occur at the same time and $Z = 0$ when at least two seeds could germinate, one at each time.

I.2.4.3.4. Seed viability versus germination

Actually, literature differs with what a viability test actually measures. The term viability can be defined as ‘‘having the capacity to live, grow, germinate or develop’’. Seed technologists on the other hand define viability as the ability to germinate under a set of defined conditions (Lincoln et *al*., 1982). This actually means that a seed can be viable but will not germinate if certain conditions are not fulfilled. Viability testing involves the use of rapid biochemical assays to determine the germinability of a seed lot in the place of slower germination assays which may require more than one week (Moore, 1976). The tetrazolium assay is a colourless dye composed of 2, 3, 5 triphenyltetrazolium chloride (TTZ).

In this test, viable ungerminated seeds that are fully imbibed in TTZ absorb the colourless dye. Endogenous dehydrogenase enzyme present only in the embryo of viable and never in dead seeds converts TTZ into a stable none diffusible red chemical called triphenylformazan. Living embryo stain red while dead ones will not, endosperm staining is not considered part of the TTZ test. The pattern and intensity of staining indicate viability of embryo (William, 1994). This viability in TTZ assay has been reported to correlate with seed germination (Harty et *al*., 1972). The main limitation of TTZ is the fact that visual evaluation of degree of embryo staining may also be inconsistent.

I.2.5. Seed dormancy

I.2.5.1. Definition

Dormancy is a seed characteristic, manifesting as a block or series of blocks that prevent germination under otherwise favourable moisture, temperature and gaseous conditions. Dormancy is an adaptive life history trait to seasonally unfavourable environmental conditions

(Denohou et *al*., 2010). This inhibition of germination is caused by one or more of the following three seed-dependent factors: Chemical inhibitors, physical barriers and incomplete development of embryo prior to dispersal. In the later factor, the embryo needs extra time after dispersal to ripen (Bewley & Black, 1994; Baskin & Baskin, 1998).

According to Hilhorst (1997) and Bewley (1997a), dormancy is one of the least understood processes in seed biology. According to these authors the concept of dormancy is still to be elucidated. Partisan to this view, Rees (1981) and Bewley (1997b) reported that there is yet no consensual definition, therefore many definitions and classifications exist. A simple, straight forward definition is that of Gordon (1973): a viable seed which does not germinate though given presumably favourable conditions. Dormancy in a strict sense will refer to the inability of an intact viable seed to resume growth even when the environmental conditions are most favourable (Nikolaeva, 1977; Bewley, 1997a). A very valuable, empirical definition, which permits experimental verification of dormancy, is that of Simpson (1990): "temporary failure of a viable seed to germinate, after a specified length of time, in a particular set of environmental conditions that later evoke germination when the restrictive state has been terminated by either natural or artificial means." Considering dormancy and germination as concurrent rather than independent processes in seed development, Eira & Caldas (2000), define dormancy as a state in which the development or germination of a viable seed is blocked by one of many possible limitations located within the seed itself. Any one of a whole set of seed characteristics, morphological as well as physiological, can act, individually or in combination, at one of the many critical control points (Fig. 13) to block germination under environmental conditions.

I.2.5.2. Classification of dormancy

Owing to the fact that the notion of dormancy still needs to be elucidated, several classifications exist, but the most common are those proposed by Nicolaeva (1977), Hilhorst (1995) and that of Baskin & Baskin (2004) which is universally accepted (Silveira, 2013).

I.2.5.2.1. Classification according to Nicolaeva (1977)

One of the earliest classifications of dormancy is that proposed by Nicolaeva (1977) who based his classification on barrier factor and distinguished three main types of dormancy;

I.2.5.2.1.1. Exogenous dormancy

Dormancy is due to some features of the seed located outside the embryo. Exogenous dormancy could be due to the following; impermeability of seed coat to water owing to seed coat structure, which is hard enough to restrict the entry of moisture into the seeds. Besides, seed coat may be impermeability to gases. Thirdly, extremely hard seed/fruit structure such as seed coat, endosperm as in Acacia species can impose mechanical resistances on further development of the embryo. Finally, the testa and or embryo may produce some biochemical substances that serve as inhibitors, blocking germination of embryo.

I.2.5.2.1.2. Endogenous dormancy

The cause of this dormancy type is present within the embryo and can be as a result of; incomplete embryo development after ripening period, in such cases, germination does not occur until the embryos develop to their normal size. Common in *Palmaceae* and *Amgnoliaceae* families, inhibitors are present within the embryo.

I.2.5.2.1.3. Combined dormancy

This type is provoked by a combination of two or more exogenous and endogenous factors which act in complementary fashion.

I.2.5.2.2. Classification according to Hilhorst

Hilhorst (1995) based his classification on time when dormancy is induced and distinguished only two types of dormancy: primary and secondary dormancy.

I.2.5.2.2.1. Primary dormancy

Primary dormancy is induced during the seed maturation phase and reaches a high level in freshly harvested seeds. During subsequent dry storage of seeds, dormancy slowly reduces (Holdsworth et *al*., 2008). Primary dormancy is maintained by the accumulation of the phytohormone abscisic acid (ABA) during seed maturation to prevent precocious seed germination (Bolingue et *al*., 2010) and requires a period of after-ripening before seeds have the capacity to germinate under favourable conditions. Primary dormancy is initiated during seed development hence seeds with primary dormancy are dispersed in a dormant state from the mother plant (Bewley, 1997b). All of these factors may cause physiological variability

which is matched with differences in seed morphology (size, weight, colour *etc*.) or simply heterogeneity in degree of dormancy (Bewley & Black, 1994).

I.2.5.2.2.2. Secondary dormancy

It is the re-induction of dormancy by after-ripened non-dormant seeds in response to certain environmental conditions (Bewley, 1997b) particularly unfavourable ones (Hilhorst, 1995). Geneve (2003) indicated that secondary dormancy is induced when changing environmental conditions cause undesirable germination conditions, such as unfavourable temperature, extended light or darkness, water stress, or lack of oxygen. This type of dormancy does not only decrease with time, but it can also be re-induced in non-dormant seeds when conditions for germination like light are lacking. Secondary dormancy occurs essentially after dispersal and is basic to seasonal dormancy cycling in soil seed banks (Karssen, 1980).

I.2.5.2.3. Classification according to Baskin and Baskin

Baskin & Baskin (2004) based on an initial dormancy classification scheme proposed by Nikolaeva (1977) and put forward a more comprehensive hierarchical classification system made up of five classes of seed dormancy which are in turn sub divided into levels and types.

I.2.5.2.3.1. Physiological dormancy (PD) or class A

Physiological dormancy is present among species distributed over the entire phylogenetic tree of gymnosperms, basal angiosperms, monocots and eudicots (Baskin & Baskin 2004; Finch-Savage & Leubner-Metzger, 2006). PD is caused by low growth potential of embryo, which cannot overcome mechanical constraint of seed coat. It is the most abundant form of dormancy common in seeds of angiosperms and all major angiosperm clades. Hartmann et *al*. (1997) reported that physiological dormancy in seeds is closely related to the proportion between inhibitors ‘mainly ABA’ and growth regulators ‘especially gibberellins’. Class A has three levels: deep, intermediate and non-deep.

I.2.5.2.3.1.1. PD deep

Embryos excised from seeds with deep PD either do not grow or produce abnormal seedlings. GA treatment does not break the level of PD dormancy. 3-4 months of cold (subtype a) or warm (subtype b) stratification are needed before germination can occur. Examples of plant family of subtype a, is *Aceraceae* and *Ericaceae* for subtype b (Baskin et *al*., 2005).

I.2.5.2.3.1.2. PD intermediate

Embryos excised from such seeds produce normal seedlings. GA treatment promotes germination in some but not in all species. Seeds require 2-3 months of cold stratification. Dry storage after-ripening can shorten the cold stratification period. Example of a plant with PD intermediate is *Acer pseudoplatanus* (*Aceraceae*) (Finch-Savage et *al*., 1998).

I.2.5.2.3.1.3. PD non-deep

Embryos excised from such seeds produce normal seedlings. GA treatment can break this dormancy but can also be overcome by scarification, after-ripening in dry storage, and cold (0-10 ^{o}C) or warm (> 15 ^{o}C) stratification. The sensitivity of seeds to light and GA increases as non-deep PD is released. A great majority of seeds have non-deep PD.

I.2.5.2.3.2. Morphological dormancy (MD) or class B

Class B dormancy is evident in seeds whose embryos are under developed in terms of size but well differentiated cotyledon and hypocotyl-radical. The embryos are not physiologically dormant but simply needs time to grow before seed germinates, i.e., growth period of embryo is equal to the duration of dormancy.

I.2.5.2.3.3. Morpho-physiological dormancy (MPD) or class C

It is common in seeds whose embryos are underdeveloped in terms of size in addition to one or more physiological components that impose dormancy. These seeds therefore need a dormancy-breaking treatment that will take in to account both morphological and physiological constrains. For example a defined combination of warm and/or cold stratification which in some cases can be replaced by GA treatment.

I.2.5.2.3.4. Physical dormancy (PY) or class D

Class D dormancy is provoked by one or several layers of palisade cells in the seed or fruit coat. According to Baskin & Baskin (2001), seeds with PY possess fruit coats ‘pericarps’ or seed coats ‘testa’ that are water impermeable. The nature of the impermeability may be due to multiple layers of tightly packed, thick-walled cells in the pericarp and testa, the presence of waxes, heavy lignifications, suberin-cutin matrix, and pectins, or a combination of these factors, that hinders water uptake (Baskin et *al*., 2000). The seeds will remain dormant until

some factor(s) render the covering layer(s) permeable to water. In seed technology, mechanical or chemical scarification can break PY dormancy (Baskin, 2003).

I.2.5.2.3.5. Combinational dormancy (PY + PD) of class E

Class C dormancy is found in seeds with water impermeable seed or fruit coat (PY) synchronized with physiological embryo dormancy (PD none deep). Baskin & Baskin (2004) reported that release from PY and PD of PY+PD-dormant seeds appears to be independent events and the timely order can be species specific.

I.2.5.3. Multiple control points in dormancy induction and breaking

Simpson (1990) has explicitly made the point that a block to germination may occur at more than one point in the sequence of steps leading to visible growth. For example a dormant seed without a hard seed coat will imbibe water but remain blocked at some stage of metabolic activation or growth until other blocks are removed (Fig. 13) (Wareing, 1969). This shows that dormancy induction and maintainance can occur in more than one control point which can act either singly or in combination at a given stage of the germination depending on the plant species (Eira & Caldas, 2000).

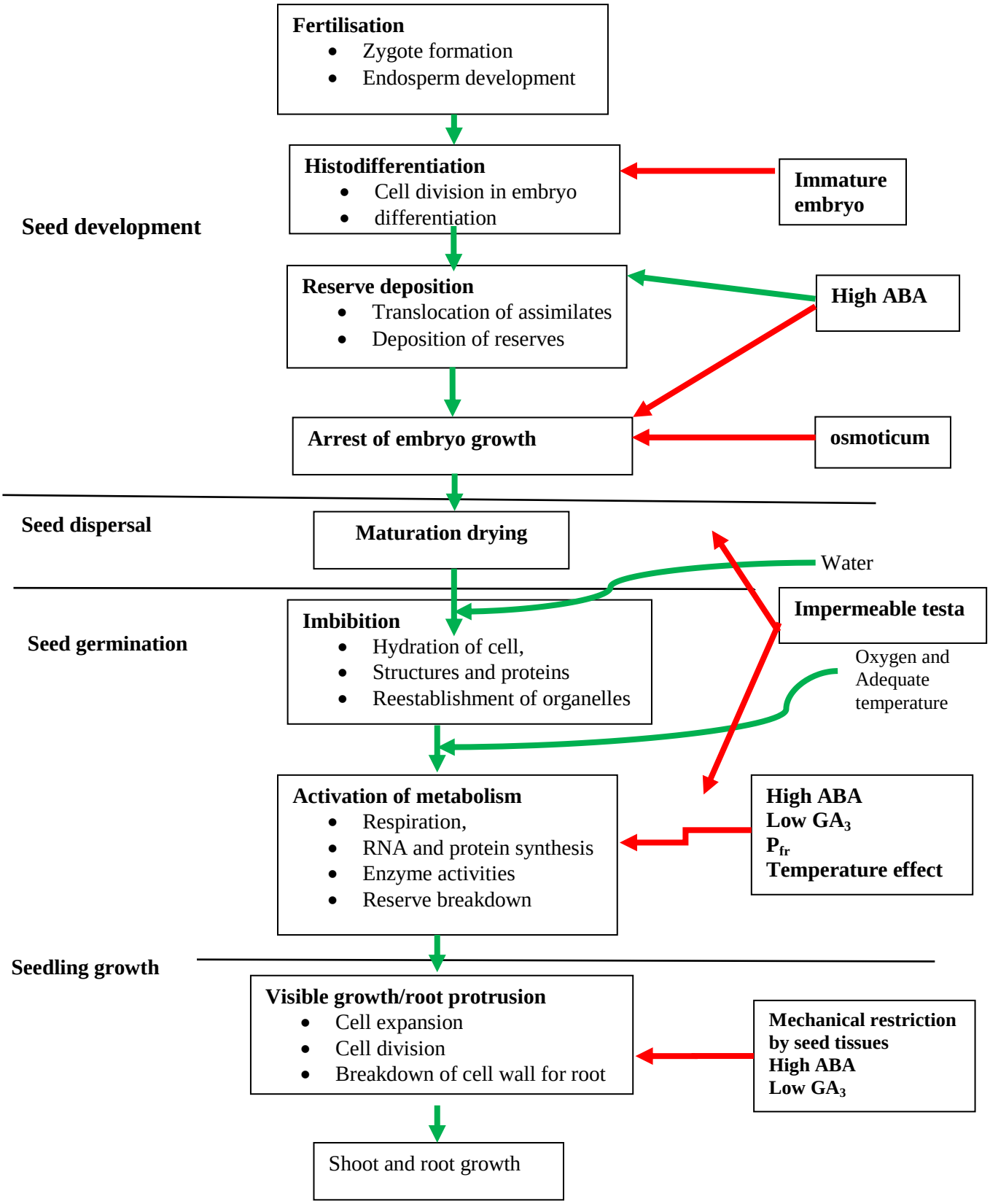

Fig. 13. Dormancy blocks during different stages in seed development and germination (Eira & Caldas, 2000).

Stimulus of a given process

Blockage of development (dormancy compelled)

I.2.5.4. Significance of dormancy

Whether a seed should remain dormant or proceed to germination under certain circumstances is important in two aspects; first with respect to its survival as a species under specific ecological conditions and secondly relating to its economic and agronomic importance.

From an ecological viewpoint, dormancy is an important survival mechanism that favours propagation and dissemination of seeds to establish plant populations. Because specific conditions are required to break dormancy, it may favour germination and seedling emergence under more favourable conditions (Hilhorst, 1995). In support of this view, Kassen et *al*. (1983) argued that seeds that require cold stratification to break dormancy for example may avoid germinating in winter. In the same way, seeds that require light for germination may avoid germinating when they are too deep in the soil or under plant shadows that would compete with the seedling, giving it little or no chance to survive.

Heterogeneous and asynchronous seed germination in the soil over years due to dormancy is an extra advantage to the survival of some species for example some weeds continue to emerge in the field even after years of rigorous weed control. This is because individual seeds in a seed population usually have different levels of dormancy, spreading their germination over time. This delay avoids unfavourable environmental events such as a drought that would eliminate the population if all the seeds germinated at one time on one hand and intra-specific competition for the available resources within the ecosystem on the other hand (Gutterman, 1980). This explains why weeds are difficult to eliminate in a field because the seed banks provide a vast array of seeds with differing levels of dormancy.

Although deep seed dormancy is considered problematic in agricultural species, some level of dormancy is desirable to prevent vivipary, a phenomenon rampant in cereals in which precocious seed germination or pre-harvest sprouting occur. Vivipary causes losses in seed quality and quantity in agricultural plants.

Regardless of all the advantages of dormancy for natural plant populations, it is an undesirable trait for most economic crops because it makes rapid and uniform germination during crop establishment difficult. Pérez (2009) reported that, although seed dormancy is widespread in the palm family and advantageous from an ecological perspective, it can frustrate palm growers and enthusiasts.

Summarily, under natural conditions, seed dormancy can be beneficial for propagation and dissemination of plant populations while in agronomic systems, dormancy is a problem for seed evaluation and seedling establishment.

I.2.5.5. Different methods of breaking dormancy in seed technology

Different types of dormancy exist and a given type of dormancy can be overcome only by identifying the cause. For example, in nature, physical dormancy is broken by changing temperatures. For horticultural and agronomic purposes it can easily be relieved by mechanical and chemical scarification, or submersion in hot water (Geneve, 2003).

I.2.5.5.1. Scarification

Scarification is a technique used to break physical dormancy which acts by impeding the perception of germination elicitors like water, light, temperature and oxygen by the embryo. Scarification techniques used commonly include; mechanical, thermal, hot water and chemical scarification. This type of dormancy has been documented in *Anacardiaceae, Arecaceae, Cornaceae, Elaeagnaceae, Empeteaceae, Juglandaceae, Meliaceae, Nyssaceae, Oleaceae, Rhamnaceae, Rosaceae and Santalaceae* plant families (Baskin et *al.*, 2002).

I.2.5.5.1.1. Mechanical scarification

This method can be accomplished, by abrading the surface of the seed until the endosperm becomes visible, or by using a knife to scrap out the hair plug at the micropylar opening. According to Cavanagh (1975), this method gives the possibilities to bring the best of germination capacity of seeds, although its application is difficult for significant quantities of seeds. Yang et *al.* (2007) reported that mechanical scarification accelerates germination and some chemical treatments significantly increased germination speed of the mechanically scarified seeds.

I.2.5.5.1.2. Thermal scarification

Thermal scarification can be by dry heat or hot water. This can be realized by subjecting seeds under a high and steady temperature for a given duration depending on the seed type. In the case of oil palm, dormancy is only broken when seeds are exposed to a constant thermal scarification temperature of 40 ^{o}C for 80 days, (Hussey, 1958).

I.2.5.5.1.3. Hot water treatment

This treatment involves soaking seeds in water at 40 to 100 °C depending on the species and seed coat thickness, for a specific period of time or until the boiling water cools to room temperature (Khasa, 1992). For example, a brief soak in 80 °C water for 10 minute

resulted to 91.26 % germination of *Acacia catechu* while soaking in 100 °C water for a period of 12 min for *Elaeocarpus floribundus* gave 84 % seed germination success rates (Das, 2014). Higher seed germination due to soaking in hot water might be due to the weakening of seed coat by distributing and dissolving the lignins and pectins present on epidermal layer of the seed coat, which render them impermeable to water and oxygen (Ameer et *al*., 2013).

I.2.5.5.1.4. Acid scarification

This method is recommended only for those seeds that are very hard to germinate, as damage to the embryo during the process can be high (Meerow, 1990). The treatment requires soaking seeds in 95 % pure (1.84 specific gravity) sulphuric acid in an acid-resistant container such as thick plastic, for various periods depending on the species, draining the acid over a screen, then washing and drying the seeds. The drained acid can be re-used. The effectiveness of the treatment can be judged by the high percentage of swollen seeds and their dull, pitted appearance (Bonner et *al*., 1974). The timing of this treatment is critical therefore the soaking period and the post soak washing have to be precisely controlled to avoid seed injury (Khasa, 1992).

I.2.5.5.2. Stratification

This is a simple, inexpensive, and effective technique for overcoming seed dormancy of temperate tree species depending upon the type of dormancy involved: warm stratification is applied for seeds that have immature embryos; cold stratification is used to break physiological dormancy; and a combined warm and cold stratification is effective for seeds that have both immature embryos and physiological dormancy (Baskin et *al*., 2000).

I.2.5.6. Key factors in the maintenance of dormancy and initiation of germination

I.2.5.6.1. Environmental factors

It is important to keep in mind when dealing with dormancy and germination that a seed is never just under the control of one factor in nature, but many factors concurrently (Bewley & Black, 1994).

I.2.5.6.1.1. Temperature

Seed germination is a complex process involving many individual reactions and phases, each of which is affected by temperature. Because temperature influences both the percentage and rate of germination of seeds, it is one of the most critical factors affecting seed

germination. Although seeds of each species have optimal temperatures for attaining maximum germination, between 30-40 °C for most palms (Addae-kagyah, 1988), most species can reach their maximum germination at an alternating temperature regime of an 8-hour day at 30 °C with light and a 16-hour night in darkness at 20 °C. In the wild, most tropical tree seeds generally require higher temperatures to germinate; for example soil temperatures above 38 °C, but below 42 °C can reduce the time required for germination of seeds of *E. guineensis* from years to weeks (Rees, 1960b, 1962).

I.2.5.6.1.2. Water

Water is a basic requirement for germination and its role as a medium for biochemical processes leading to germination, such as weakening the seed coat, activating enzymes, and breaking down food reserves, scarcely requires emphasis. Physical dormancy has been attributed to a hard or water-impermeable seed cover (Baskin et *al*., 2000), such as a fibrous mesocarp and / or a stony endocarp, which are very common among palms (Holmquist & Popenoe, 1967). It is generally recognized that seed germination is more sensitive to moisture stress than is subsequent seedling development (Mayer & Poljakoff-Mayber, 1989). While inadequate moisture in the medium will result in delayed and poor germination, excessive moisture will hinder germination due to decreased aeration (Rees, 1960b).

I.2.5.6.1.3. Oxygen

Seeds of many species will not germinate well at an oxygen level considerably lower than present in the atmosphere (Mayer & Poljakoff-Mayber, 1989). From the physiological point of view it has not been demonstrated that lack of oxygen induces dormancy (Bradbeer, 1988). Nevertheless, it has been proposed that oxygen is required to break chemical dormancy caused by substances in the endocarp that inhibit or delay seed germination (Hussey, 1958).

I.2.5.6.1.4. Light

Light is an important factor for seed germination, with both positive and negative effects. The promotional effect of light is through a single photoreaction controlled by the blue pigment phytochrome (Geneve, 2003). The phytochrome is known to exist in two photo convertible forms: Pr, which absorbs light at 660 nm; and Pfr, the far red light which absorbs light at 730 nm. Seeds of many temperate tree species are known to be light sensitive, and their germination is promoted by red light and inhibited by far red light (Toole, 1973). Germination is triggered by increases in light as well as by the ratio of red to far-red light and temperature

(Denslow, 1987). However, while light has been reported to be a germination inhibitor i.e., show negative photoblastism in some Palmae as it's the case of *Sabal palmetto* (Brown, 1976); others are indifferent to light for their germination. Germination of seeds covered by soil suggests that most seed palm species are indifferent to light (Orozco-Segovia et *al*., 2003).

I.2.5.6.1.5. Soil factor

Edaphic factors like soil structure, texture, humidity, pH and temperature among others influence seed germination.

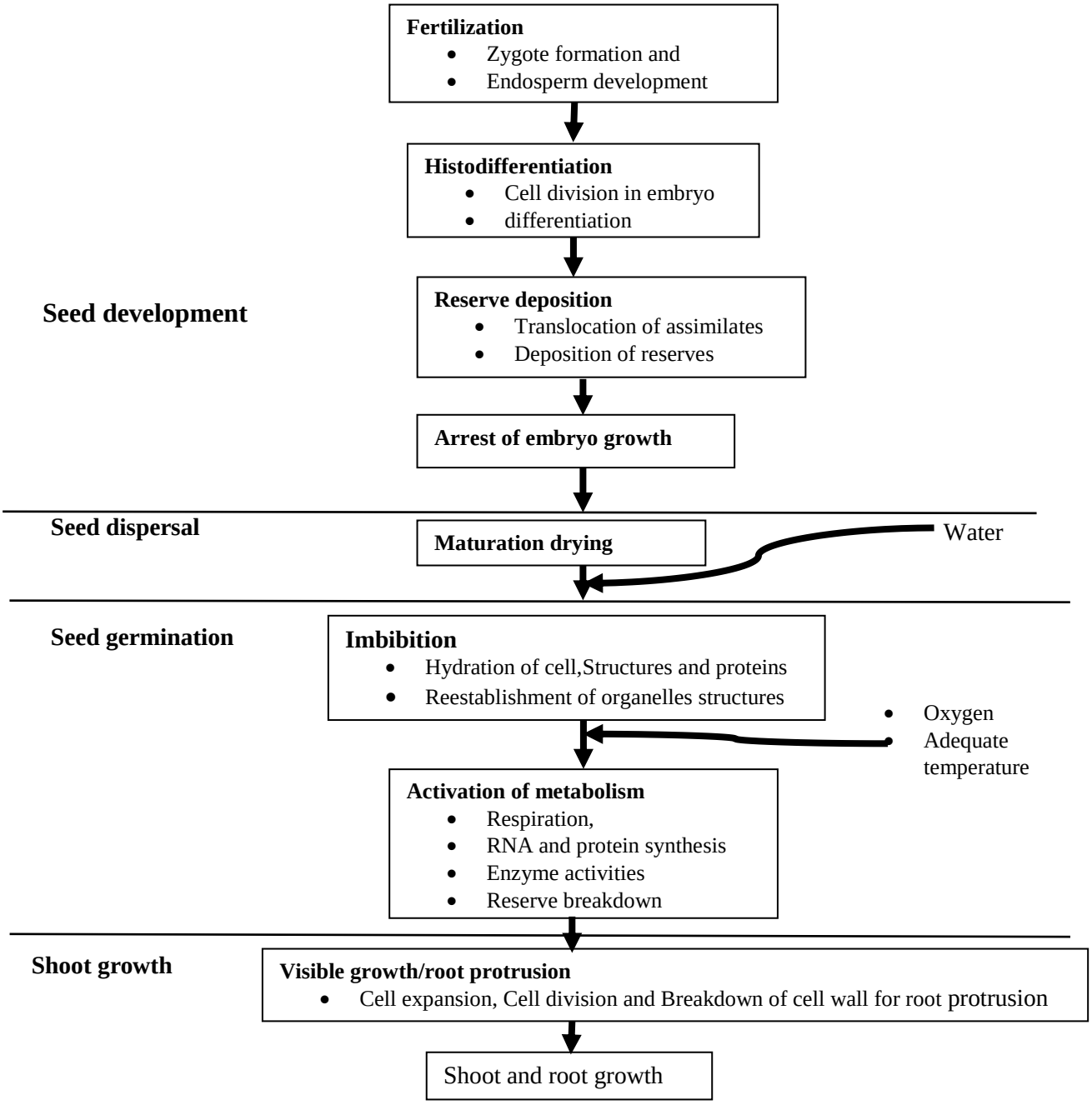

Fig. 14. Stages of seed development and germination with some associated processes

I.2.5.6.2. Internal factors

I.2.5.6.2.1. Seed maturity

Seed maturation is the second phase of seed development after embryogenesis during which food reserves accumulate and dormancy and desiccation tolerance develops (Goldberg et *al*., 1994). ABA content of seed commonly increases during development; and declines during late maturation. For seeds developing within fleshy fruits, ABA content + low water potential of fruit tissues both prevent precocious germination. Hence immature seeds will not germinate because of high ABA content (Bewley & Black, 1994). The size of seeds, may influence germination timing and success (Castro, 2006), and within species, seed mass is often associated with probability or time of germination (Hendrix, 1984).

I.2.5.6.2.2. Plant growth regulator

ABA, GA, ethylene, brassinosteroids, auxins, and cytokinins have a tremendous effect on plant development, even at low concentrations (Kucerna et *al*., 2005). Among all plant growth regulators, ABA and GA have a considerable influence on seed maturation, dormancy, and germination and act antagonistically (Finch-Savage & Leubner-Metzger, 2006).

I.2.5.6.2.3. Regulation of dormancy / germination by GA and ABA

The two main plant growth regulators that control dormancy-germination cycling are ABA and GA_3. Cadman et *al*. (2006) proposed a model for the regulation of dormancy by ABA and GA in response to the environment. According to this model, ambient environmental factors like temperature affect the ABA / GA balance and sensitivity of embryo to these hormones. ABA synthesis and signalling (GA catabolism) dominates the dormant state whereas GA signalling (ABA catabolism) dominates the transition to germination (Fig. 15). The complex interplay between hormone synthesis, degradation and sensitivity in response to ambient environmental conditions can result in dormancy cycling. GA / ABA ratio during seed development determines, to a large extent, whether seed dormancy and maturation can be induced and maintained. In addition, GA / ABA ratio is also of great importance in the control of seed germination. Accumulation of GA is accompanied with reduction of ABA during seed imbibition, suggesting that GA and ABA play antagonistic roles in germination process (Nambara & Marion-Poll, 2005). GA counteracts the effect of ABA by promoting the embryo growth potential and the weakening of tissues covering the embryo (Bentsink & Koornneef, 2008).

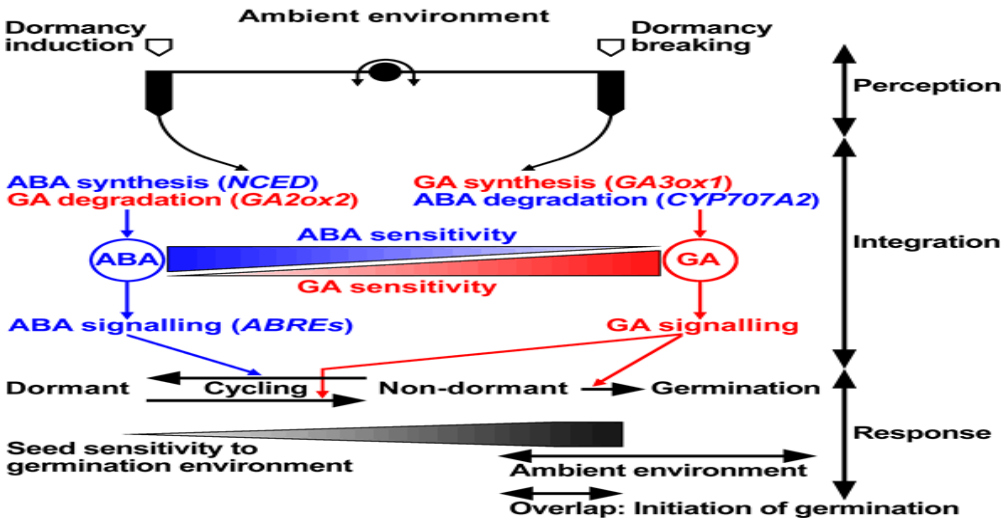

Fig.15. Regulation of dormancy and germination by ABA / GA in response to the environment (Cadman et *al*., 2006)

*Target genes are in parenthesis

I.2.6. Generalities on regeneration of plants by *in vitro* culture

Plant tissue culture also referred to as, *in vitro* or axenic is the aseptic culture of cells, tissues, organs and their components under defined physical and chemical conditions *in vitro*. The notion of tissue culture had its foundation from the initial conception of Scheilden and Schwann in the 1830s that the cell is capable of autonomy and it is totipotent (Gautheret, 1983). In practice, 5 basic areas can be distinguished in tissue culture; callus culture, cell suspension protoplast culture, anthers culture and organ or meristem culture (Neunamm et *al*., 2009).

I.2.6.1. Factors affecting the success of tissue culture

The empirical approach has shown three main factors, namely; explants choice, media composition and control of physical environment, responsible for a successful tissue culture (Molnar et *al*., 2011).

I.2.6.1.1. Explants

The explants is any plant part isolated from an intact plant body put in culture to regenerate the whole plant. It can be part of leaf, root, stem, flower, pollen, embryo etc. Youmbi et *al*. (1998) found that the physiological state of the explants influences its response to attempt to initiate tissue culture. The choice of a an explants is usually to solve a given problem for example, the pollen grain is used to regenerate haploid male plants, zygotic embryo culture is a useful tool to bypassing seed dormancy (Tabi et *al*., 2016), rescue F1 hybrid of a wide cross and inducing a faster growth in a breeding program (Das et *al*., 1999).

I.2.6.1.2. Culture media (CM)

A culture medium is a complex growth substrate on which the explants is grown. Basic media that are frequently used include those developed by Murashige and Skoog (1962) or MS medium, Linsmaier & Skoog (1965) or LS medium, Gamborg (1968) or B5 medium and Nitsch & Nitsch (1969) or NN medium.

I.2.6.1.3. Media composition

Plant tissue culture media should generally contain some or all of the following components: macronutrients, micronutrients, vitamins, amino acids or nitrogen supplements, source(s) of carbon, undefined organic supplements, growth regulators and solidifying agents. The optimum concentration of each essential nutrient for achieving maximum growth rates in a CM is species specific (Molnar et *al*., 2011).

I.2.6.1.3.1. Macronutrients and micronutrients

Any element, whose concentration is greater than 0.5mM l^{-1} is a macroelement (De Fossard, 1976). Besides C, H and O, there are six essential macroelements in plant cell media, namely: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S) for satisfactory growth and morphogenesis. Culture media should contain at least 25-60 mM of inorganic nitrogen for satisfactory cell growth. Most media contain K in the form of nitrate chloride salts at concentrations ranging from 20-30 mM (George, 2008).

Microelements are elements in concentrations lesser than 0.5m M L^{-1}(De Fossard, 1976). The essential micronutrients for plant cell and tissue growth include iron (Fe), manganese (Mn), zinc (Zn), boron (B), copper (Cu) and molybdenum (Mo) (Epstein, 1965). Iron is usually the most critical of all the micronutrients. Ethylene diaminetetraacetic acid (EDTA)-iron chelate (FeEDTA) is the best ion type because unlike other iron sources, it does not form a precipitate after media preparation (Murashige & Skoog, 1962).

I.2.6.1.3.2. Carbon source

Sucrose is frequently used as carbon source at a concentration of 2-5 %. However, other carbon sources like lactose, galactose, maltose and starch are used but were found to be less effective than either sucrose or glucose. The latter was similarly more effective than fructose considering that glucose is utilized by the cells in the beginning, followed by fructose. Sucrose was reported to act as morphogenetic trigger in the formation of auxiliary buds and branching of adventitious roots (Vinterhalter & Vinterhalter, 1997).

I.2.6.1.3.3. Vitamins

Vitamins play an essential role in stimulating cell growth (Gamborg et *al*., 1968); increases callus weight, embryo number and embryo length (Al-khayri, 2001); stimulate rooting (Trindade & Pais, 1997). They may act as limiting factors for cell growth and differentiation when plant cells and tissues are grown *in vitro.* Thiamine (B1), nicotinic acid, and pyridoxine (B6) are the most commonly used. Myo-inositol classification either as a water-soluble plant vitamin or as a sugar alcohol remains controversial (George, 2008).

I.2.6.1.3.4. Amino acids

The exogenous addition of certain amino acids or amino acid mixtures is particularly important for establishing cultures of cells and protoplasts in certain species (Benson, 2000). Amino acids provide plant cells with a source of nitrogen that is easily assimilated by tissues and cells faster than inorganic nitrogen sources (Rao et *al*., 1995) due to the high mobility of organic nitrogen than inorganic nitrogen (Kim & Moon, 2007). Amino acid mixtures such as casein hydrolysate, L-glutamine, L-asparagine and adenine are frequently used as sources of organic nitrogen in culture media (Asad et *al*., 2009).

I.2.6.1.3.5. Activated charcoal

The use of activated charcoal (AC) can make a major difference in the success or failure of a given tissue culture attempt (Pan & van Staden, 1998). Globally, AC has been used in plant tissue culture media to improve and / or promote morphogenesis in a wide variety of species (Fridborg et *al*., 1978; Teixeira et *al*., 1993; Madhusudhanan & Rahiman, 2000; Alves et *al*., 2011). According to Cattelan et *al*. (2007), perennial plants such as oil palm are rich in substances of secondary metabolism origin, like polyphenolic compounds. The presence of such phenolic substances in *in vitro* culture causes tissues to suffer oxidation due to polyphenolic oxidase enzyme. Teixeira et *al*. (1993) found that, AC can adsorb phenolic compounds and their products of oxidation, such as quinones, avoiding the oxidation process.

I.2.6.1.3.6. Solidifying agent

Gelling or solidifying agents are commonly used in preparing semi-solid or solid tissue culture media. Hardness of the culture medium greatly influences the growth of cultured tissues. There are a number of gelling agents such as agar, agarose and gellan gum. Semi-solid media provide a fixed anchorage that determines polarity and vertical growth for plant tissue

(Dunwell, 1986). Agar has several advantages over other gelling agents; mixes with water, easily melts in a temperature range 60-100 ^{o}C, solidifies at approximately 45 ^{o}C and forms a gel that is stable at all feasible incubation temperatures (Miller & Murashige, 1976).

I.2.6.1.3.7. Growth regulators

Auxins, GA and cytokinins are the three main growth regulators in tissue culture. Each has a specific role to play. In tissue cultures, auxins are usually used to stimulate callus production and cell growth, initiate shoots and rooting, induce somatic embryogenesis, stimulate growth from shoot apices and shoot stem culture (Gamborg et *al*., 1976). In culture media, Cytokinins stimulate cell division, induce shoot formation and axillary shoot proliferation and retard root formation. The different Cytokinins vary in action for example BAP is more effective on shoot induction whereas Kinetin was more effective on shoot elongation (Mehdi et *al*., 2014). These compounds enhance growth of callus and help elongation of dwarf plantlets (Anagnostakis, 1974).

I.2.6.2. Tissue culture in the oil palm

The tissue culture technique for oil palm was developed in the 1970s (Jones, 1974; Rabechault & Martin, 1976) to increase availability of elite oil palm plantlets. Since then many laboratories embraced this method to clonally propagate elite oil palm by means of somatic embryogenesis on calli derived from various origins (Rival et *al*., 1999). Whole plants successfully regenerated in the 70s from various explants were planted in hundreds of hectares. Unfortunately some of the clones were not flowering normally, instead had a high incidence of flowers with ‘mantled’ character (conversion of stamen whorl into a carpel whorl) (Corley et *al*., 1986). Successful cloning of oil palm from various explants has been reported from young leaves (Te-chato, 1998b; Te-chato & Muangkaewngam, 1992), roots (Smith & Thomas, 1973), shoots (Starisky, 1970) and zygotic embryos (Rabechault & Cas, 1974; Te-chato, 1998a).

Vegetative propagation of the oil palm by cloning has significant advantages over traditional breeding. It enables rapid multiplication of uniform planting materials with the desired attributes. It also offers a new opportunity in oil palm breeding. By obtaining haploid palms from pollen/anther culture, the process of hybridization to produce homozygous diploids is considerably reduced (Sogeke, 1998). Limiting the time of the ‘embryoid’ culture phase and avoidance of ‘fast-growing’ callus have been suggested to solve the notorious constraint of inflorescence mantling of mature oil palms clones (Eeuwens et *al.*, 2002; Soh et *al.*, 2001).

CHAPTER II. MATERIALS AND METHODS

II.1. Materials

II.1.1. Study sites

One part of this study was carried out at the improved oil palm seed production unit of the Institute of Agricultural Research for Development, Specialized Centre for Oil Palm Research (IRAD-CEREPAH) of La Dibamba, located at 3.948848°N, 9.762726°E and 55m above sea level in the Sanaga Maritime Division, Littoral Region of Cameroon. The other part took place at the African Centre for Research in Banana and Plantain (CARBAP) Njombe found in the Mongo Division of the Littoral Region of Cameroon.

II.1.2. Plant materials and plant growth promoting chemicals

The biological material used in this study (Table II) constituted of the ten cultivars (Cv1-10) of commercial hybrid tenera-type *E. guineensis* seeds obtained through control crosses of D x P parent palms of CEREPAH breeding program.

Table II. Plant materials and plant growth promoting chemicals used

Tenera cultivars	Plant growth promoting chemicals
1. C1001II	90 % pure GA_3 (Scharlab S.L. Spain)
2. C1501II	
3. C1901II	
4. C2001II	30 % w/w (110) extra pure H_2O_2 (Sigma-Aldrich Germany)
5. C2101II	
6. C2301II	
7. C2501II	50 % wt CH_2N_2 (Sigma-Aldrich Germany)
8. C1001F	
9. C2301IIF	
10. C2501FX	
Nine open pollinated FFBs from different plants of block A99	

II.2. Methods

II.2.1. Determination of causes of low germination and germination variation among Cv

II.2.1.1. Determination of fators accounting for germination variation between cultivars

II.2.1.1.1. Seed preparation

Six months after controlled pollination, FFB were harvested, striped, allowed to ferment for 5 days and depulped using a mechanical depulper. Parthenocarpic and cracked seeds were eliminated by hand picking. Seeds were then treated with 2.5 g / L contact fungicide solution (penncozeb) for 2-5 min. Samples were then air dried under shade and once seeds got rid of all free water, they were stored in well labelled drawers in an air conditioned room at 20-22 °C and 60 % relative humidity. All seeds used in this rubric had been under the storage conditions for at least 3 months. Biometric parameters like average shell thickness and weight of the seed, beside quantitative analysis of Palm kernel oil, were assessed to attempt an answer to differences in germination capacity between the oil palm seed cultivars.

II.2.1.1.2. Assessment of biometric parameters

Twenty-five seeds randomly chosen from each of the ten cultivars obtained from assisted pollination were used. For each Cv, a Vernier callipers reading to 0.05 mm was used to measure the length and diameter of 25 seeds for each seed cultivar. The weight of the twenty five randomly selected seeds from each Cv was taken using an electronic balance. To assess the average shell thickness each of the 25 seeds / Cv was wrapped in a kaki material and upon one or two knocks with the aid of a small hammer, the shell was separated from the kernel. The folding of the seed on a kaki material prior to the knock reduced the impact of the knock thereby avoiding shattering of the nut. In fact with this technique, the shell was separated into mostly 2-3 pieces. The calliper was used to measure the thickness of the shell at the micropylar end given that the germ pores are the site for imbibition. Each time the shell was separated from the endosperm for the 25 repetitions / Cv; the resulting number of endosperms or kernels was noted.

II.2.1.1.3. Quantitative analysis of Palm kernel oil (PKO) in the ten cultivars

Endosperms of 25 ungerminated seeds / Cv with their embryos initially removed were crushed in a traditional mortar and pestle. Using an electronic balance (RADWAG, AS 220/X), a sample kernel cake of 5 g / Cv was weighed, carefully parcelled in porcelain paper and dried

in an oven at a temperature of 40 ^{o}C. The DW of each of the ten parcels was recorded at intervals of 3 hours until there was no further change. Using the Soxhlet simple distillation extractor, palm kernel oil was extracted from the cake for 4 hours using hexane as solvent. PKO in 5 g of cake of each category was determined by subtracting the DW of the cake after extraction from its DW before extraction.

II.2.1.2. Determination of factors accountable for general low annual germination

II.2.1.2.1. Position dependent effect of oil palm seeds on germination

All seeds used here were collected from 9 open pollinated bunches in block A99 of CEREPAH's seed garden. The criteria of FFB readiness for harvesting was 2-5 loosed fruits.

II.2.1.2.1.1. Bunch analysis

Bunch analysis is a process used by oil palm breeders to estimate FF and oil components in the oil palm FFB. The method used for bunch analysis was a modification of that proposed by Blaak et *al*. (1963). The whole FFB was weighed on the Beckel's scale, after which it was pruned using a small axe. For each FFB pruned, the seeds were manually separated from the spikelets. Parthenocarpic fruits were eliminated and mature seeds were separated into two lots, lot 1 made up of seeds that were deep red in colour 'usually positioned at the apex of spikelets' and lot 2 seeds that were orange in colour 'usually positioned at the base of spikelets' (Fig. 16). The seeds of each lot were counted and their weight taken. 60 seeds were randomly taken from each lot to constitute the sample for germination test. Mesocarp of selected seeds was depulped manually using a well sharpened knife. The weight of depulped seeds was measured using an electronic balance, while the length and diameter of 20 randomly selected seeds for each lot were taken using a digital Vienna calliper (150mm digital callipers, Nankai Japan technology). The callipers had specificities like measuring range of 150 mm/6″, maximum measurement speed of 1.5 m / sec and an accuracy of ± 0.02 mm ± 0.001″. Physical analysis was carried out immediately because if allowed to ferment, colour based separation would not have been possible given that orange seeds gradually get a deep red colour over time. The seeds were treated with 20 % Pencozeb solution and introduced into the DHT circuit.

II.2.1.2.1.2. Dry heat scarification (DHT)

The seeds were then put in double layer plastic bags and treated under dry heat at 40 ^{o}C. After 80 days, the dry heat scarified seeds were submerged in water for 5 days. This was

followed by treatment with fungicide as described above and finally the seeds were put in polythene bags and kept for germination at room temperature.

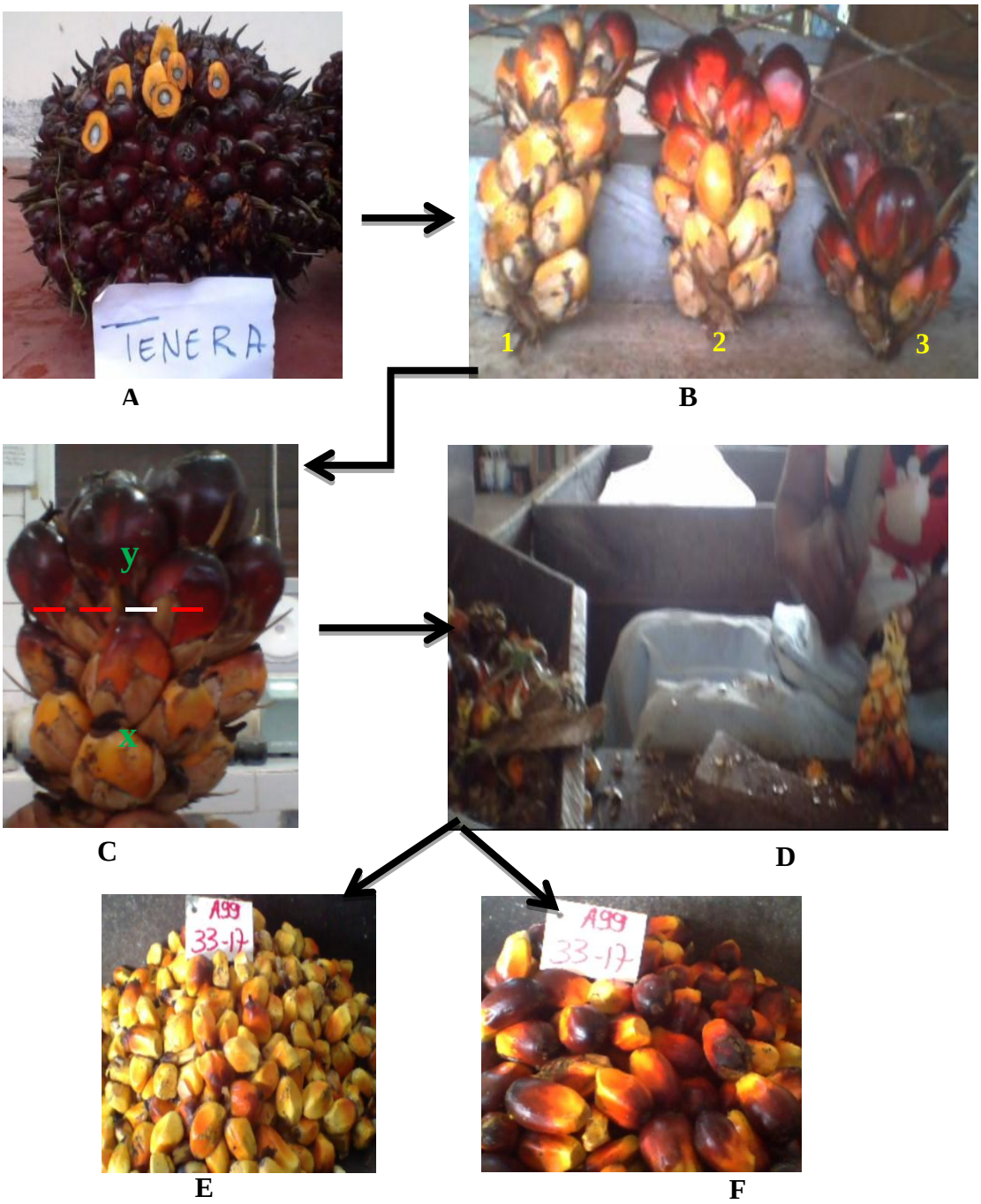

Fig. 16. Pictographic layout of FFB preparation for position based analysis

A. Tenera type FFB, B. types of spikelets: 1. Basal, 2. Central, 3. Apex, C. spikelet showing fruits position and colour, x. Orange coloured fruits at the base of spikelet, y. Red coloured fruits positioned at the apex of the spikelet, D. manual separation of fruits from spikelet, E. Lot of basal fresh fruits, F. Lot of apical fresh fruits

II.2.1.2.2. Harvesting date and germination capacity

Eight FFB constituted the sample size for this aspect of study. Four were harvested after six months following controlled pollination, while the remaining 4 were harvested 30 days later. As a precaution, the jute bags were maintained on the pollinated bunches throughout the defined harvesting date so as to prevent any FF loss. Once the defined date per sample elapsed, FFB were harvested, with the jute bag still intact. The FW was measured using the Beckel scale and the number of abscised fruits / FFB recorded. FFBs were then pruned, and kept in special boxes to facilitate fermentation. After 7 days, depulping was carried out using a mechanical depulper. Hand picking was used to eliminate cracked and immature seeds. The seeds were then treated with 20 % Penncozeb, air dried on jute bags under shade and preserved in an air conditioned room of 20-22 °C and 60 % humidity. After 30 days, the seeds were introduced into the conventional DHT circuit. Cumulative germination capacity was noted after 8 weeks.

II.2.2. Optimisation of the conventional DHT by incorporating GPC

The methodology applied in breaking oil palm seed dormancy by DHT in this study was a synchronised modification of different stages of oil palm seeds treatment to initiate germination proposed by Ree (1962); Addae-Kagyah et *al*. (1988); Corrado & Wuidard (1990). The sample size of this experiment was 4000 seeds initially depericarped, treated with fungicides and conserved in the storage room for at least three months.

II.2.2.1. Soaking

From each Cv, 400 seeds collected randomly constituted the sample size. The seeds were weighed, put in bags permeable to water, tied firmly and soaked in a water bath filled with tap water for 7 days at ambient temperature. The water was renewed daily to avoid growth of mould. Prior to daily re-immersion in renewed water, the seeds were weighed in order to appraise variation in rate of imbibition. The seeds were then treated with 20% Penncozeb for 3-5 min to prevent fungal growth and air dried under shade.

II.2.2.2. Heating of seeds

The seeds were then put in strong transparent polyethylene bags and positioned on wooden shelves in a heating room at a constant dry heat temperature of 40 ^{o}C. The heating room was air tight and furnished with temperature adjustable heaters to provide heat and

ceiling fans to ensure the equal and full distribution of hot air in all parts of the room. After 80 days, samples from the heating room were further subjected to the following sub treatments;

II.2.2.3. Treatments

II.2.2.3.1. Control

Sub sample of 20 seeds / cultivar were selected randomly and re-soaked in water for 5 days in order to raise the MC to 22 %. This was followed by soaking for 3-5 min in a solution of penncozeb prepared by dissolving 5 g of the fungicide in 10 L of water; with the aim to prevent the growth of mould fungus on the seeds. Samples were then air dried under shade until they got rid of all free water. They were again put transparent polyethylene bags and kept on wooden shelves under ambient temperature pending germination. Meantime, seeds were misted with water on weekly bases. Germination parameters were accessed fortnightly over a period of 8 weeks.

II.2.2.3.2. Preparation of GPC (GA_3, H_2O_2 and CH_2N_2) stock solutions

For GA_3**,** three stock solutions of different concentrations 500, 1000 and 1500 mg. L^{-1} were prepared by dissolving the respective masses of 90 % pure GA_3 powder in a litre of pipe borne water. Given that GA_3 dissolution is very slow at ambient temperature, the solutions were prepared 5 hours before application time and were agitated regularly to enhance a homogenous solution. For H_2O_2, three different concentrations (0.5, 1.0 and 1.5 %) were prepared from the mother solution. As per hydrogen cyanamide (CH_2N_2), three stock solutions with different concentrations of 1.0, 1.5 and 2.0 % were prepared from the 50 % commercial formula.

II.2.2.3.2.1. DHT+ GA_3

A randomly selected sample of 20 seeds / Cv was made, weight taken and finally were re-soaked in 200 ml of the respective GA_3 stock solutions for 24 hours and then in water to raise the MC to 22 %. The water was renewed daily for 3 days just after the weight was taken. The experimental designed was random with a 20 x 10 x 3 x 2 factorial, including 10 seed Cv, 20 seeds / Cv, three GA_3 concentrations (500, 1000 and 1500 mg/l) repeated 2 times.

II.2.2.3.2.2. DHT+ H_2O_2

A sub sample of 20 seeds / Cv were removed and re-soaked in the corresponding concentrations of 0.1, 1.0 and 1.5 % of H_2O_2 for 24 h and then in water renewed daily for 3

days with successive measurement of MC until 22 %. The same experiment was carried out except for the fact that the duration of soaking was 48 hours. The treatments with H_2O_2 could easily be distinguished by the presence of air bobbles adhered on the seeds. The intensity of the bobbles varied with concentration of H_2O_2 in the treatments (Fig. 17). The bobbles disappeared over time and by the 24th hour they were completely absent in some. The experimental designed was random with a 20 x 10 x 3 x 2 factorial, including 10 seed cultivars, 20 seeds / cultivar, and 3 H_2O_2 concentrations (0.5, 1.0 and 1.5 %) replicated 2 times.

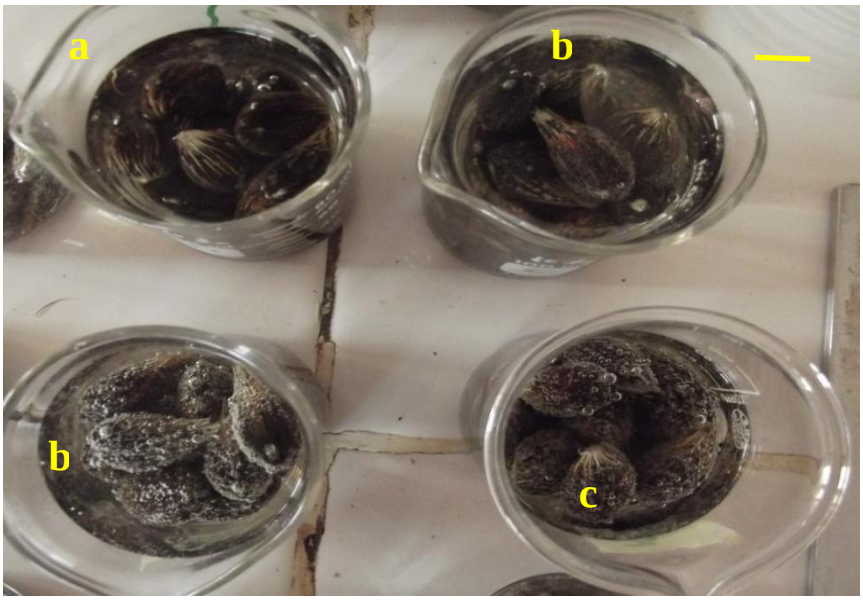

Fig. 17. Treatment of seeds with H_2O_2

a. Control, b. 0.5%, c. 1% and d. 1.5 %

Bar: 1cm

II.2.2.3.2.3. DHT+ CH_2N_2

A sub sample of 20 seeds / cultivar was removed and re-soaked in the respective concentrations of 1.0, 1.5 and 2.0 % CH_2N_2 for 24 h and then in water renewed daily for 3 days. The experimental designed was random with a 20 x 10 x 3 x 2 factorial, including 10 seed cultivars, 20 seeds / cultivar, 3 concentrations (1.0, 1.5 and 2.0 %) of CH_2N_2 and 2 replications.

After each treatment, samples were then air dried under shade and after they got rid of surface water, they were again put in strong transparent polyethylene bags and kept on wooden shelves under ambient temperature pending germination. Meantime, seeds in the plastics were misted once a week. Germination capacity (%) was assessed fortnightly for 8 weeks.

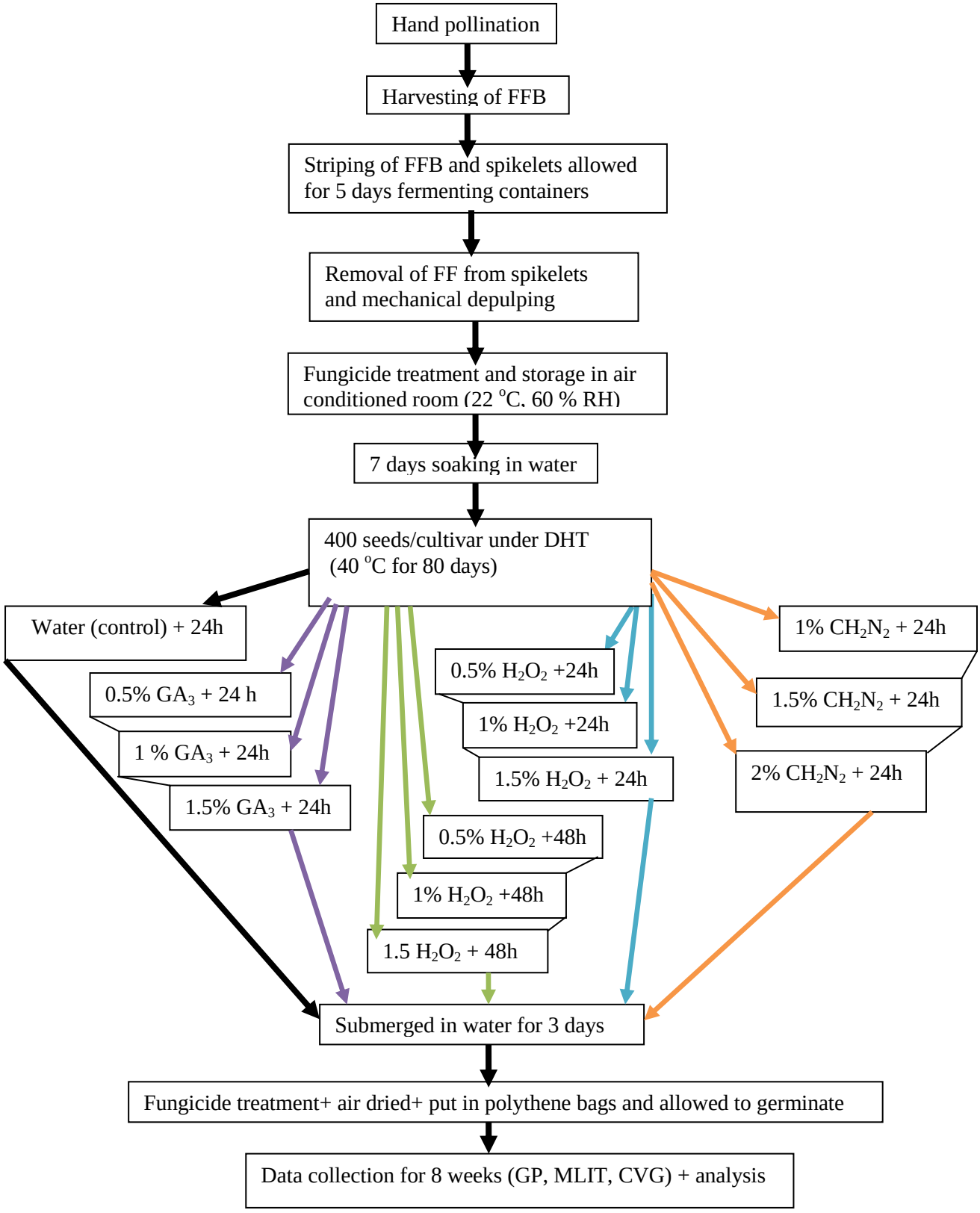

Fig. 18. Schematic layout of methodology for incorporation of some GPC to DHT (Ree, 1962; Addae-Kagyah et *al*., 1988; Corrado & Wuidard, 1990 *modified*)

Contro, DHT scarification + H_2O_2 (24h), DHT scarication+H_2O_2 (48 hrs),
DHT scarification + CH_2N_2, DHT scarification +Ga_3,

II.2.3. Potentials of acid and hot water scarifications as alternatives to conventional DHT

II.2.3.1. Effect of Acid scarification (AS)

The sample size was 420 seeds / Cv. The methodology adopted here is a modification of approaches put up by Herrera et *al.* (1998), Dewir et *al*. (2011) and Médjati et *al*. (2013). Three rubber bowls were labelled with 50 %, 70 % 95 % corresponding to three acid concentrations. The H_2SO_4 was diluted according to the respective labels and pour in their respective bowls. 420 randomly selected seeds / cultivar were immersed in the different acid concentrations for 10 min in one experiment and 20 min in another. At the end of each immersion time, the acid was decanted and the seeds rinsed 5 times with running tap water, air dried under shade and treated as follows.

II.2.3.1.1. Control

A sub sample of 20 seeds / Cv / acid concentration / immersion duration was soaked in water. Daily moisture content (MC) of the samples was obtained by subtracting the formal FW from the new FW just before water was renewed. Once a MC of 22 % was attained, the seed were removed in water and air dried.

II.2.3.1.2. Effect of Acid scarification (AS) supplemented with GPC

II.2. 3.1.2.1. AS + GA_3

A random sample of 20 seeds / Cv / acid concentration / incubation time was submerged in three distinct solutions of GA_3 diluted to the following concentrations 500, 1000 and 1500 mgL^{-1} for 24 h. Treated seeds were later incubated in water until MC of 22 % .

II.2. 3.1.2.2. AS + H_2O_2

A sub sample of 20 seeds / Cv / acid concentration / incubation time was soaked in beakers containing solution of H_2O_2 at concentrations of 0.5, 1.0 and 1.5 % for 24 hours and then in water until MC of 22 % was attained.

II.2.3.1.2.3. AS + CH_2N_2

A random sample of 20 seeds / Cv / acid concentration / duration of soaking were immersed in solutions of 1.0, 1.5 and 2.0 % CH_2N_2 for 24 hours and then in tap wateruntil MC of 22 %. Raising the MC in all treatments with GPC involved 24 hours submergence in water, until a MC 22 % reached.

At the end of each treatment above, the seeds were then spread over jute bags under shade to air dry. They were then put in strong transparent polyethylene bags and kept on wooden shelves under ambient temperature pending germination. Meantime, films of water were sprayed in the plastics on weekly bases to prevent the seeds from getting dry. Germination rate was assessed at two weeks interval basis for 8 weeks and the germination capacity recorded. The experimental designed was random and made up of a 10 x 20 x 3 x 2 x 4x 3 factorial including 10 seed cultivars, 20 seeds / Cv, 3 acid concentrations, 2 acid incubation durations, 4 growth enhancing treatments and 3 repetitions per treatment.

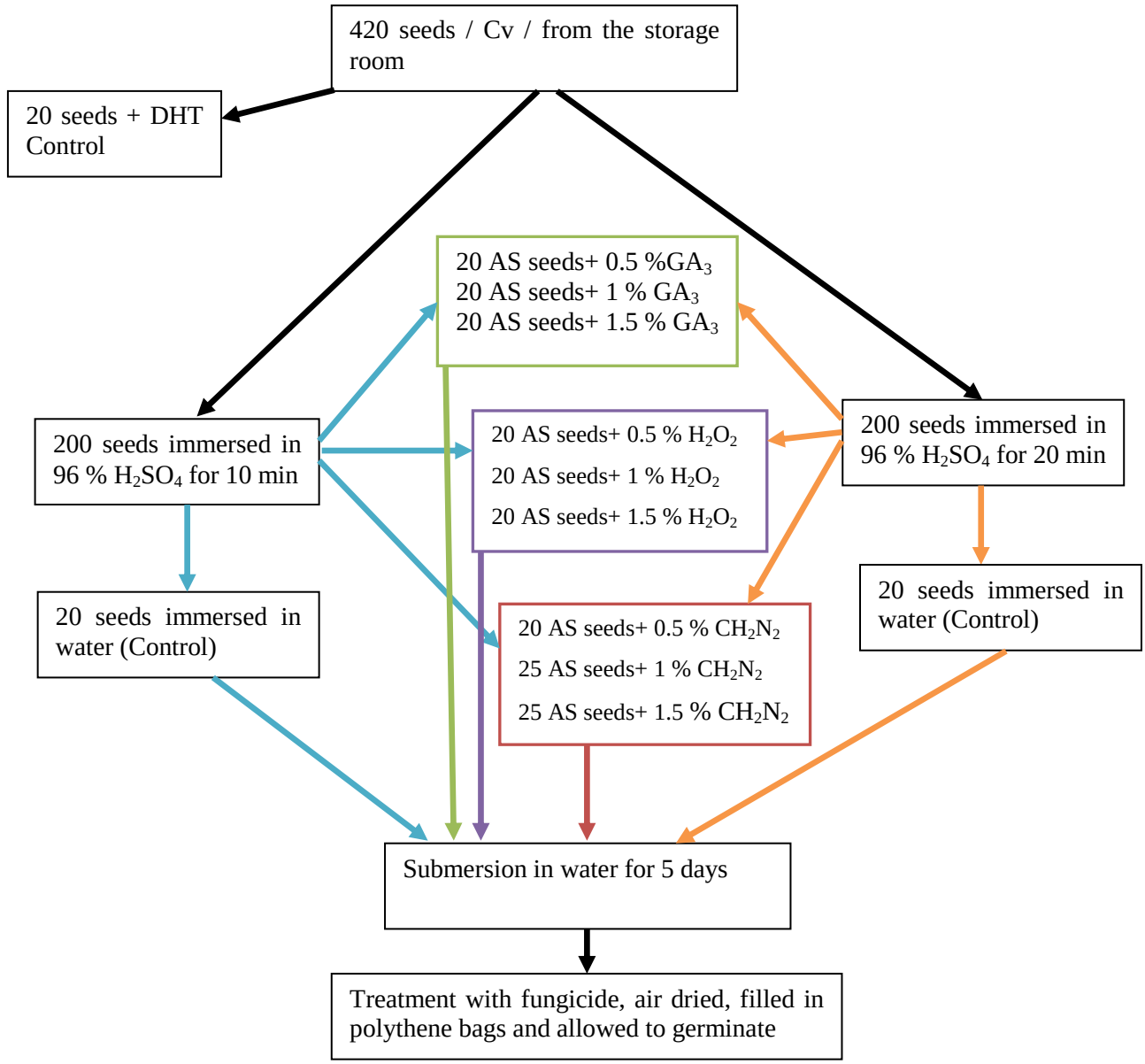

Fig. 19. Flow diagram of acid scarification methodology

Control, 10 minutes treatment, 20 minutes treatment,
acid scarication + Ga_3, acid scarification + H_2O_2, acid scarification + CH_2N_2

II. 2.3.2. Effect of hot water scarification (HWS)

The sample size was 80 seeds / Cv. Initially, the DW of the seeds was taken using an electronic balance (PG5002-S, METTLER TOLEDO). Using an electrical heater (STUART), tap water was heated in a clean aluminium pot. From time to time, a thermometer was dipped inside the water to get the temperature. Once a temperature of 90 ^{o}C was recorded, the heater was put off and all the seeds submerged in the hot water and allowed for 48 h after which the FW was taken again. Water was decanted and seeds treated with Penncozeb for 10 min and finally air dried under shade. The sample was then put under the following treatments.

II. 2.3.2.1. Control

A sub sample of 20 seeds / Cv immersion duration was soaked in water. The moisture content (MC) of the samples was taken daily just before water was renewed. Once a MC of 22 % was attained, the seed were removed in water and air dried.

II. 2.3.2.1. Effect of HWS complemented with plant growth proting chemicals

II. 2.3.2.1. HWS + GA_3

A sub sample of 20 seeds / Cv were treated with three concentrations of GA_3 being 500, 1000 and 1500 mg / l for 24 h and then in water until MC of 22 %.

II.2.3.2.2. HWS + H_2O_2

A sub sample of 20 seeds / Cv soaked in three distinct concentrations (0.5, 1 and 1.5 % of H_2O_2 for 24 h and then in water until MC of 22 %.

II.2.3.2.3. HWS+ CH_2N_2

A sub sample of 20 seeds / Cv was immersed in a solution of CH_2N_2 at the concentrations of 1, 1.5 and 2 % for 24 h and then in water until MC of 22 %.

II. 2.3.3. Data indicators and collection

Out of the several parameters used to study the dynamics of germination process (Ranal & Santana, 2006); germination capacity or final germination, time and rate were chosen in this study. The choice of the latter was based on their importance not only to physiologists, seed technologists and ecologists, but most especially to policy makers because these parameters enable predictions on the degree of success of a species for agronomic and socioeconomic reasons over time. Germination capacity (GP) refers to the total number of seeds germinated in

a seeds lot. The GP value is proportionate to the number of seeds with complete germination in a seed population.

GP was calculated as:

$$GP = \frac{\text{number of germinated seeds}}{Numberoftestedseeds} x\ 100$$ (Labouriau, 1983a)

As for germination time, the mean length of incubation time (MLIT) was preferred to the median time or time to 50 % germination due to the asymmetric nature of germination frequency. MLIT is a measurement of the average length of time required for maximum germination of a seed lot, and is expressed in terms of the same unit of time used in making germination counts (hours or days). It is denoted as:

$$\text{MLIT} = \frac{(G1T1+G2T2+\cdots+GnTn)}{G1+G2+\cdots+Gn)}$$ (Czabator, 1962)

Where *G*: germination count on any counting period; *T*: time.

The lower the MLIT, the faster a population of seed has germinated (Orchard, 1977). Germination rate represented as coefficient of velocity of germination (CVG) gives an indication of the rapidity of germination. CVG increases when the number of germinated seeds increases and the time required for germination decreases (Jones & Sanders, 1987).

$$\text{CVG} = \frac{A1+A2+\cdots+Ax}{A1T1+A2T2+\cdots+AxTx} x\ 100$$

Where, A_1, $A_{2...}$ A_x: number of seedlings counted on the first day, second day, and so on until the last day, and; T_1, T_2... T_x: number of days between sowing and the first collection, between the sowing and the second collection, and so on until the last collection.

II. 2.3.4. Determination of viability vigour of none germinated seeds

II. 2.3.4.1. TTZ test and mechanism

All seeds that did not germinate in all the treatments above after 8 weeks were subjected to a viability test using 95 % pure 2, 3, 5- triphenyltetrazolium chloride (TTZ) purchased from Sigma-Aldrich, Germany. The mechanism of the test (Fig. 20) is based on the fact that all living tissues must respire, thus capable of reducing a colourless TTZ in to a red coloured compound called Formazan. The change in colour from colourless to red is enhanced by H^+ transfer reactions catalysed by the dehydrogenase enzyme found only in living tissues. The Formazan being non-diffusible stains the living tissue red. Therefore only the living parts of viable seeds turn red when incubated in TTZ (Anonymous, 1970).

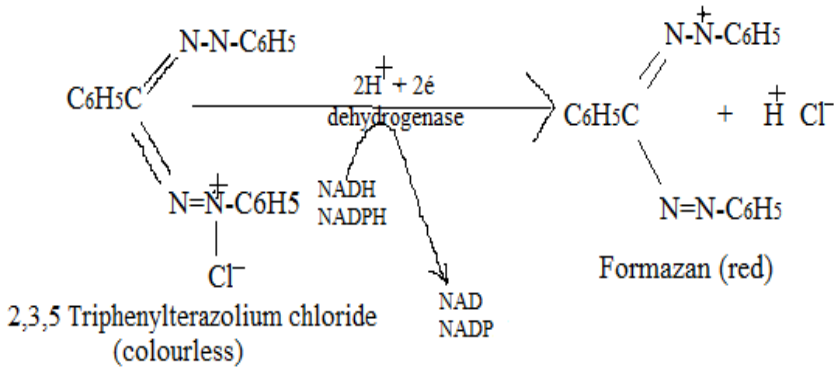

Fig. 20. Reaction mechanism of TTZ

II. 2.3.4.2. Preperation of a TTZ stock solution

A stock solution of 1.0 % TTZ was prepared by dissolving 1.0 g of 95 % pure TTZ powder in a graduated cylinder and distilled water added to the 100 ml level. The prepared solution was stored in a bottle coated with brown-colored paper to prevent deterioration from light. The bottle was tightly capped and stored in the refrigerator at 5 °C.

II. 2.3.4.3. Excission of embryo and testing

The seed viability of all none germinated seeds / treatment was estimated by TTZ Test following the guidelines of the International Seed Testing Association (Anonymous, 1976). All seeds from all treatments that did not germinate after 8 weeks constituted the sample size of this test. The seeds were wrapped in a kaki material and cracked upon a knock or two with a small hammer to collect the endosperms. A surgical blade was used to scrap off the micropylar scar found at the polar end of the endosperm and by shaking over a white piece of paper; the embryo fell out of the endosperm. A 0.1 % TTZ solution was prepared by dissolving 1 ml of the stock solution in 100 ml of water. The embryos were immersed in 0.1 % solution of TTC in Petri plates (90 cm dia.), wrapped with a dark paper and incubated at 25 °C.

After 24 h, they were washed properly with distilled water to remove excess stain. The embryos were checked for colour development under a microscope. Any embryo that was unfractured, turgid, and evenly stained entirely was considered to be viable and the staining was described as normal staining. On the other hand, any embryo that was completely unstained, flaccid and damaged on embryo axis was considered to be non-viable and such staining, described as abnormal or not stained (Anonymous, 1970).

II. 2.4. Direct regeneration of *vitro* oil palm plantlets through MZE rescue

The approach for the direct regeneration was divided into two main phases; the first phase was to assess *in vitro* germination potential of MZE while the second phase was to assess survival rate of germinated MZE at acclimatization. The methodology employed was a modification of Thawaro & Te-chato (2010) and Suranthran et *al*. (2011).

II. 2.4.1. Assessment of germination potential of MZE

II. 2.4.1.1. Preparation of culture media (CM)

For 1 L stock solution of MS prepared, 4.4 g of MS powder was gradually added in to 750 ml of distilled water initially containing 30 g of sucrose obtained from Merck, Germany at a temperature of 15-20 ^{o}C. In the course of adding MS powder, the mixture was continually agitated to allow complete dissolution of the powder. For solidification of media, 2 g / L of phytagel (P8169, Sigma-Aldrich) was added. In respective media (Table III), 2 ml of Morel vitamin complex was supplemented / L of culture medium (Morel & Wetmore, 1951). 10 ml of Fe EDTA were added to each media. Distilled water was then added to raise the volume to 1 L and the solution was then put in 2 L beakers, placed on a non-heater magnetic agitator (PROLABO SR 100) and the temperature and PH electrode of a P^{H} meter (HI 2211 PH/ORP, Hanna instrument) were inserted and the stirring speed adjusted to medium. In the course of stirring, a dropper was used to add drops of 1 M NAOH mother solution in the stirring medium while readings on the meter were read upon the addition of each drop. The P^{H} was adjusted from initial 4.5-4.99 in different full strength based MS media and 5.4-5.62 in all ½ MS based media to 5.7 ± 0.1 in all the media at 25 ± 1 ^{o}C. The media were dispensed in to 2500 mL beakers, capped severally with aluminium foiled paper (Fig. 21) and autoclaved (SMI, Montpellier France) for 20 min after 121 ^{o}C and 105 Pa, using a slow exhaust cycle. The media were then allowed to cool, and in a laminar air flow cabinet, each of the 10 media was filled in labelled Petri dishes in 30 repetitions (Fig. 22). After 30 min, the media became solidified, and each Petri dish was sealed to its cover by scotch, followed by folding the repetitions in groups of tens with aluminium foil paper and stored in a refrigerator at 2 to 8 ^{o}C pending embryo sowing. Given that GA_3 is thermolabile, it was not added to respective media prior to autoclave treatment. GA_3 was only added at the Laminar flow chamber and for its sterilization; 2 mL removed from the mother solution with a syringe was added to the respective media through the use of a 0.2 µm absolute filter (Sartorius Minisart). In all, 10 culture media were prepared (Table III).

Fig. 21. Different stock culture media ready for sterilization in the autoclave

Bar: 1 cm

Fig. 22. Petri dishes filled with different sterilized culture media
Bar: 1cm

Table III. Composition of the two base culture media

	For full strength MS	For ½ MS
In a1000 ml cylinder	500 ml of distilled water	500 ml of distilled water was put
MS Powder (macro and micro elements)	4.4 g/l	2.2g /l
Fe EDTA	10 ml/l	10 ml/l
Morel vitaminecomplex	2 ml/l	2 ml/l
Sucrose	30 g/l	30 g/l
Add distilled water in cylinder	1000 ml	1000 ml
Phytagel	2 g/l	2 g/l
pH ajustment	5.7 ± 0.1	5.7 ± 0.1

II. 2.4.1.2. Preparation of explants

Mature oil palm fruits of ten commercial hybrids seed cultivars obtained from inter cross of D x P were obtained from CEREPAH seed garden 180 days after controlled pollination. For each seed cultivar, 200 randomly selected seeds constituted the sample size. FFs were then depulped using an electrical moto-driven industrial depericarper. Later the seeds were surface sterilised by immersion in a fungicide solution prepared by dissolving 10 g of Penncozeb in 10 L of water. After 2 min, the solution was decanted and seeds were spread on bags dried in an air conditioned room for 10 days under a control temperature of 20 ^{o}C and a relative humidity of 60 %.

The seeds were then transported to the tissue culture laboratory of the African Centre for Research in Banana and Plantain (CARBAP) Njombe. Each seed was wrapped in a strong kaki cloth and upon a knock or two with a hammer the shell was cracked to release an intact kernel. The latter were washed repeatedly with pipe born water and Cyteal antiseptic foaming solution (Pierre Fabre medicament, Boulogne, France) and sterile water for five times until no type of visible dirt existed. After which they were placed in a laminar air flow chamber for a more rigorous sterilisation. The sterilization process began with immersion in 70 % ethanol for 1 min. The seeds were rinsed once with sterile water and once again submerged in 1 % silver nitrate ($AgNO_3$) for 10 min. The acid was decanted and a solution of 0.5 % NaCl was added to get rid of the excess $AgNO_3$, a white precipitate was observed following the addition of NaCl. The seeds were then meticulously rinsed with sterile water 4 times.

II.2.4.1.3. Inoculation of explants on culture media

MZE served as the explants of this study and its excision and inoculation was done in a laminar air-flow cabinet. The explants were aseptically isolated from the sterilized endosperms using a surgical blade initially sterilized by immersion in 90 % alcohol and burnt over a flame. The seed was held in a steady position with one hand while the surgical blade held with the other hand was used to scrap away the micropylar scar. Once the latter was removed, the micropylar aperture was opened and a white embryonic axis could be seen. By lifting the kernel such that the micropylar scratched mark faces the working surface, an embryo fell out after a knock or two on the opposite end of the micropyle or by shaking in the up-down direction. MZE measured between 1-3 mm in length, with a whitish shoot apex encroached in to the endosperm and a light greenish terminal where it attached to the micropylar scar. Once the embryo fell out, a hole measuring the same length as the MZE could be seen on the micropylar edge of the endosperm. A scalpel was dipped in the culture medium and the

medium stock at the edge permitted the MZE to be picked up and inoculated on its respective medium. Explants were sown in such a way that their cut end were in direct contact with the culture medium. Once sown, the Petri dish was covered with its lid and sealed with Cling films paper (Falcon pack, Sharjah, UAE) several times such that no air enters the medium. Incubation was done in the dark while controls were incubated in 16/8 hours photoperiod.

For each of the ten oil palm seed cultivars, five MZE were inoculated / Petri dish containing 10 ml of one of the two culture media: full strength MS and ½ strength MS, and in 8 other experiments, in which the two culture media above were supplemented with 2 ml morel vitamin complex, 2 g / L activated charcoal (AC) and 0.1 mg / L GA_3. All culture media were supplemented with 30 g / L saccharose but void of PGR (Table III). The pH was adjusted to 5.7 ± 0.1 before autoclaving. All inoculated cultures were incubated in a growth room. The experiment made up of ten growth media types (Table IV) was set up to investigate the effects of MS medium strength, activated charcoal treatments, vitamin supplementation and GA_3 on the *in vitro* germination of embryos.

Table IV. Media for *in vitro* culture of MZE of *E. guineensis*

Medium no	Composition + 30 g /l sucrose + 2 g /l phytagel
1.	Full strength MS salt
2.	Full strength MS salt + 2 g /l AC
3.	Full strength MS salt + vitamin*
4.	Full strength MS salt + 0.1 mg / l GA_3
5.	Full strength MS salt + 2 g / l AC + vitamin* + 0.1 mg /l GA_3
6.	½ strength MS salt
7.	½ strength MS salt + 2 g / l AC
8.	½ strength MS salt + vitamin*
9.	½ strength MS salt + 0.1 mg / l GA_3
10.	½ strength MS salt + 2 g / l AC + vitamin* + 0.1 mg / l GA_3

*Morel vitamin supplement was a complex composition of 5000 mg myo inositol, 50 mg Thiamine, 50 mg Pyridoxine, 50 mg nicotinic acid and 1 ml of biotine all dissolved in 100 ml of distilled water (Morel & Wetmore, 1951)

II.2.4.2. Evaluation of survival rate of germinated MZE during acclimatization

II.2.4.2.1. Sub culture of germinated embryos

Each germinated embryos was sub cultured or transferred in to a test tube containing 10 ml of semi-solid freshly prepared respective culture media (1-10) used during the germination phase and incubated under similar photoperiods cycle and temperature as in germination. All transfers were carried out after 4 weeks from the germination date under aseptic conditions in a laminar air flow cabinet. The sub cultured test tubes were capped and treated under light conditions (3000 lux illumination for 16 h photoperiod and 8 h dark cycle) at 25 ± 2 °C for 2 months. It was noticed that using the above media, the shoot bud developed into leaves but no growth was observed at the level of the micro root bud i.e. no new roots were induced nor where existing once prior to sub culture showing any signs of development. There was therefore a need to stimulate rhizogenesis so as to increase the success rate during the process of acclimatization.

II.2.4.2.2. Stimulation of rhizogenesis in *vitro* oil palm plantlets

In an attempt to optimize root induction and development, the effect of incorporating different concentrations of auxin, a plant growth regulator known to stimulate rhizogenesis (Blythe et *al*., 2007) to full strength MS medium was tested. The three auxins used were Indole-3- acetic acid (IAA), Indole -3-butyric acid (IBA) and 1-Naphthalene acetic acid (NAA). For each auxin type, three concentrations were prepared (Table V).

Table V. Concentrations of rooting medium

Auxin type	medium composition
IAA	MS+0.5 mg / l
	MS+ 1 mg / l
	MS+1.5 mg / l
IBA	MS + 0.5 mg / l
	MS + 1 mg / l
	MS + 1.5 mg / l
NAA	MS + 0.5 mg / l
	MS + 1 mg / l
	MS + 1.5 mg / l

Only healthy and robust *vitro* plantlets obtained from MZE culture of Cv 1, 2, 4, 5 and 9 were used owing to their high response in rate of germination *in vitro.* Two Lots were made; Lot 1 constituted all plantlets derived from direct shoot bud proliferation of MZE i.e. germinated only to a micro-shoot (Fig. 24A) and Lot 2 was comprised of plantlets that showed complete differentiation in to a shoot and a root directly from MZE explants (Fig. 24B). In each experiment (1-9), three randomly selected explants from Lot 1 and Lot 2 were inoculated in two repetitions. In all, three partially differentiated MZE (shoots only) and three completely differentiated MZE (shoot + root) were then inoculated in two replications for each of the medium. The experiment was incubated at a temperature of 26 ± 2 ^{o}C under a 16 / 8 hours photoperiod, and after a period of 40 days, the rate of root initiation was assessed for explants of Lot 1 while root elongation and development of root hairs was evaluated for Lot 2 explants.

II.2.4.2.3. Hardening, acclimatization and nursery development of *vitro* plants

Plantlets with well-developed root system were removed from the rooting medium, washed properly with running tap water to get rid of any adherent gel and transferred to plastic cups containing sterilized substrate made out of coffee residue. Hardening and acclimatization was done under a shade with diffused light condition to maintain high relative humidity. For four months, the plants were watered daily and gradually exposed to natural ambient conditions. After 6 weeks, the plantlets were transferred in to earthen pots containing garden soil and gradually exposed to environmental conditions.

II.2.4.3. Parameters analysed in production of oil palm platlets via embryo rescue

Parameters assessed for direct regeneration of oil palm *vitro* plants from MZE were; speed of shoot induction, shoot length, root number and length, number and leaf length, degree of embryo discoloration and degree of media discoloration were collected at weekly intervals. Based on visual observations, the degree of media and embryo discoloration (entire explants and at the media contact point) was rated on a scale of 1-5 (1 = No discoloration and 5 = Extreme discoloration), modified from the rating scale given by Ziv & Halevy (1983). For rhizogenesis, the effect of the different auxin types was assessed on rate of induction for MZE that germinated to micro shoots only and root elongation for MZE that directly completed differentiation in to a shoot and root primordia at germination.

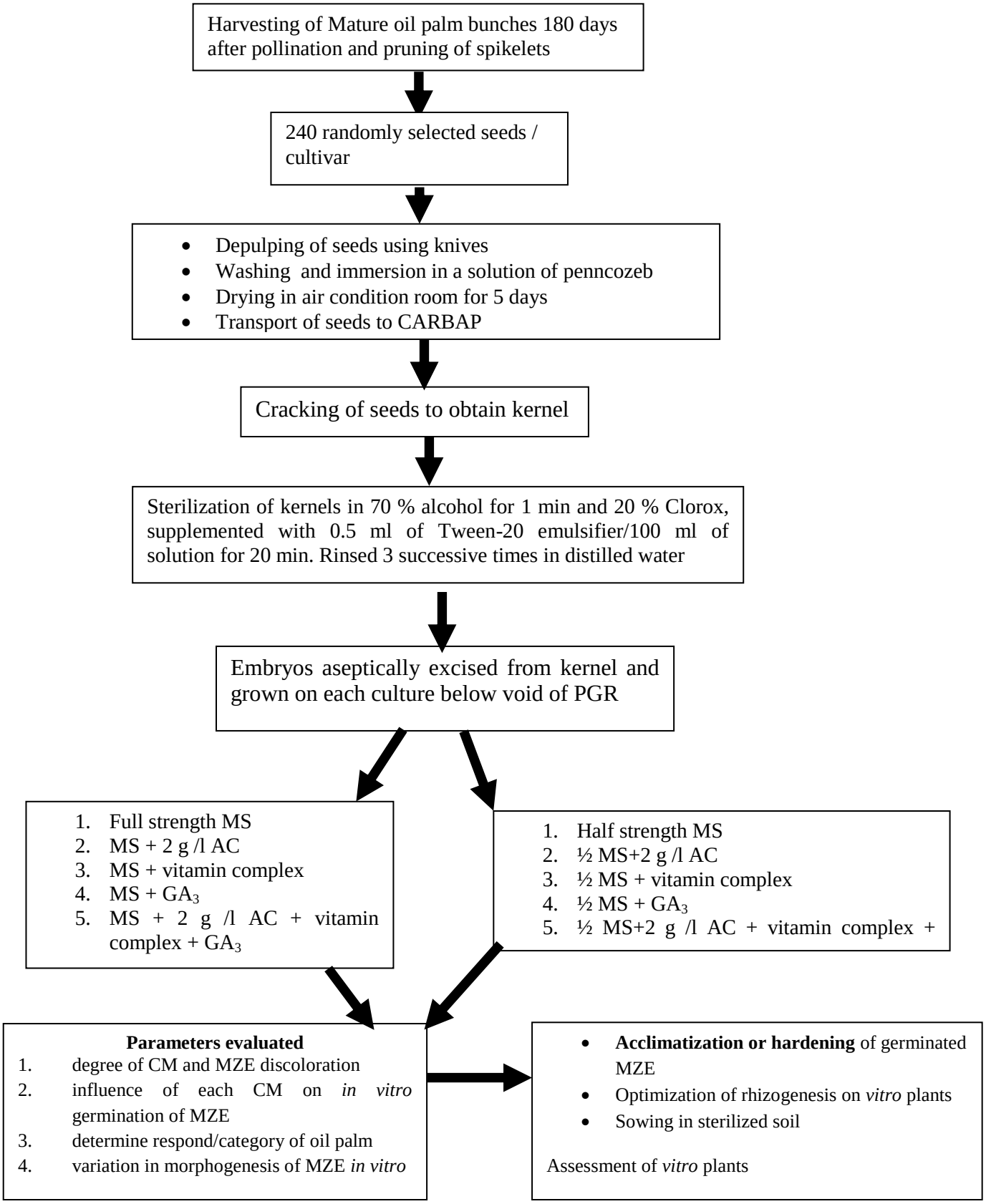

Fig. 23. Protocol for direct regeneration of oil palm plantlets from MZE (Thawaro & Te-chato, 2010; Saldanha & Martins-Corder, 2012 *mordified*)

II.2.5. Statistical analysis

II.2.5.1. Determining factors responsible for low annual and variation in germination / Cv

As concerns causes of germination variation between cultivars, the design was random split block sampling with 4 levels (FW, number of kernels, shell thickness at micropylar end, FW of endosperm and quantity of PKO). Data was analyzed using SPSS Version 20 and the Student t test at 95 % probability was used to compare means at 95 % significant level.

As for position dependent effect, the experimental design was random with two main factors (fruit position on spikelet and germination capacity) tested for 9 open pollinated FFB and eight controlled pollinated FFB. Mean values of parameters studied for the different samples where analyzed using SPSS version 20. The means for treatment combinations for biometric parameters and GP were compared using the student t test at a probability of 0.05.

II.2.5.2. Effect of supplementing DHT with GPC

Three out of the ten seed cultivars used in this study did not register any germination in all the treatments and control; hence they were eliminated during statistical analysis. The experimental design was therefore a two factorial treatment combination of three GPC (GA_3, H_2O_2 and CH_2N_2), three concentrations for each GPC and seven oil palm seed cultivars arranged in a randomized complete block design repeated thrice. The data was analysed using the IBM SPSS 22. The means for treatment were compared using the Duncan multiple range test at a probability $P < 0.01$.

II.2.5.3. Production of *vitro* oil palm seedling by embryo rescue

The experimental design for *in vitro* germination was two factorial treatment combinations of culture media and oil palm seed cultivar arranged in a randomized complete block design for each sowing date made up of two factors repeated thrice (10 x 10 x 15 factorial). There were 10 levels of the first factor (culture medium) and 10 levels of the second factor (seed cultivars), repeated 15 times with 5 MZE / Petri dish in 3 repetitions.The data were analyzed using the IBM SPSS 22. The Turkey Honestly Significant Difference test was used to group culture media and the cultivars according to mean germination. The means for treatment combinations were compared using the DMRT at a probability of 5 %.

CHAPTER III. RESULTS AND DISCUSSION

III.1. Results

III.1.1. Factors accounting for variation in seed germination among cultivars

III.1.1.1. Comparison of some physical parameters of the ten cultivars of oil palm

Biometric parameters evaluated in this study revealed significant differences between the ten cultivars in parameters like weight of whole kernel, weight of endosperm and shell thickness at $P < 0.05$ (Table VI). This implies that each planting material presents some variations in physical characteristics even though all of them were tenera Cvs derived from dura x pisifera crosses. As concerns average weight of whole kernel, the greatest (3.45 g) was observed in C1501II while the least (1.99 g) was noticed in C1001 II (Fig. 24A). Analysis as far as FW of endosperm is concerned (Fig. 24B), revealed variations, with cultivar C1901 II showing the highest (0.89 g) while the least was obtained from C1001 II. So many significant differences were observed for the latter parameter (Table VI). Significant differences were also recorded between the ten oil palm Cvs for the number of kernels/nut, with C2001 II having over 40 % of its nuts with more than one kernel (Table VI). In another sample, 86 % of both kernels in each nut had a viable embryo following the TTZ test. As concerns shell thickness (Fig. 24C) at the level of the micropylar end, only two out of the ten cultivars showed significant differences at $P < 0.05$ (Table VI) in spite of the range of 3.3 mm for the highest observed in C1001 II to 2.5 mm for the least observed in C2101 II (Fig. 24C).

Concerning the quantity of PKO present in the endosperm, the 10 samples could be grouped into three; Cvs C2301 II, C2101 II, C2001 II, C2301 II and C1001 IIF constituted the first group, the 2nd group constituted of C2301 IIF, C1901 II, C1501 II and C2501 FX, while Cv C1001 II was alone in the third group. No significant differences were observed within groups, rather significant differences were observed between groups and at $P < 0.05$ (Table VI).

It was observed that Cv C1001 II presented the least values in three of biometric parameters assessed except for the number of kernels per nut.

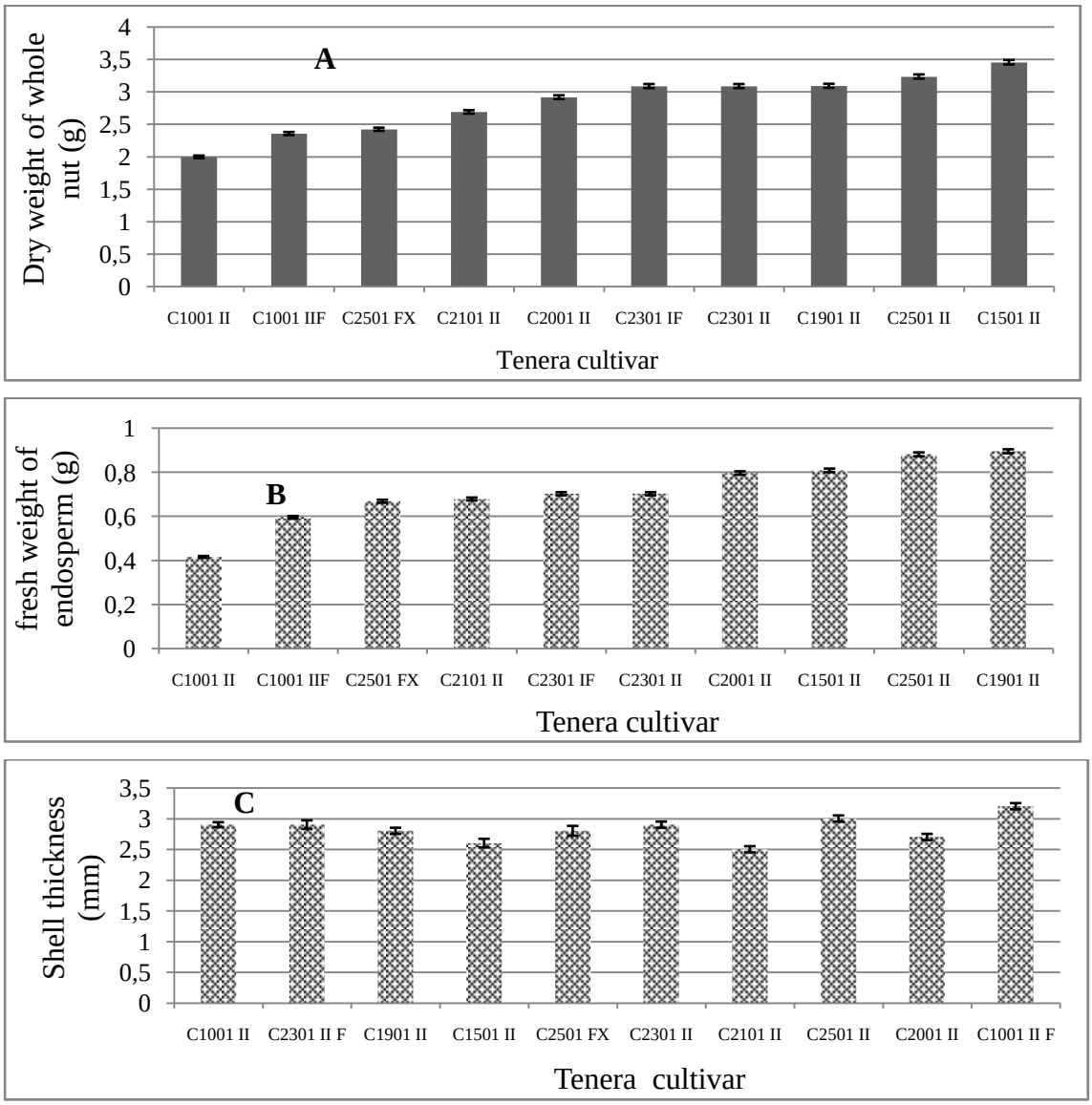

Fig. 24. Variation in morphology of seeds of the ten oil palm cultivars produced by CEREPAH

A. Fresh weight of whole kernel B. Fresh weight of Endosperm C. shell thickness.

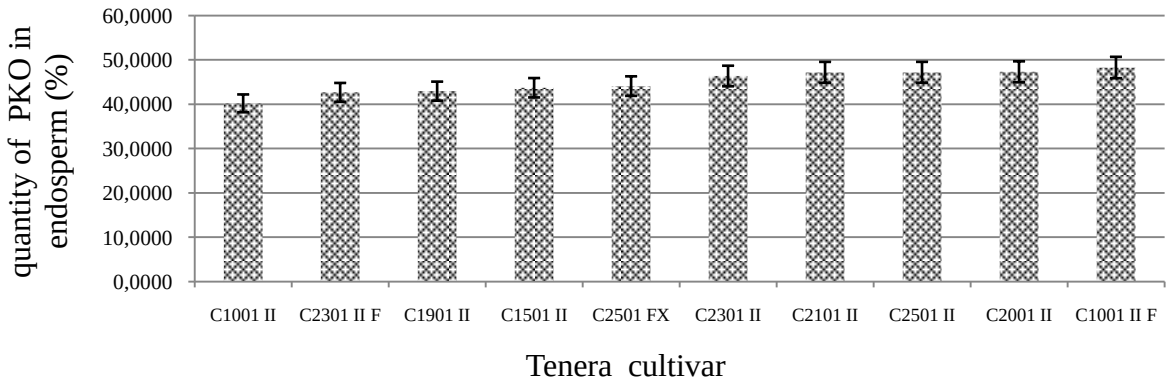

Fig. 25. Average quantity of PKO per oil palm cultivar

Table VI. Comparison of some biometric features and quantity of PKO of the ten oil palm Cv

	weight of whole nut (g)	>1 kernel/nut (%)	shell thickness (mm)	weight of endosperm (g)	Quantity of PKO (%)
C1001 II	1.99±0.69d	16±0.59b	2.9±0.04a	0.42±0.19d	40.18±2.2c
C2301 II F	2.46±1.08c	8±0.53c	2.9±0.07a	0.70±0.24bc	42.65±2.2b
C1901 II	3.09±0.86b	8±0.27c	2.8±0.05a	0.89±0.29a	42.94±2.2b
C1501 II	3.45±0.81a	0±0.0d	2.6±0.07ab	0.81±0.33b	43.70±2.2b
C2501 FX	2.42±0.58c	0±0.0d	2.8±0.08a	0.67±0.21c	44.07±2.2b
C2301 II	3.08±0.46b	8±0.43c	2.9±0.07a	0.70±0.24	46.35±2.2a
C2101 II	2.68±0.62c	0±0.0d	2.5±0.05b	0.68±0.25c	47.18±2.2a
C2501 II	3.23±0.64b	0±0.0d	3.0±0.05a	0.88±0.29a	47.18±2.2a
C2001 II	2.91±1.17b	40±0.76a	2.7± 0.05a	0.79±0.35b	47.29±2.2a
C1001 II F	2.35±0.52cd	12±0.33c	3.2±0.05a	0.42±0.20	48.26±2.2a

Values are means of 25 seed/cultivar ± standard deviation

Different letters for a given column indicate significant differences at $P < 0.05$

III.1.1.2. Position based effect of oil palm seeds on germination

III.1.1.2.1. Effect of seed position on biometric parameters in open pollinated FFBs

III.1.1.2.1.1. Number of fruit / FFB

In all FFBs analyzed, the number of basal seeds (orange coloured) was generally greater than that of apical (deep red coloured) seeds (Fig. 26). Significant variations were observed between the number of apical and basal seeds / FFB analyzed.

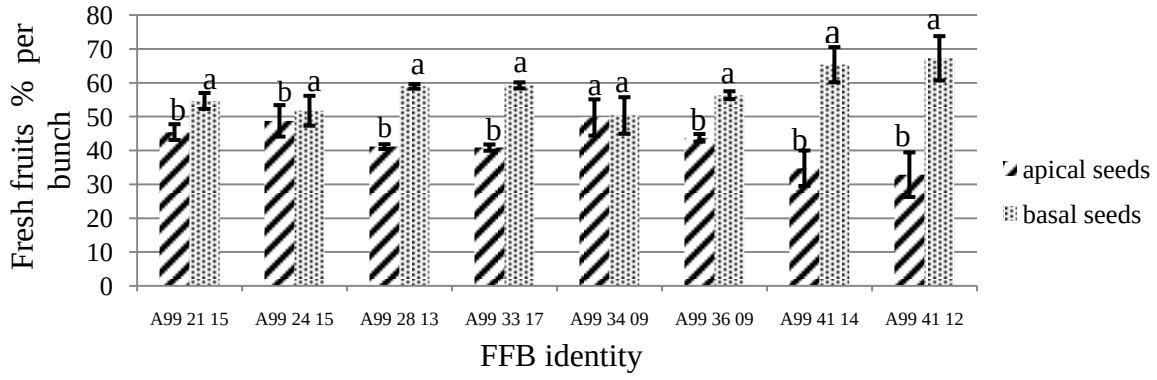

Fig. 26. Percentage of apical and basal fruits in an oil palm FFB

Different letters on histograms for a given FFB indicates significant difference at $p < 0.05$

III.1.1.2.1.2. Fresh biomass of seeds

It was observed that the weight of the apical seeds was generally greater than that of basal seeds in 75 % of the FFB analyzed (Fig. 27) with glaring statistical differences. Even in FFBs A99 41 14 and A99 41 12 where basal seeds recorded a higher weight than apical seeds, no significant differences were however found between the two.

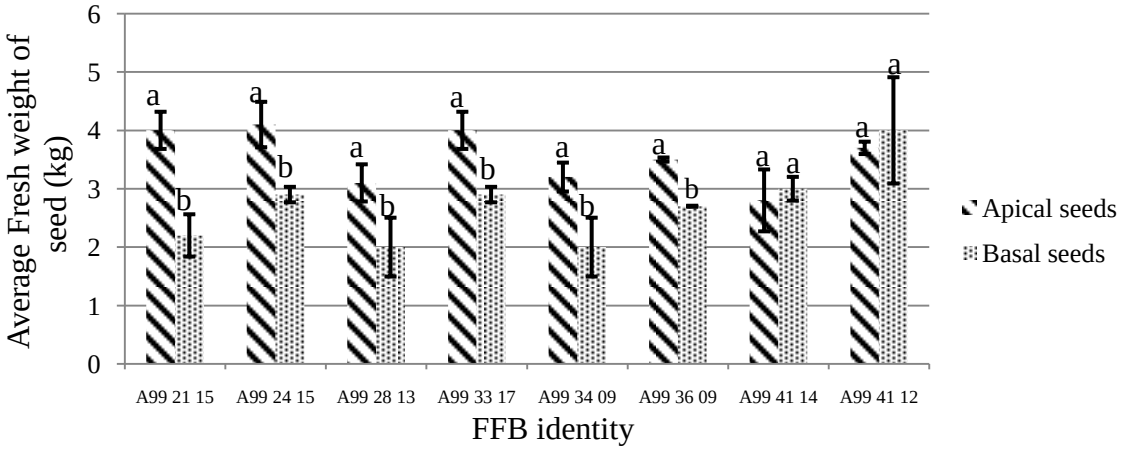

Fig. 27. Variation in FW of oil palm seeds found on apex and base of the spikelet
Different letters on histograms for a given FFB indicates significant difference at $P < 0.05$

III.1.1.2.1.3. Length

Generally, apical seeds presented greater length than that of basal seeds for all FFB sampled. Significant differences in length of seeds were observed in five out of the eight FFB analyzed (Fig. 28). The average length of apical seeds was 2.5 cm while for basal seeds, a mean length of 2.2 cm was observed.

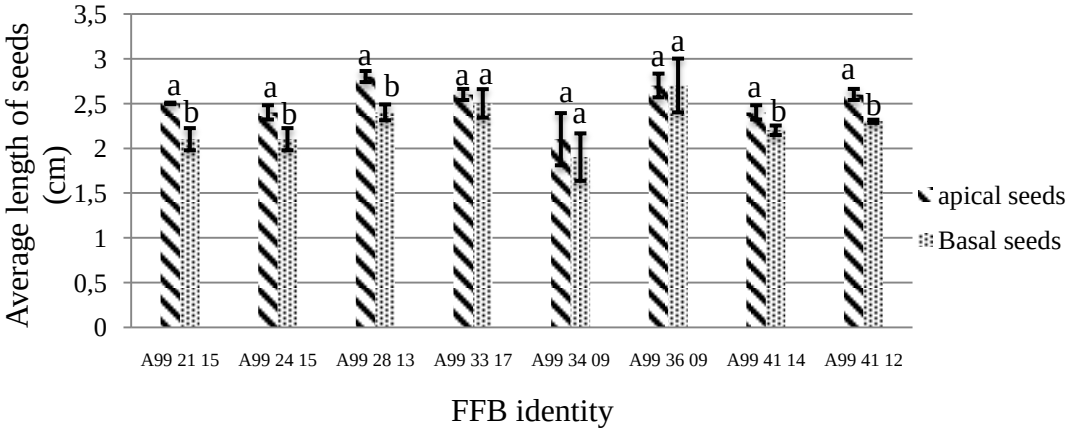

Fig. 28.Variation in length with seed position on the spikelet
Different letters on histograms for a given FFB indicates significant difference at $P < 0.05$

III.1.1.2.1.4. Effect of seed position on germination of open pollinated seeds

No seed germination was observed in 3 of the 8 FFB sampled. Globally, a significant difference was found when the mean germination of all apical seeds (5.625) was compared to the mean germination of basal seeds (2.625) at $p < 0.05$. However, at the level of individual FFB, no significant difference was noticed in 2 of the 5 samples (Fig. 29) eventhough apical seeds showed a relatively higher germination than basal seeds.

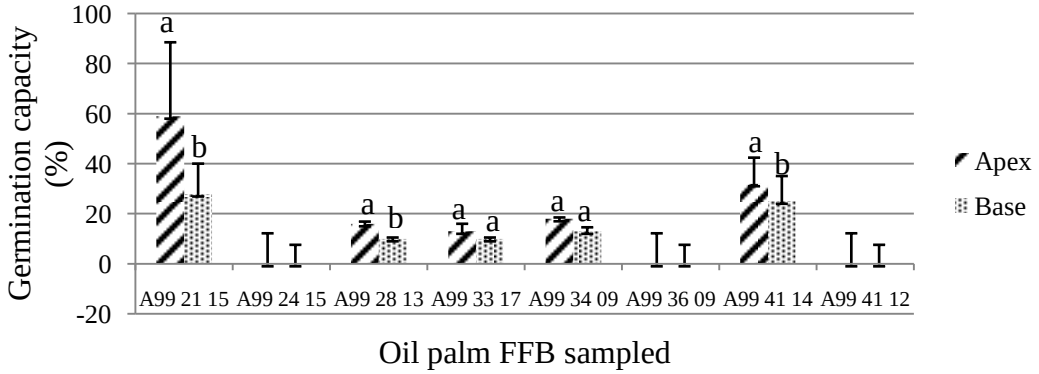

Fig. 29. Effect of seed position on germination capacity of open pollinated seeds
Different letters on histograms for a given FFB indicates significant difference at $P < 0.05$

III.1.1.2.2. Position dependent effect of seeds obtained from assisted pollination

III.1.1.2 .2.1. Effect on fresh biomass

As far as the fresh biomass is concerned, significant differences were noticed in 2 out of the 5 FFBs studied eventhough 80 % of apical seeds presented a FW greater than that of basal seeds (Fig. 30).

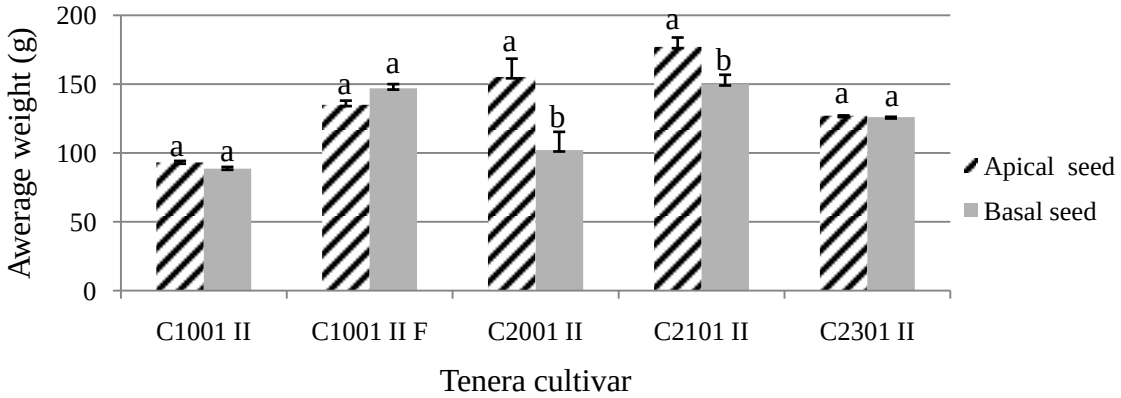

Fig. 30. Variation in FW of apical and basal seeds derived from assisted pollination FFB
Different letters on samples indicate significant differences

III.1.1.2 .2.2. Effect on germination

Globally the rate of germination was comparatively higher in seeds obtained from controlled pollination compared to germination rate in open pollination both for apical and basal seeds. The highest percentage recorded was 59 % for apical seeds in open pollination while in controlled pollination (Fig. 29), three out of the five samples scored at least 80 % germination for seeds at the apex (Fig. 31). As concerns seeds derived from the spikelets base, the highest germination scored in open pollination was 25 % (Fig. 29), while 3 out of the 5 sampled FFB from assisted pollination gave a germination rate greater than 60 % (Fig. 31). As observed in open pollination, seeds at the apex also presented a germination capacity higher than basal seeds in assisted pollination in all the five cultivars tested. Highly significant differences were obtained in all the five samples at $p < 0.05$.

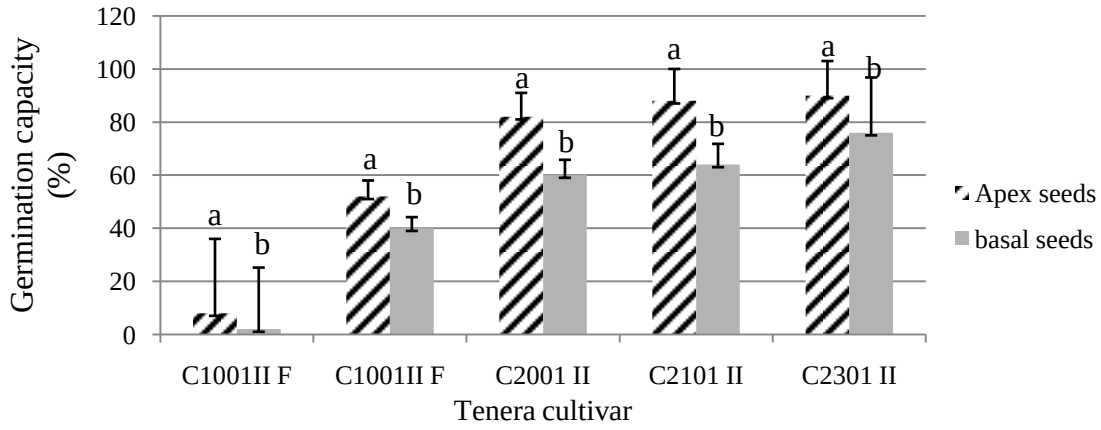

Fig. 31. Position dependent effect on germination of seeds from assisted pollination

Different letters on each sample indicate significant differences at P <0.05

III.1.1.2.3. Influence of harvesting date on seed germination

It was also noticed that prior to harvest of FFBs allowed for 7 months, an average of 120 FF had abscised on their own while at 6 months harvesting, only an average of 7 FF had abscised. Variations in final germination scores were noticed between FFB harvested 6 months and 7 months after hand pollination for the two FFBs weight range. Significant germination scores were noticed between 6 and 7 months FFBs with FW ≥ 11kg while no significant differences were observed between the latter for FFB of FW ≤ 11kg (Fig. 32). The FW of FFBs had an influence on the germination rate (Fig. 33). It was observed that germination scores increased with increase in FFB biomass. FFB ≥ 11 kg in FW showed germination scores

greater than 60 % while FFBs with ≤ 11kg FW could not score up to 20 % germination in all the samples tested (Fig. 33).

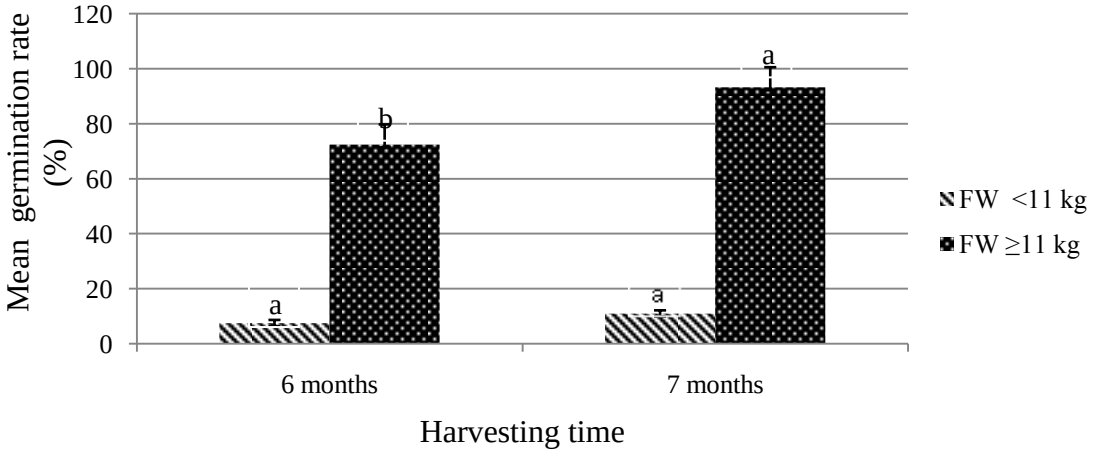

Fig. 32. Influence of harvesting date on mean germination capacity of oil palm seeds

Different letters between FW of both harvesting dates signifies significant differences at P <0.05

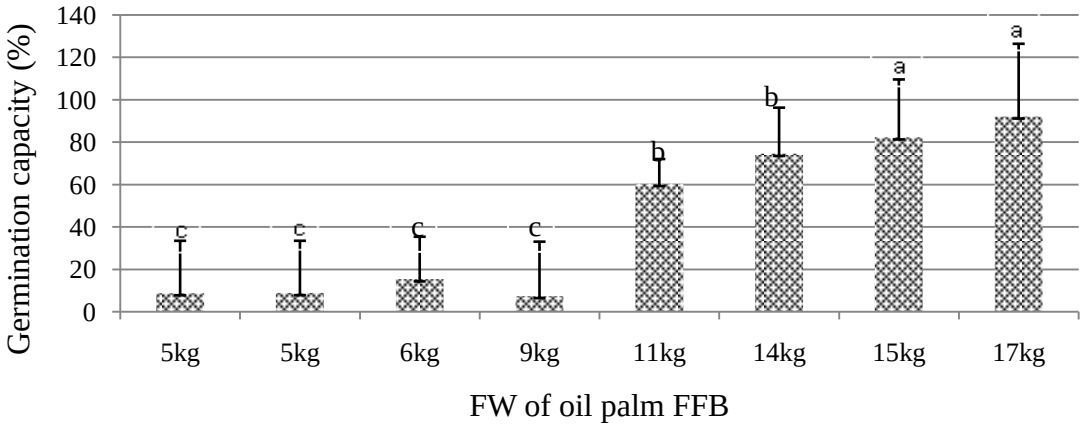

Fig. 33. Effect of FFB weight on seed germination capacity

Different letters between FW of each sample indicates significant differences at P <0.05

III.1.2. Optimization of the conventional DHT by incorporation of GPC

III.1.2.1. Germination Capacity

III.1.2.1.1. Effect of DHT + GA

All concentrations of GA_3 had a positive impact on the rate of germination compared to the control. However variations were noticed with respect to concentrations (G1, G2 and G3). Significant differences were recorded between GA and control and within the different concentrations of different GA_3 for all cultivars with concentration G2 recording overall best

germination capacity (Fig. 34). The effects of the different GA_3 concentrations were mainly significant where the control responded positively.

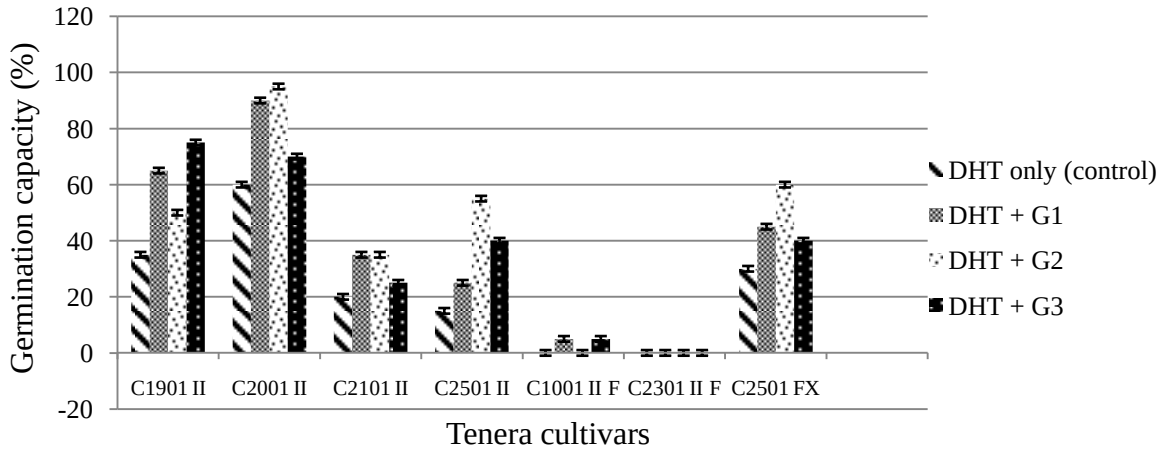

Fig. 34. Effect of GA+DHT on germination of oil palm cultivars

III.1.2.1.2. Effect of DHT + H_2O_2

Hydrogen peroxide enhanced germination than the controls at all concentrations used. Nonetheless variations in germination capacity were noticed between the different concentrations of H_2O_2. The response to different H_2O_2 concentrations also varied with the seed cultivar. Globally, H2 stood out as the best concentration recording a germination capacity of 75 % in Cv C2001II which scored the highest germination capacity of all the Cvs used in this study (Fig. 35). Significant differences were obtained between H2 and H1, H3 ($p<0.01$) (Table VII) in Cvs (C1901II, C2001II and C2501FX) that responded with more that 50 % of total germination (Fig. 35).

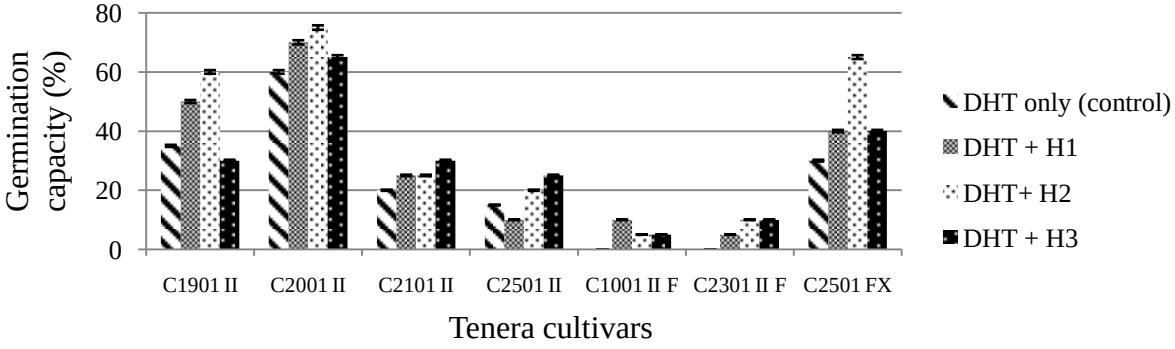

Fig. 35. Effect of DHT+H_2O_2 on germination of oil palm seeds

III.1.2.1.3. Effect of DHT + CH_2N_2

Germination capacity was higher in controls than in all concentrations of CH_2N_2 except in C2501 FX were no significant difference was found between control and C1 (Table VII).

Among the different concentrations, C3 showed a negative influence on germination capacity with germination occurring only in C2501IIFx (Fig. 36).

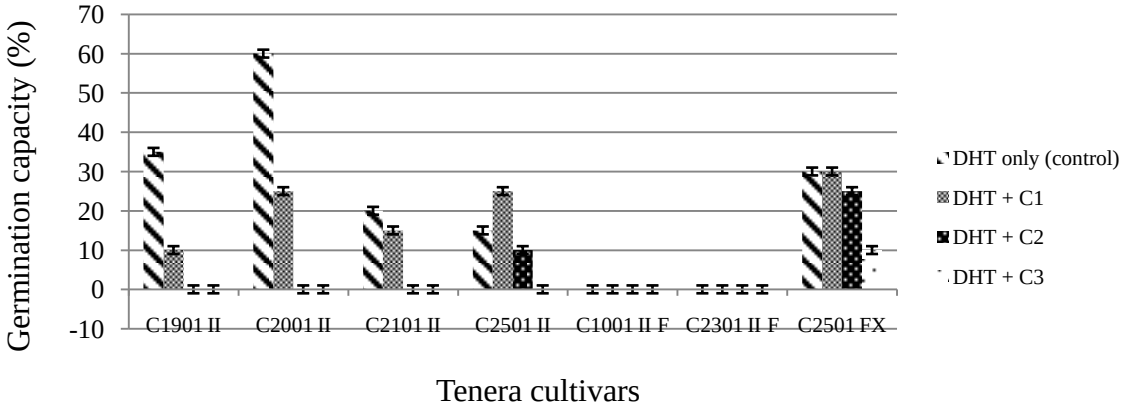

Fig. 36. Effect of DHT + CH_2N_2 on germination of oil palm seeds

III.1.2.2. Germination time

Significant differences were obtained in MLIT between the different treatments and controls as well as between the different seed cultivars studied (Table VIII). Generally, H_2O_2 treatments presented the least MLIT among the GPC in all the cultivars. The longest MLIT was noticed mostly in CH_2N_2 treatments. Even though seeds treated with CH_2N_2 did not germinate, when their embryos were subjected to 0.01 % TTZ test, it was however noticed that over 70 % of the embryos were still viable after 120 days.

The general trend comparing results of germination (Table VII) and MLIT (Table VIII), shows that for each Cv, seeds treated with 1 % H_2O_2 presented a better germination over short period of time than other treatments. For example in cultivar C1901 II, 45 ± 0.5 days were required to obtain 60 % germination in 1 % H_2O_2, 64.3 ± 5.9 days for 35 % germination in control, 48 ± 0.5 days for 75 % germination in 1.5 % GA and 90 days to give 10 % germination in CH_2N_2.

III.1.2.3. Coefficient of velocity of germination (CVG)

Comparison made at the level of each cultivar revealed that significant differences in CVG were observed between the different treatments (Table IX). Generally, DHT + H2 treatment showed a higher germination speed than the control and other treatments at the same concentrations.

Table VII. Effect of DHT + GPC on germination capacity (%)

Cultivar	Treatment									
	control	DHT + G1	DHT + G2	DHT + G3	DHT + H1	DHT+ H2	DHT+ H3	DHT+ C1	DHT+ C2	DHT+ C3
C1901 II	35±0.8d	65±9.16ab	50±4.16c	75±12.5a	50±4.16c	60±7.5bc	30±2.5d	10±9.1e	0±0.0f	0±0.0f
C2001 II	60±1.6c	90±11.6a	95±13.3a	70±5.0bc	70±5.0bc	75±6.6ab	65±3.3bc	25±10.0d	0±0.0e	0±0.0e
C2101 II	20±0.3d	35±4.6a	35±4.6a	25±1.3c	25±1.3c	25±1.3c	30±3.0b	15±2.0e	0±0.0f	0±0.0f
C2501 II	15±2.5cd	25±0.8b	55±10.8a	40±5.8ab	10±4.16d	20±0.8bc	25±0.8b	25±0.8b	10±4.1d	0±0.0e
C1001 IIF	00±0.0b	5±1.15a	0±0.0b	5±1.15a	10±2.33a	5±1.15a	5±1.15a	00±0.0b	0±0.0b	0±0.0b
C2301 IIF	0±0.0b	0±0.0b	0±0.0b	0±0.0b	5±1.0a	5±1.0a	10±2.66a	0±0.0b	0±0.0b	0±0.0b
C2501 FX	30±2.8cd	45±2.16b	60±7.16a	40±0.5b	40±0.5b	65±8.83a	40±0.5bc	30±2.8cd	25±4.5c	10±9.5d

Values are mean germination percentage ± SD. Values with the same letter in each row are not significantly different at 0.01

Table VIII. Effect of DHT + GPC on germination time: Mean incubation duration (days)

Cultivar	Treatment									
	control	DHT + G1	DHT + G2	DHT + G3	DHT + H1	DHT+ H2	DHT+ H3	DHT+ C1	DHT+ C2	DHT+ C3
C1901 II	64.3±5.9b	49.3±0.9c	65.5±6.3b	48±0.5c	48±0.5c	45±0.5c	55±2.8c	90±14.5a	0±0.0d	0±0.0d
C2001 II	47.5±5.2b	40±7.7c	44.2±6.3bc	47.2±5.3b	42.9±6.7c	40.7±7.4c	39±8.0c	90±8.9a	0±0.0d	0±0.0d
C2101 II	60±6.1c	51.2±3.2cd	48±2.19d	36±12.0e	36±12.0e	48±2.1d	45±1.1d	90±16.1b	120±13.8a	0±0.0d
C2501 II	67±1.6b	54±2.71c	65±0.95b	63.3±0.3b	30±10.71c	60±0.7b	36±8.7c	66±1.2b	90±9.29a	90±9.2a
C1001 IIF	0±0.0b	71±8b	0±0.0c	76±3b	60±14a	60±14a	60±14a	0±0.0c	0±0.0c	0±0.0c
C2301 IIF	0±0.0b	0±0.0b	0±0.0b	0±0.0b	60±14a	60±14a	60±14a	0±0.0b	0±0.0b	0±0.0b
C2501 FX	76.6±3.3b	70±1.1b	55.7±3.5d	72.3±1.9b	41.2±8.3c	36±10.14c	37.5±9.6c	98.8±10.7a	86.2±6.6a	90±7.8a

Values are means of three repetitions ± SD. Values with the same letter in each row are not significantly different at the threshold level 0.01. MLIT of 0 indicates that no germination was observe after the 120 days study period even though the 0.01% TTZ test showed that >70 % embryos were still viable.

Table IX. Influence of DHT + GPC on coefficient of velocity of germination

	Treatment									
Cultivar	DHT only (control)	DHT + GI	DHT + G2	DHT + G3	DHT + H1	DHT + H2	DHT + H3	DHT + C1	DHT + C2	DHT+ C3
C1901 II	1.5 ±0.03b	2± 0.18a	1.5 ± 0.03b	2 ± 0.18a	2 ± 0.18a	2.2 ± 0.25a	1.6 ± 0.05a	0.8 ± 0.21c	0.8 ± 0.21c	0 ± 0.0d
C2001 II	2.3 ± 0.12b	2.5 ± 0.18ab	2.2 ± 0.08b	2.1 ± 0.05b	2.3 ± 0.12b	2.4 ± 0.15ab	2.9 ± 0.32a	1.1 ± 0.28c	0.8 ± 0.38c	0.8 ± 0.38c
C2101 II	1.9 ± 0.1c	1.4 ± 0.06cd	1.2 ± 0.12d	2.7 ± 0.44b	3.3 ± 0.57a	2 ± 0.14c	2.2 ± 0.2c	1.1 ± 0.16d	0 ± 0.0e	0 ± 0.0e
C2501 II	1 ± 0.08c	1.8 ± 0.18b	1.3 ± 0.01bc	1.5 ± 0.08b	0 ± 0.0d	1.6 ± 0.11b	2.7 ± 0.48a	1.5 ± 0.08b	1.1 ± 0.05c	0 ± 0.0d
C1001 II F	0 ± 0.0a	0.03± 0.0a	0 ± 0.0a	0.03 ± 0.0a	0.03 ± 0.0a	0 .05±0.0a	0 .05± 0.0a	0 ± 0.0a	0 ± 0.0a	0 ± 0.0a
C2301 II F	0 ± 0.0c	0 ± 0.0c	0 ± 0.0c	0 ± 0.0c	2.2 ± 0.55a	1.1 ±0.18b	2.2 ± 0.55a	0 ± 0.0c	0 ± 0.0c	0 ± 0.0c
C2501 FX	1.3 ±0.09b	1.1 ± 0.16c	1.7 ±0.03b	1.1 ± 0.16c	2.4 ±0.27a	2.7 ± 0.37a	2.6 ± 0.33a	0.9 ± 0.23c	1.1 ± 0.16c	1 ± 0.19c

Values are means of three repetitions ± SD. Values with the same letter in each row are not significantly different at the level 0.01. CV of 0 indicates that no germination was observed for the indicated treatments throughout the study period even though the 0.01 % TTZ test showed that > 70 % embryos were still viable.

III.1.3. Effect of alternative scarification methods to break oil palm seed dormancy

III.1.3.1. Effect of chemical scarification on seed germination

Treatment of oil palm seeds in different concentrations of sulphuric acids and different soaking time without prior DHT did not show any germination after 60 days. However, when none germinated acid scarified seeds were further subjected to DHT for 80 days at 40 ^{o}C, followed by GPC incorporation, significant germination capacity was observed (Fig. 37). Aesthetically, the germinated seeds were more presentable compared to seeds that were scarified by the DHT as the acid corroded all the fibres and rendered the shell smooth.

Variations in germination rate were noticed between the different GPC incorporated to the acid scarified seeds and in time (10 and 20 min) of soaking in acid. As concerns duration of soaking seeds in concentrated H_2SO_4, significant differences were noticed in germination capacity between 10 and 20 min at $P < 0.05$ for each treatment (Fig. 37).

Comparison of the effect of different GPC incorporated to the initially acid scarified seeds after DHT, shows that the highest germination capacity of 45 % was observed with H_2O_2 while the least (0 %) was noted in CH_2N_2 (Fig. 37).

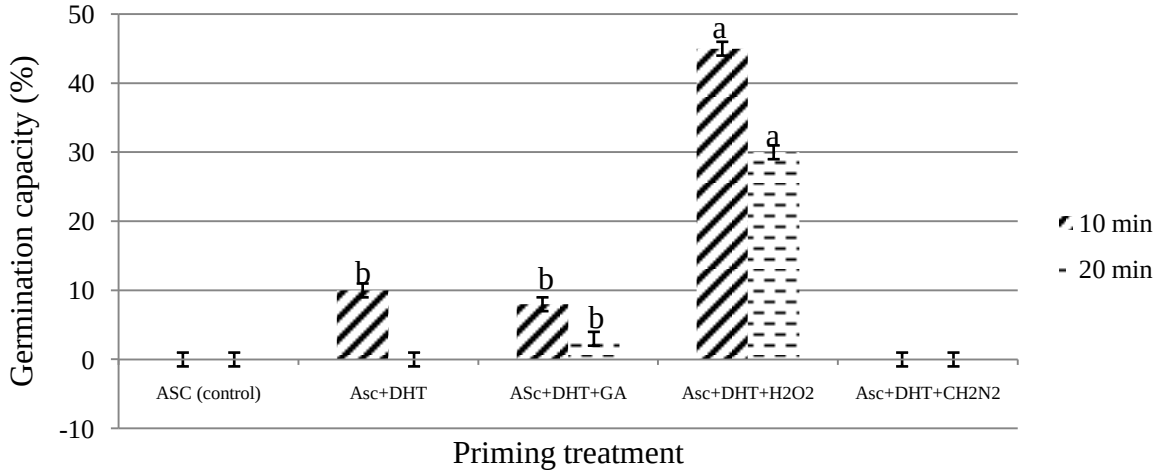

Fig. 37. Effect of incorporating DHT and GPC to different periods of acid scarification
Different letters on bars indicate significant differences ($P < 0.05$) between treatments for a specific time

III.1.3.2. Impact of pre-soaking in hot water on seed germination

No seed germination was observed after 60 days for all seeds that were pre-soaked in 100 ^{o}C hot water for 10 min followed by treatment with or without GPC.

III.1.4. Seedling production through embryo rescue

III.1.4.1. Embryo and culture medium browning

Throughout the present study, no colour changes in embryo or culture media were observed regardless of whether AC was supplemented or not.

III.1.4.2. Effect of light on *in vitro* germination

MZE were considered to have germinated if the haustoria developed into at least a shoot or a root axis (Fig. 38, and 42) throughout the study. Light had a negative impact on germination of MZE. Germination was positive in all culture media incubated in the dark 24 / 24 hours while all explants cultured under 16 / 8 hours photoperiod did not even germinate, rather the explants turned green, indicating the process of photosynthesis by MZE.

Table X. Effect of culture media and seed cultivar on germination potential of MZE

Culture medium	Mean germination/culture medium	Seed cultivar	Mean germination/oil palm cultivar
CM1	2.4 ± 0.15^{c}	Cv 1	4.0 ± 0.37^{a}
CM2	2.8 ± 0.01^{b}	Cv 2	2.9 ± 0.01^{c}
CM3	2.7 ± 0.05^{bc}	Cv 3	2.5 ± 0.12^{c}
CM4	3.1 ± 0.08^{b}	Cv 4	2.2 ± 0.22^{cd}
CM5	3.6 ± 0.25^{a}	Cv 5	4.0 ± 0.37^{a}
CM6	3.0 ± 0.05^{b}	Cv6	3.6 ± 0.24^{ab}
CM7	3.2 ± 0.11^{ab}	Cv7	3.5 ± 0.21^{b}
CM8	2.4 ± 0.15^{c}	Cv 8	0.8 ± 0.69^{e}
CM9	2.8 ± 0.01^{b}	Cv 9	4.0 ± 0.37^{a}
CM10	2.5 ± 0.11^{bc}	Cv 10	$1.2^{e} \pm 0.55$

Values are mean ± SD of five MZE cultured per Petri dish repeated thrice. Different letter superscripts in each column represent significant differences at $P < 0.05$ (DMRT).

III.1.4.3. Effect of culture medium on *in vitro* germination

Germination was observed in all the ten MS culture media (CM1- CM10) tested. Nonetheless, significant differences were observed between the culture media at $P < 0.05$ (Table X). All germinated embryos followed the same pattern of morphogenesis in all CM and seed Cvs (Fig. 38). However variations were noticed on three major aspects namely: speed of germination, rate of germination and level of organogenesis in the various culture media.

As concerns speed of germination, by the 7^{th} day, haustoria had formed in 60 % of explants that responded in CM5 while in CM1, the first haustoria were observed on the 10^{th} day after inoculation. This same pattern was noticed with the rate of germination. CM5 showed the highest germination rate of 72 % while the lowest (48 %) was noticed in CM1 (Fig. 39).

The rate of organogenesis was recorded 35 days after inoculation. Based on morphogenic response of MZE *in vitro*, five different levels of differentiation (Ld1-5) were categorised (Fig. 40 and 42). Ld1 consisted of explants that did not differentiate at all; the second level of differentiation (Ld2) comprised of those that differentiated into haustoria but did not develop further; the third level (Ld3) was MZE whose haustoria developed into a shoot axis only. Ld4 consisted of those whose haustoria completed development into a shoot and root axes while in Ld5, the haustoria developed only a root axis (Fig. 41). The medium with the highest percentage of Ld1 was CM1 (20 %) and the least was noticed in CM5 (3 %). For Ld2, the highest score was observed in CM10 (13 %) while the least was recorded in CM5 (1 %). Concerning Ld3, the optimal was found in CM3, CM6, and CM8 (100 %) while the lowest was noted in CM5 (10 %). CM4 and CM9 produced the longest but thin and lanky shoots measuring up to 2 cm. As for Ld4, positive results were found in six out of the ten culture media, precisely CM2, CM4, CM5, CM7, CM9 and CM10. However, the maximum scores were observed in CM5 (26 %) and CM10 (28 %). In all, Ld4 observed in CM5 and CM10 appeared highly compacted and robust while those of CM4 and CM9 were elongated and lanky. Regarding Ld5, the maximal of 10 % was observed in CM10 while the least of 0 % was observed in CM1, CM2, CM3, CM4, CM6 and CM8.

Generally, *in vitro* germination of MZE was more synchronized in all culture media and seed categories. Any undifferentiated MZE from stage 1 to 2 between 7-14 days of inoculation (Fig. 38) never showed any further development.

Fig. 38. *In vitro* morphogenesis developmental stages of MZE of oil palm

1. Excised embryo 2. Haustorium (7-10 days) 3. Shoot develops (14-21 days) 4. Shoot put under 16/8 h photoperiod (21-30 days) 5. Young seedling (31-45 days) ready for acclimatization. Bar= 5 mm

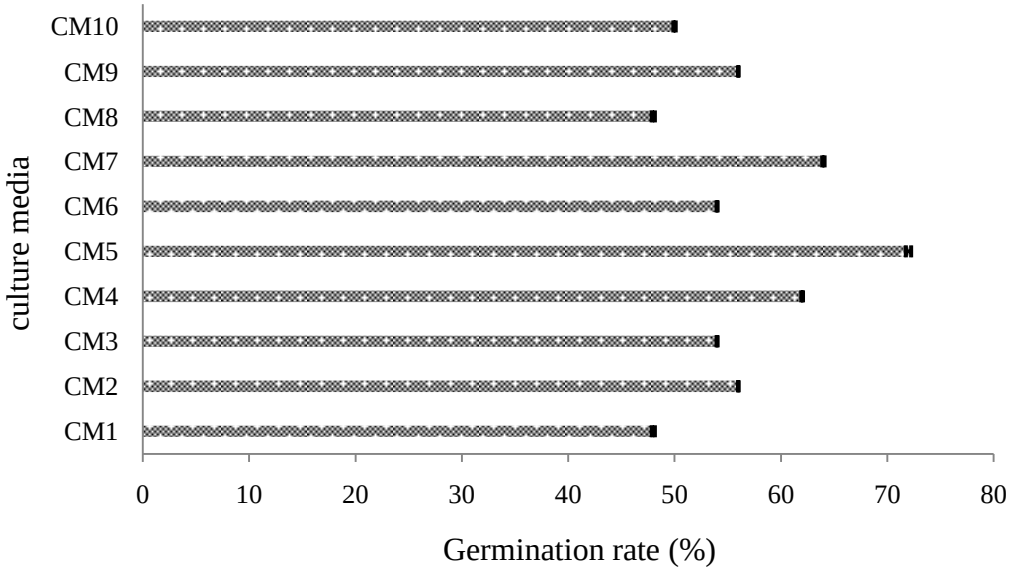

Fig. 39. Cumulative rate of *in vitro* germination of MZE per culture medium

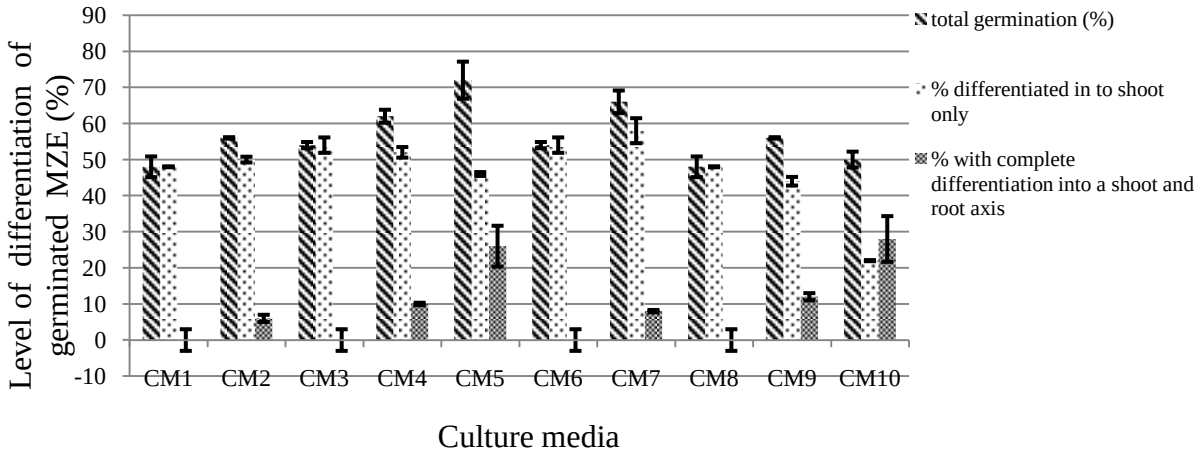

Fig. 40. Effect of culture medium on organogenesis of MZE of *tenera* cultivars

III.1.4.4. Reassessment of the four best CM in the first experiment

After identifying CM2, CM5, CM7 and CM10 as growth media that maximized embryo development with respect to organogenesis, a new experiment repeated 4 times was carried out in these four media. Three seed cultivars used were Cv1, Cv5 and Cv9 owing to their high germination potential. The results indicated that CM5 and CM10 did present higher % of both primary root and root hairs compared to CM7 while all germinated embryo in CM2 presented only the primary root without any root hairs (Fig. 41).

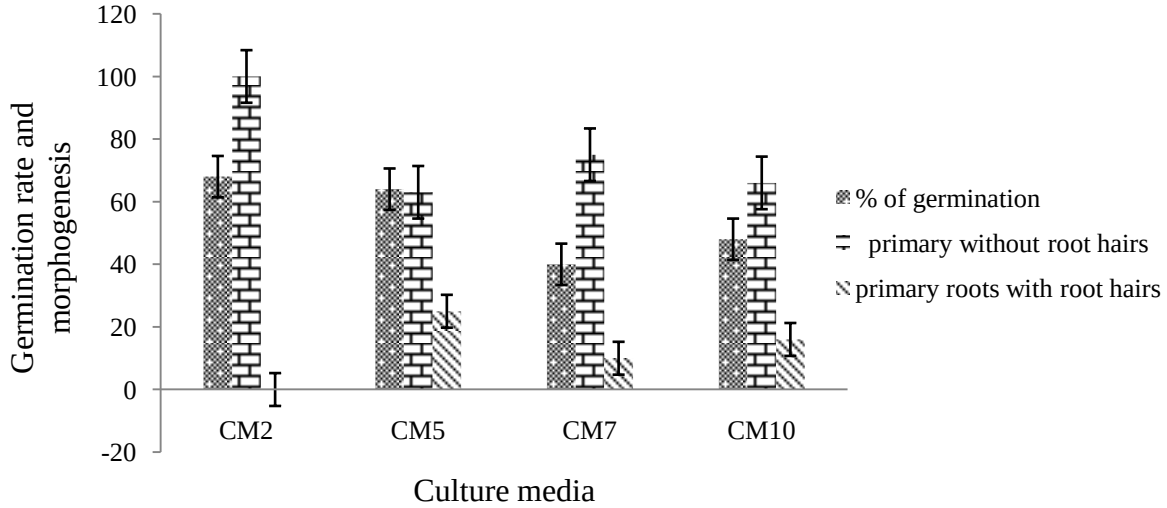

Fig. 41. Level of organogenesis in the four optimal culture media

III.1.4.5. Effect of media strength and supplements on MZE differentiation

III.1.4.5.1. Strength of MS medium

MZE germinated in both the full and half strength MS growth media (Fig. 40 and 41). Globally, MS strength had a significant impact on the level of germination, with ½ strength MS (CM6-10) averagely showing same germination rate as full strength MS (CM1-5). However, the effect of MS strength was better noticed in the two basic MS, CM1 (for full strength MS) and CM6 (for half strength MS) with sucrose being the lone additive. A significant difference in mean germination was recorded at $P < 0.05$ (Table X) between the two controls with CM6 recording a higher mean germination rate.

III.1.4.5.2. Activated charcoal (AC)

In the present study, MZE from between and within cultivars cultured in the presence of AC presented better results in terms of germination percentage and organogenesis. The latter were observed both in CM where AC was lone supplement (CM2 and CM7) and where other supplements were present alongside AC (CM5, and CM10) compared to their counterparts in CM void of AC (Fig. 39, 40 and 41). However, significant differences in mean germination were noted among CM supplemented with AC (CM2, CM5, CM7 and CM10) (Table X). In spite of these differences, plantlets that resulted from the AC supplemented CM did not only show complete differentiation into a shoot and root axis but were especially robust and produced root hairs (Fig. 40).

III.1.4.5.3. Vitamin

It was noticed that CM3 and CM8 in which lone supplement in MS was Morel vitamins complex, didn't show any significant difference to the control (CM1 and CM6) in terms of the total germination percentage and morphogenesis (Fig. 40).

III.1.4.5.4. GA_3

In the present study, no significant difference was observed in mean germination between CM6 (control for ½ MS) and CM4 or CM9 that were supplemented exclusively with GA_3. However, rhizogenesis was observed in CM4 and CM9 while it was completely absent in CM1 and CM6 (Fig. 40). Even though CM4 and CM9 presented complete germination, the shoot and root axes were extraordinarily lengthy and had an etiolated appearance.

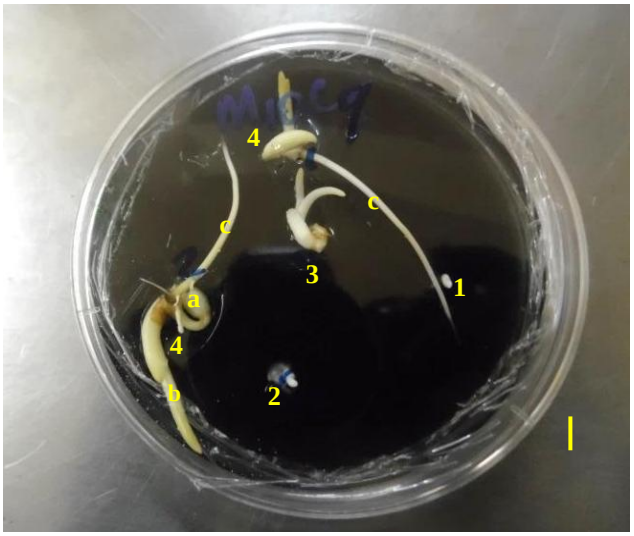

Fig. 42. Different levels of differentiation for MZE of the same seed category

1: Embryo failed to differentiate; 2: Differentiation stopped at haustorium stage; 3: Embryo differentiated only to a shoot axis; 4: Complete differentiation into a shoot and root axes. a. remnants of haustorium, b. differentiated shoot axis, c. differentiated root axis.
Bar= 5mm

III.1.4.6. Effect of cultivar on *in vitro* germination

All the ten seed cultivars responded to germination but at different rates (Table X) that ranged from 14 % (Cv8) to 90 % in Cv1 and Cv5 (Fig. 43). No significant differences were found among Cv1, Cv5, and Cv9, while significant differences were found to exist between the latter and the rest of the Cvs ($P < 0.05$) (Table X). Even though Cv1 had a high germination percentage, no roots were induced. MZE of cultivars (Cv1-7) whose pollen source was LM18106 appeared to generally give a better germination than in Cv8, Cv9 and Cv10 which were pollinated by LM19029 (Fig. 43).

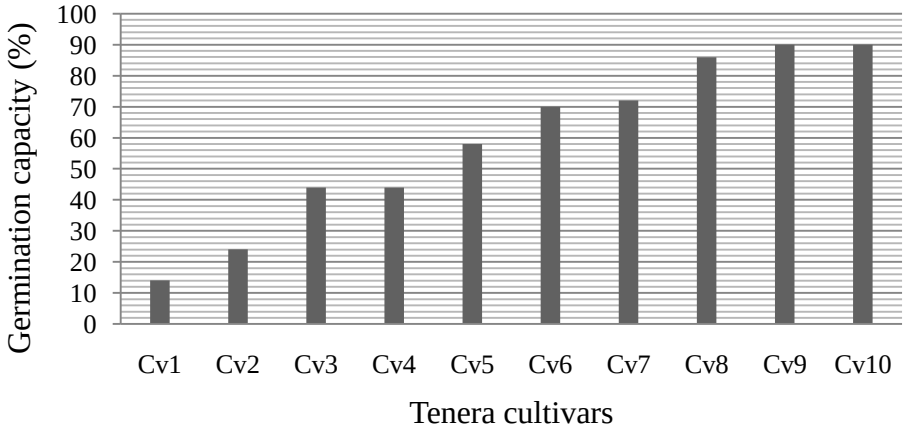

Fig. 43. *In vitro* germination responses of MZE of ten oil palm cultivars

III.1.4.7. Evaluation of survival rate of germinated *vitro* plantlets

III.1.4.7.1. Effect of some auxins supplements on rhizogenesis improvement

The problem of poor rhizogenesis (Fig. 44A) for over 70 % of germinated MZE was observed to be a major constrain in the applicability of embryo rescue tissue culture technique to produce oil palm *vitro* plantlets. Emergence of roots (Fig. 45A and B) in explants that were not completely differentiated i.e. MZE whose germination resulted only to micro shoots, as well as the growth of roots from MZE that completed differentiation during germination was highly influenced by the type and concentration of auxin supplement in full MS. Out of the three types of auxins employed, only IBA let to the initiation and emergence of roots from MZE with incomplete differentiation. In all culture media that received IAA and NAA, no root emergence was observed within the study period as in the controls where no auxin was added. However, significant differences were observed in rate of root emergence between the different concentrations of IBA, with the highest emergence (50 %) observed in culture medium supplemented with 1.5 mg L^{-1}, 25 % was observed in 1 mg L^{-1} and 0 % recorded in 0.5 mg L^{-1} (Table XI). The emerged roots from the raised *in vitro* micro shoots of oil palm were healthy thick and contained many secondary roots as well (Fig. 45B).

As concerns continuation of growth and development of roots from plantlets of Lot 2, no increase in root length was observed in explants inoculated in the control and IAA supplemented media (Fig. 45A). On the other hand, 1.0 and 1.5 mg L^{-1} NAA showed an average increase in initial length of 5 %. The samples inoculated in 1.5 mg L^{-1} IBA showed a significant increase of 100 % in their initial length (Table XI). Similarly, the rate of proliferation of root hairs was more profuse in 1.5 mg L^{-1} IBA (Fig. 45C) compared to other auxins regardless of the concentrations. No root hair development was observed in the control and culture media supplemented with IAA.

Fig. 44. *In vitro* germinated MZE

A. With incomplete differentiation into a shoot primordia only (Lot 1 explants)

B. With complete differentiation in to a root and a shoot bud (Lot 2 explants).

1. Remnants of haustorium 2. Shoot bud 3. Primary horizontal root, Bar = 5 mm

Table XI. Effect of auxin types on root induction and development of *vitro* plantlets

	control		IAA		IBA			NAA		
	0	1	2	3	4	5	6	7	8	9
Shoots (Lot1) that rooted (%)	0	0	0	0	0	16	50	0	0	0
Mean increase in initial length (%) for Lot 2	0	0	0	0	10	25	100	0	10	10
Proliferation of root hairs for Lot 2	0	0	0	0	+ +	+ + +	+ + + + +	+	+	+ +

Numbers 0-9 represent different experiments

\+ rate of root hair proliferation

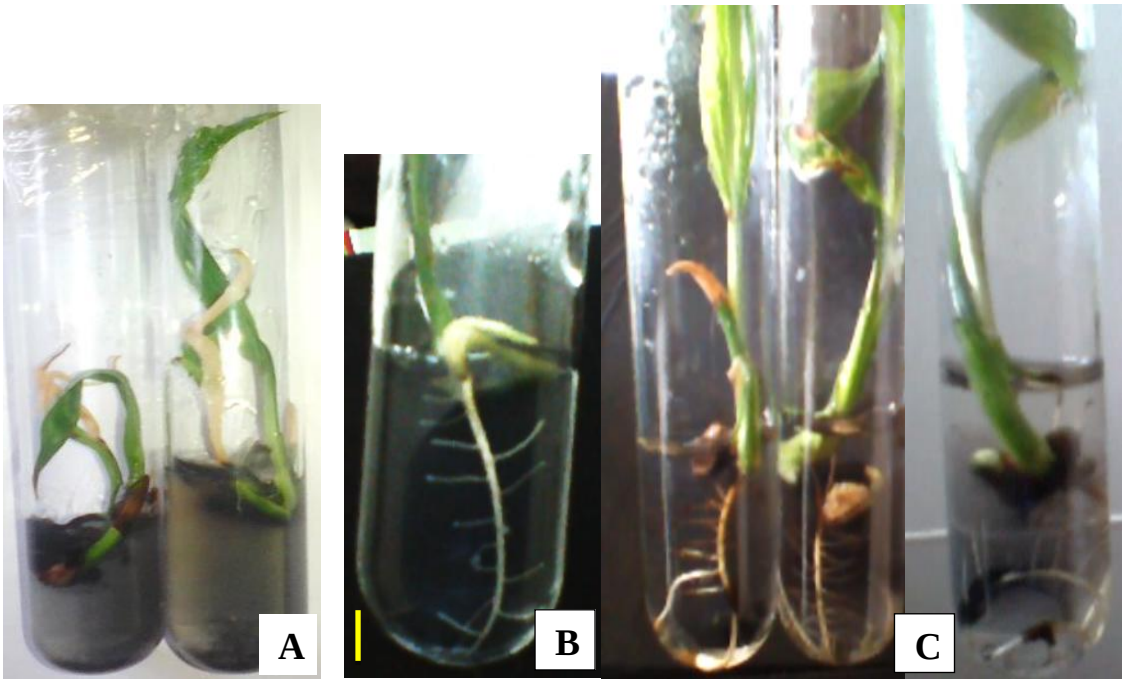

Fig. 45. Influence of different auxin types on root initiation and development of *vitro* plant:

A. Failure to induce roots in Lot 1 B. Emergence of roots from Lot 1 (notice the complete whitish nature of the root) C. Elongation of roots in Lot 2 (notice the brownish colour of the upper part which is the older root and the whitish colour of the lower part of the root which represents the new root). Bar = 1cm

Within 3 months, the rooted *vitro* plantlets under acclimatization had developed 3 new leaves (Fig. 46C). The success rate following acclimatization was averagely 70 %.

III.1.4.7.2. Assessment of *vitro* plantlets vigour in the nursery and in the field

Survival rate in the nursery was 70 %. All survived *vitro* plantlets showed normal growth with robust collar and well developed leaves in the nursery. After 7 months in the nursery, an average of 6 leaves out of which 4 had completely differentiated in to fronds appeared ready for the field. The *vitro* plantlets were later transplanted in the CEREPAH seed garden for growth parameters to be taken in perspective.

After transplanting, field survival was rated at 90 %. Besides after fifteen months in the field, preliminary growth parameters revealed that the *vitro* plants emanating from embryo rescue of MZE presented normal growth (Fig. 47A) as plants issued from the conventional practice. A note worthy observation was the production of the first inflorescences (Fig. 47B) in 40 % of the total population of *vitro* plants 15 months after transplanted.

A

B

C

Fig. 46. Acclimatization of *vitro* oil palm plantlets

A. One month old *vitro* plants under photoperiod 16/8h ready for hardening
B. *vitro* plants transferred to a natural substrate (sterilized soil + coffee chaffs)
C. Four months old hardened *vitro* plantlets

Bar = 1 cm

A B

Fig. 47. *Vitro* plants fifteen months after transplanting

A. Whole plant
B. Bole of *vitro* plant with first inflorescence
   Arrow: Inflorescence still enclosed by the bract

III.1.4.8. Comparison of oil palm seedling production through DHT and MZE rescue

Averagely, 120 days are required to obtain germinated seeds through the conventional DHT circuit, while an average of 40 days was needed to obtain *vitro* plantlets from direct regeneration via rescue of MZE. In addition, regeneration of *vitro* plantlets through embryo rescue was more synchronized than the DHT. Unfortunately, out of the 90 % MZE that germinated, merely 10 % *vitro* plantlets could survive at the time of transplanting to the field. While in the field, preliminary growth parameters of *vitro* oil palm plantlets showed normal growth with oil palm seedlings derived from conventional method.

III.2. Discussion

III.2.1. Probable causes of low germination in oil palm

III.2.1.1. Variation in germination capacity among cultivars

Generally, the slow and low seed germination in oil palm is due to intensive dormancy. Besides, its erratic nature of germination is caused by variations in level of dormancy among seeds of the same FFB (Rees, 1962). Hussey (1958) attributed slow and low germination in oil palm on the endocarp's hard and dense consistency which gives mechanical strength to resist the absorption of oxygen. Analogically, the thicker the diameter of the shell and endosperm at the micropylar end, the more the difficulty faced by the embryo to perceive external factors of germination, the more severe the dormancy which then culminate in slower germination capacity. In a recent study, Norsazwan et *al*. (2016) has shown that oil palm seeds have a combination of both morphological and physical dormancy.

The results of biometric parameters in the present study indicate that great variations exist in the ten Cvs of commercial oil palm seeds produced by CEREPAH. Variations noticed in terms of shell thickness of the different cultivars (genotype sh^{+}/sh^{-}) could explain variations obtained in germination capacity. The phenotype and genotype of a given tenera hybrid depends on the origins of dura and pisifera parents (Hartley, 1977). Though all the oil palm Cvs produced at La Dibamba are derived from crosses between two BPROs (Deli x La Mé) (Rosenquist, 1986), there are many origins, implying that the tenera variety may have several sub forms or cultivars. In an earlier study, Rajanaidu (1987) reported the existence of variability and morpho-agronomic similarities between tenera material originated from Tanzania and Madagascar. According to the author, one of the most significant variations among the populations was related to fruit weight, which averaged 1.6 g for the accessions from Madagascar and 8.5 g for those from Tanzania. This great variability in weight was also observed in the morphological analysis of both seeds and kernels in the present study. The biometric analysis showed high variance in seed mass, with mean values ranging from 1.9 to 3.4 g and from 0.3 to 0.8 for kernels. Similar results were obtained by Akinoso & Raji (2011) in which the mean mass of seeds varied between 1.9 and 3.7 g, and that of kernels between 1.1 and 1.7 g. The latter was higher than registered in the present study. The present study revealed a shell thickness range of 2.4 - 3.2 mm. The results are different from that reported by Akinoso & Raji (2011) who observed a mean endocarp thickness for tenera to vary from 0.5 to 2 mm.

Variations in biometric analysis of morphological parameters noticed in seeds of different tenera cultivars in the present study could therefore be associated to the different origins of their parents. Given that all the seeds produced in La Dibamba have the same broad background derived from Deli x La Mé crosses, it is clear that from the genetic base and diversity points of view, Deli and La Mé alongside Yangambi, AVROS, Binga, Calabar constitute Breeding Populations of Restricted Origins (BPROs) (Rosenquist, 1986). Within each BPRO, there are many origins and within the same origin there are many progenies that can be traced back to distinct, often small groups of wild or unimproved ancestral palm. Differences in origin could therefore explain the variation in level of germination which is directly proportionate to the degree of dormancy among other factors.

III.2.1.2 Effect of seed position on oil palm seed germination

In commercial seed production process, the heterosis effect is exploited by crossing Dura (D) x Pisifera (P) parents to produce improved oil palm hybrids characterised with high quality and quantity palm oil (Hartley, 1988). The disparity that exists between demand and supply of improved oil palm seeds can only be bridged by improving on germination capacity which is generally very low, ≤ 20 % under natural conditions (Rees, 1962).

Apical seeds significantly showed a higher germination capacity than basal seeds both in open and assisted pollinated FFBs sampled. This indicates that the position of seeds on the spikelet influences germination rate in oil palm. Many factors have been highlighted in an attempt to explain seed position-dependent variations in germination capacity. Amongst which are; unequal allocation of resources to all seeds on same fruit or bunch (Datta et *al*., 1970), seeds produced at one position (e.g. at the base of an inflorescence), developing under different micro environmental conditions than those produced at another position (e.g. at the top of an inflorescence) and differences in physiological age of the mother plant at the time seeds are produced (Baskin & Baskin, 2001). The fact that within a given oil palm FFB, some seeds undergo abscission before others could be an indication that ripening time of FF on the same bunch does also vary (Kingsley et *al*., 2016). This may account for the significant difference in germination capacity between apical seeds that usually naturally abscised before basal seed in the oil palm. The result obtained in this study seem to be consistent with the work of Kaida & Zulkefly (1992) who reported that in the oil palm, ripening begins from the apical to the basal part of the FFB and from the outer to the inner halve of the spikelet. Based on the latter report seeds that abscised early analogically lost their dormancy before those abscised later, explaining the disparity in germination. Besides, the higher germination capacity in apical seeds could be

explained by the fact the seeds attained maturation dryness, characterized by reduction in water and ABA before basal seeds at the time of harvest. Bewley & Black (1994) reported that, low ABA concentration and water during drying phase of seed development decreases dormancy. This probably accounts for significant germination capacity observed in apical seeds.

The results also revealed a higher germination capacity in assisted pollinated samples than open pollinated samples. This result corroborates with the findings of Santos et *al.* (2015) who reported higher germination in passion fruits seeds derived from assisted pollination than in seeds derived from open pollination. Better germination performance in seeds derived from controlled than open pollinated FFBs could be explained by the fact that the parents (Pisifera x Dura) crossed have been carefully studied and identified to show hybrids vigour in seed emergence, biometric parameters and resistance to environmental conditions. Three out of the five controlled pollinated samples appreciably distinct their germination scores from the two other samples with low GP. This variation could be attributed to the origin of the pollen source, given that the three samples with higher GP both in apical and basal seeds had the same pollen source. This variation observed ties with the work of Breure (2006) who reported that even though tenera is the main planting material, there exist several Cvs of tenera whose variation in form is due to the diversity in origin of D x P parents.

It was also noticed that seed position does not only affect germination capacity but also morphology of seeds. Significant variations were observed both in number, biometric parameters and GP between apical and basal seeds in open pollinated FFB. In spite of the higher % in terms of numerical strength (57.9 % against 42.1 %), seeds from the base of the spikelet presented a lower average fresh biomass 2.71 kg (≈ 43.3 % in a FFB) compared to 3.65 kg ≈ 56.7 % FW of all seeds in a FFB for apex seeds. This result ties with other findings in which heteromorphism in seed, number, mass, dormancy and germination had been reported for seeds found at different positions within an inflorescence in some plant families like *Asteraceae* (Baskin & Baskin, 2001) and *Poaceae* (Datta et *al*., 1970). Similar variations had been reported by Breure (2006) who attributed variations observed to different origins, genetic and selection criteria of the pisifera and dura type. Teo et *al*. (2004) confirmed that there are many types of dura and pisifera in the world which are also known as parent material. The variations noticed in open pollinated samples in the present study endorse the assertion that a natural hybrid between different parent materials produces large variety of planting material (Esnan et *al*., 2004). In different plant species, maternal factors, such as the position of the inflorescence on the mother plants or the position of the seeds in the inflorescence or in the fruit, can markedly influence the germinability of seeds (Gutterman, 1992a; 1994a).

III.2.1.3. Influence of harvesting date on oil palm seed germination

Some earlier studies on this subject hold that the dynamics of dormancy / germination depend on stage of maturation and ripeness of seeds at the time of harvest (Do Cao et *al*., 1978). In the present study it was observed that FFB samples harvested 7 months after controlled pollination presented a higher germination capacity compared to FFB samples harvested one month earlier. This result could be explained by the fact that the degree of dormancy or germinability of a seed lot depends on the physiological maturity of the embryo at harvesting (Gutterman, 1980). The fact that a mean of 115 FF had abscised prior to harvest of FFB allowed for 7 months, compared to an average of 7 loose FF for sampled FFB left for 6 months could mean that embryos from FF harvested after 7 months were more mature. This latter accession corroborates with Gutterman (1992) who reported that ripe fruits or seeds that abscise off from the mother plant or bunch on their own appear to have a high germination capacity than those harvested before their natural abscission.

There seem to be a wide scope as far as appropriate harvesting date of oil palm FFBs is concern among oil palm researchers. However, Mesocarp colour is one of the key indicators among the several grading methods used to gauge ripeness of oil palm FFB (Singh et *al*., 2014). Generally, mesocarp colour changes from green to orange for *virescens* at maturity while in *nigrescens* type the colour change goes from black or brown cheek colour for immature fruits to red. The use of mesocarp colour as a criterion to guage ripeness is based on the stage at which optimal quantity and quality crude palm oil can be extracted from FF (Mohd et *al*., 2012). Nonetheless, ripeness of the mesocarp does not necessarily mean maturity of the embryo, which actually affects the rate of progeny production for the next generation through seed germination. Another method that appears to give more accurate gauge for ripeness is the number of empty fruitlet sockets. Somehow researchers and plantations also defined oil palm FFB ripeness based on the number of fruit detached from a bunch. If more than 10 % of total FF in one bunch detach, the bunch is categorised as ripe (Choong et *al*., 2006). On the other hand, Ng & Southworth (1973) considered 20-25 % detached fruit / FFB for ripeness. It should however be recalled that, oil palm fruit abscission depends on several factors amongst which are, normal ripening, sudden changes in physiological conditions associated with environmental changes and chemical changes, artificial termination of bunch development and genetic make-up of plants which produce FFB that detach FF before maturity (Flingoh & Zukarinah, 1985).

In all, naturally abscission decreases water and ABA content of FF as well as maximize maturity of the embryo. These are critical internal factors for an embryo to attain

optimal germination potentials and survival of seedling (Bewley & Black, 1994). Nonetheless, variation in level of dormancy / germination within a seed lot remains a strategy for survival of the species. It will therefore be very difficult if not impossible to have all seeds in a lot germinate at the same time regardless of the pre-treatment method used.

III.2.2. Effect of incorporating GPC to the conventional DHT

Based on criteria used by Baskin & Baskin (2004) to classify dormancy types, the oil palm seed can be categorized under physical and non-deep physiological dormancy. The routine use of scarification by the DHT appears to rupture mainly the physical barrier and to some extent, the physiological aspect. Halimah et *al*. (2014) holds that heat treatment is essential for breaking the dormancy of oil palm seeds but that for germination to occur, an optimum moisture content, temperature and oxygen level are needed. All control experiments in the present study were subjected exclusively to DHT and significant variations were recorded in germination capacity, time and rate between the seed cultivars. Nonetheless, germination capacity was lower in controls than in GA_3 and H_2O_2 but higher than in CH_2N_2.

Anatomical studies have revealed that the endocarp of seeds with physical dormancy is made up of three water impermeable palisade layers namely brachysclereid, osteosclereid and macrosclereids from outside to inside bound by an outer layer called the crystalliferous layer (Wannan & Quinne, 1990). Apart from the scar, the carpellary micropyle i.e. site of rupture from where the radicle protrudes during germination is the only areas on the endocarp that differ anatomically from the remainder of the endocarp. This is because brachysclereid, and osteosclereid are shorter while the monosclereids are not elongated compared to other parts of the endocarp. It is for this reason that carpellary micropyle is said to be anatomically weaker portion of the endocarp besides its thinnest (Li et *al*., 1999). The major mechanism of breaking physical dormancy by DHT is centred on the fact that heat causes the formation of a blister near the carpellary micropyle via uplifting of the osteosclereid and brachysclereid layers. During blister formation, a slit is formed in these layers, thereby creating an opening for water entrance. Once broken, the seed coat becomes permeable to water and the seeds can germinate over a wide range of ambient conditions (Baskin et *al*., 2000). Besides, heat probably causes modifications at the level of the endosperm that enable the embryo to start making use of the reserves. The fact that germination capacity was lower in control than when DHT was complemented with GA_3 and H_2O_2 probably indicates that DHT removes only physical dormancy and when GA_3 and H_2O_2 were incorporated, non-deep physiological dormancy was relieved enhancing thus a higher germination than in the control treatment.

However significant variations observed between the three GPC used in this study is an indication that there is some divergence in their degree of action. This may be because they are implicated in different physiological processes and metabolic pathways of growth and development. Among all plant growth regulators, ABA and GA have a considerable influence on seed maturation, dormancy and germination and they act antagonistically (Finch-Savage & Leubner-Metzger, 2006). The role of GA in breaking all forms of physiological dormancy has been documented in several studies. Hartmann et *al*. (1997) reported that physiological dormancy in seeds is closely related to the proportion between inhibitors especially ABA and growth regulators especially gibberellins. Higher ABA / GA ratio blocks germination activation processes like enzyme activation, reserve mobilisation, respiration, RNA and protein synthesis; this makes the dormancy deeper. On the other hand, higher GA / ABA ratio activates metabolic pathways that favour seed germination (Eira & Caldas, 2000). Nonetheless, it has been reported that the quantity of gibberellin that is necessary to promote germination depends on the degree of restriction of embryo elongation imposed by the endosperm and tegument (Debeaujon & Koornneef, 2000).

In the present study, incorporation of different concentrations GA_3 to DHT significantly improved oil palm seed germination than the control and some concentrations of other GPC. This increase in germination capacity might be attributed to the fact that exogenous supplementation of GA_3 elevated the endogenous GA / ABA ratios, hence increasing the sensitivity of embryo to GA_3 than to ABA thus enhancing germination. Similar results had been obtained by synchronizing GA_3 with different scarification pre-treatments like heat and acid stratification as well as removal of seed coat (Çetinbaş & Koyuncu, 2006). The promoter effect of GA_3 on germination is centred on its ability to induce synthesis of genes that up-regulate some cell wall-modifying proteins in the embryo to promote the growth potential of the embryo during seed germination. After testa rupture, endosperm must be further weakened prior to radicle protrusion. Extensive studies have been carried out on cell wall-modifying proteins involved in endosperm weakening, including expansins, xyloglucan endotransglycosylase / hydrolase (XTHs), pectin methylesterase, mannanase, cellulase, etc. (Nonogaki et *al*., 2000). Strong evidence show that de novo-synthesized GA in the micropylar endosperm after imbibition is required to induce the expression of the genes encoding the above-mentioned enzymes or cell wall proteins to weaken endosperm, thus relieving the inhibitory effect imposed by ABA (Ogawa et *al*., 2003; Muller et *al*., 2006). For example, in an earlier study, a higher GA / ABA ratio, gave GA its ability to up-adjust the expression of two expansin genes in tomato (*LeEXPA8* and *LeEXPA10)*, to promote the embryo growth potential

(Chen et *al*., 2001). Though germination was improved with incorporation of GA, variations in germination rate were noticed in the different concentrations of GA_3 applied. This may be associated with the asynchronous manner in which dormancy is overcome in each seed lot. With respect to the seeds that demonstrate physiological dormancy, the quantity of GA_3 necessary to promote germination will be dependent on the degree of restriction imposed on embryo elongation by the structural characteristics of the endosperm and tegument, which may vary in function for each individual and environmental condition as well as over time (Finch-savage & Leubner-metzger, 2006).

The use of H_2O_2 as a priming factor has been reported in a number of studies (Wang et *al*., 1998; El-Maarouf-Bouteau, 2007; Bahin et *al*., 2011). H_2O_2 is one among other Reactive Oxygen Species (ROS) like nitric oxide, hydroxyl radicals and superoxide radicals known to play a dual role in plant physiology, based on their doses. High doses are dangerous as they appear to orchestrate programmed cell death (Dat et *al*., 2003) while at low doses they present beneficial effects in vital signalling of metabolic pathways. However, ROS are continuously produced during germination of several species. To avoid ROS toxicity, plant tissues possess internal regulators that moderate ROS accumulation through the process of scavenging. It has been found that when there is a meticulous balance between production and scavenging such that ROS accumulation is tightly regulated, these toxic molecules become beneficial to plants by promoting germination (El-Maarouf-Bouteau, 2007).

In the present study, it was shown that prior subjection of seeds in DHT followed by imbibitions in 0.5, 1 and 1.5 % H_2O_2 for 48 hours positively influences germination more that the control. Similar results had been obtained in earlier works showing that exogenous application of H_2O_2 improves seed germination in both dormant and non-dormant seeds (Ogawa & Iwabuchi, 2001; Barba-Espín et *al*., 2010; Bahin et *al*., 2011). This implies that the synergistic effect of DHT and H_2O_2 is beneficial than their individual effect. While DHT overcomes physical dormancy, H_2O_2 overcomes physiological dormancy. The results of the present study contradicts the findings of Bougré et *al*. (2011) who reported lower germination capacity by soaking oil palm seeds in H_2O_2. The low germination capacity registered by Beugré et *al*. (2011) might have been provoked by very high exogenous concentrations of H_2O_2 (5 and 10 %) used in their study compared to the 0.5, 1 and 1.5 % H_2O_2 in the present study. It has been reported that the effect of ROS is dose-dependent; at high dose i.e. when their accumulation is greater than scavenging, they cause programmed dead of cells while if production is equal to scavenging, it promote metabolic activities like germination (Dat et *al*., 2003). H_2O_2 brings about specific changes at proteome, transcriptome and hormonal levels,

resulting in an acceleration of the germination process most probably because of invigoration of the seeds (Barba-Espín et *al*., 2011). The positive influence of H_2O_2 on germination is thought to adhere to the fact that H_2O_2 promotes protein carbonylation and lipid peroxidation. Protein carbonylation is considered as a potent biomarker of oxidative stress, which can play regulatory roles, such as seed dormancy alleviation (El-Maarouf-Bouteau et *al*., 2007). During lipid perodixation, lipoxygenases enzymes catalyze the hydroperoxidation of polyunsaturated fatty acids to produce unsaturated fatty acid hydroperoxides. The hydroperoxides are subsequently metabolized via secondary pathways to form bioactive compounds for the embryo (Mo & Koster, 2006).

Besides, H_2O_2 has also been reported to play an important role in decreasing the concentration of dormancy-promoting plant hormones like ABA and ethylene throughout imbibition (Wang et *al*., 1998; Barba-Espín et *al*., 2010). ABA inhibits post-germination growth water uptake, endosperm rupture, and further embryo extension and seedling growth after radicle emergence (Kucera et *al*., 2005). Ethylene on its part inhibits stem elongation, increased stem thickening and a horizontal growth habit as well as inhibition of leaf expansion and normal opening of the epicotyl hook retarded (Ross, 1992). Barba-espín et *al*. (2010) reported a 70 % decline of ABA in Pea seeds soaked in H_2O_2 while those imbibed in water recorded ABA decline of 40 % at 24 hours post-imbibition. Therefore, when imbibed in H_2O_2, the latter could act directly or indirectly by impairing ABA transport from the cotyledon to the embryo, hence, activating the germination process. A decrease in ethylene concentration in H_2O_2-imbibed seeds (Barba-Espín et *al*., 2011) could favour opening of epicotyl and radicle emergence. On the other hand, H_2O_2 treatment increases the doses of IAA, JA, and SA as the period of imbibition increases (Barba-Espín et *al*., 2011). All these hormones are known to promote cell division.

Out of the three GPC in the present study, CH_2N_2 presented the least germination capacity and the longest MLIT in all cultivars compared to GA, H_2O_2 and even the control. This result shows that CH_2N_2 has an inhibitory rather than a promoter effect on germination of oil palm seeds even at concentrations as low as 0.5 %. In a similar study, Junior et *al*. (2013) reported a lower germination percentage in *A. aculeate* seeds immersed in 0.5, 1, 1.5, 2 and 2.5 % CH_2N_2 with embryo mortality rate increasing with concentration of CH_2N_2. In a related study using peach-palm (*Bactris gasipaes*), CH_2N_2 harmed germination even for short exposure time (Villalobos et *al*., 1992). However, the results of the present study are contrary to those reported by Herrera et *al*. (1998) in oil palm where very high germination percentages were reported when seeds were treated with CH_2N_2. The low germination observed in this

study could be attributed to the higher concentration of the commercial formula used (50 %) compared to the 49 % active ingredient used by Herrera et *al*. (1998). Guevara et *al.* (2008) reported that at higher concentration, CH_2N_2 increases the concentration of ABA in the endosperm. ABA being a dormancy promoting hormone could probably be responsible for the lower germination capacity and delay observed when oil palm seeds were treated with CH_2N_2 in this study. This might be possible because over 90 % embryos were still viable when CH_2N_2 treated seeds that failed to germinate after 120 days were subjected under TTZ test. Such high embryo viability for CH_2N_2 treated seeds that refused to germinate, could mean that CH_2N_2 treatment instead aggravated the dormancy rather than break it, affirming the report of Guevara et *al.* (2008).

Out of the three GPCs integrated to DHT, H_2O_2 at concentration of 1 % appear to be the best in terms of germination capacity (Table VII), time (Table VIII) and speed (Table IX). Generally, variations noticed in germination capacity, mean length of incubation time and coefficient of velocity (Table VII, VIII and IX) within seed cultivars that received the same treatment explains the erratic behaviour of seeds and may be attributed to variations in level of seed dormancy existing within the same oil palm bunch. Meanwhile variations between the different cultivars can be ascribed to variation in genetic composition.

III.2.3. Acid and hot water scarification as alternative methods of breaking dormancy

Naturally, the oil palm seeds have a low germination rate due to the physical dormancy, caused by the endocarp's hard and dense consistency, which gives mechanical strength to resist the absorption of oxygen, thus preventing the elongation of the embryo (Hussey, 1958). It has been shown that scarification increases the germination percent of palm seeds with water-impermeable hard endocarp (Nagao et *al*., 1980; Murakami & Rauch, 1998). Acid scarification has been reported to successfully eliminate physical dormancy in several plants and it is recommended to break dormancy only for seeds that are very hard to germinate due to intense physical dormancy (Meerow, 1990). Acid treatments mimic part of a biological method of seed scarification. Seed coats can be effectively broken down by chemical means using concentrated sulfuric acid, the chemical most commonly used (Willan, 1985).

In the present study, no germination was observed after 60 days in oil palm seeds that were soaked for 10 and 20 min in 95 % H_2SO_4 without prior exposure to DHT. Even when the seeds were further treated with GPC (GA_3, H_2O_2 and CH_2N_2) no germination was observed after 60 days. These results do not tie with the work of Herrera et *al*. (1998) who reported 5 % germination capacity in oil palm seeds immersed in sulphuric acid for 10 min and 85 % when

acid scarified seeds were further submerged in 0.75, 1.5 and 2 % hydrogen cyanamide. The result of the present study do not also agree with the finding of Myint et *al*. (2010) who reported 80.66 % germination for tenera seeds that were mechanically scarified by mere removal of operculum without DHT. The complete absence of germination for seeds that received acid scarification as the lone pre-germination treatment method could be explained by the fact that only the endocarp; first barrier, was eliminated while the endosperm; the second barrier, was still intact. The several-layered oily endosperm might have therefore totally or partially prevented the embryo from perceiving the threshold of environmental factors needed to trigger the germination process. The success of acid scarification reported in other seeds could probably be due to the fact their endosperms are not stony as in oil palm.

The fact that germination only occurred when acid scarified seeds were further treated with DHT is an indication that heat probably weakens both the endocarp and endosperm allowing the embryo to access environmental factors that stimulates germination at threshold values. Similarly the higher germination capacity obtained with a third treatment (imbibitions with H_2O_2) than when DHT was supplemented uniquely could infer that oil palm also show non-deep physiological dormancy, which is eliminated by H_2O_2. Ellis et *al*. (1983) found that the effect of the GA_3 in promoting germination could be improved by an additional pretreatment in 0.5 M hydrogen peroxide (H_2O_2) before the GA_3 pretreatment.

Complete absence of germination in all seeds scarified by soaking in hot water at 100 ^oC could be an indication that this method does not allow the miniature plant to perceive environmental signals at quantities adequate enough to trigger growth.

III.2.4. Production of *vitro* oil palm planting materials through embryo rescue

III.2.4.1. *In vitro* germination potential of MZE

Generally, during the initial stages of tissue culture, excessive polyphenols are produced. The oxidation of the exuded phenolics often results to browning which blocks uptake of nutrients with consequence ranging from retarded development to death of explants (He et *al*., 2009). Browning exudates is a defence mechanism of the explants in response to wounding one or more portions of its tissue (Pan & van Staden, 1998). In the present study no browning was observed in all culture media. This result does not tie with the work of Cattelan et *al*. (2007), who stated that oil palms are rich in substances of secondary metabolism origin, like polyphenolic compounds which in *in vitro* culture cause tissues to suffer oxidation due to polyphenolic oxidase enzyme. Activated Charcoal (AC) has been reported to solve the problem

of browning in tissue culture media and its effectiveness depends on its concentration and the kind of explants (Thomas & Micheal, 2007; Yuying et *al*., 2013). The fact that embryos did not produce browning exudates even in CM void of AC in the present study ties with the work of Ozyigit (2008) who reported that a positive correlation between age of plant and exudation of phenols with explants from younger tissues producing lesser exudates. Other works have also reported that younger cells produce less phenol while older cells produce much in terms of quantity and quality (Leng et *al*., 2009). This means that explants from different parts of the same plant can display great differences in their level of response *in vitro*. However, limited wounding on the explants during the process of excision could account for complete absence of phenolic exudates production and discoloration noticed in this study.

It was also observed that, explants cultured under light did not germinate; rather, they turned green indicating, photosynthesis instead of differentiation. This implies that light is not a prerequisite for *in vitro* germination of MZE of oil palm and this may probably be due to the fact that the natural site of embryo 'inside nut' is dark. This result does not tie with that of Mohammed et *al*. (2013) who reported successful germination of *Cocos nucifera* embryos cultured under light even though with a comparatively lower germination percentage when compared to embryos germinated in the dark.

It was found that the strength and composition of CM impacted the rate of germination and organogenesis of MZE of oil palm. As concerns the strength, ½ MS (CM6) gave a higher germination than full MS (CM1). In a related study, North et *al*. (2011), reported a non-significant difference between ½ and full strength MS even though ½ MS showed a fairly higher germination rate compared to full MS in *in vitro* germination of immature embryos of bird of paradise (*Strelitzia reginae).* Higher germination % in ½ MS (CM6) than full MS (CM1) may be attributed to a relatively balanced osmotic potential of ½ MS and MZE relative to that of full strength MS.

As concerns the effect of CM composition on germination and organogenesis, variations were found when AC, GA_3, and Morel vitamin were supplemented either singly (CM2 and CM7) or in synergy (CM5 and CM10) to the base medium. AC has been widely used in tissue culture of several plant species and it has been reported to play a capital role in reducing explants browning. AC has a very fine network of pores with large inner surface area on which many substances can be adsorbed (Madhusudhanan & Rahiman, 2000; Birmeta & Welander, 2004; Wang et *al*., 2006; Thomas, 2008; Yuying et *al*., 2013). All CM supplemented with AC in the present study gave compact and robust seedlings (CM2, CM5, CM7 and CM10). Similar results had been found by Alves et *al*. (2011) who also observed that

Oil palm embryos cultured in the presence of activated charcoal showed a better development in stem length and root number. This result affirms the report of Van Winkle and Pullman (1995) who pointed out that apart from mere adsorption; AC demonstrates a greater plant survival, greater plant growth, and improved plant quality and vigour. In a similar study, rooting was promoted in culture media with AC than those without AC (Parris et *al*., 2012). Promotion of root formation by AC has also been associated to its ability to exclude light from the medium (Pan & van Staden, 1998). The promoting effects of AC on organogenesis of MZE in the present study could be attributed mainly to its irreversible adsorption of inhibitory compounds secreted by cultured tissues (Fridborg et *al*., 1978) and substantially decreasing the toxic metabolites, phenolic exudation and brown exudate accumulation (Thomas, 2008).

It was also found that in CM where Morel vitamin was the only supplement (CM3 and CM8), no significant difference with control in rate of germination was observed. This implies that vitamin supplementation is not an indispensable component in direct organogenesis of MZE of oil palm *in vitro*. This result agrees with that of Abrahamian & Kantharajah (2011) who reviewed that in as much as plant species and cultivars require different amounts of vitamins *in vitro*, others don't need any. If Morel vitamin complex supplemented in the present study did not make a significant impact on growth, it is probably because the quantity of essential vitamins produced endogenously by the explants might have just been enough to pilot physiological events that enhanced growth and development of MZE.

It was observed that supplementing GA_3 singly (CM4 and CM9) had no significant impact on germination but rather on differentiation of MZE *in vitro*. This result holds with the assertion that plant tissues can grow and differentiate without GA_3 even though the latter may become an essential ingredient when culturing cells at low densities (Stuart & Street, 1971). The fact that GA_3 did not influenced germination could mean that endogenous GA_3 produced by MZE was just enough to assist in germination while an exogenous GA_3 supplement is required for further development. Nonetheless, GA_3 supplemented CM produced root hairs. Similar results had been reported by Montoliu et *al*. (2010) in *in vitro* adventitious rooting of *Carrizo citrange* micro shoots. However, germinated explants derived from CM4 and CM9 were not desirable as they presented an elongated and lanky appearance. This observation is in line with the wide range of developmental responses in which GA_3 are implicated. Induction of hydrolytic enzymes to facilitate endosperm mobilisation, increase in stem length, seed germination, control of fruit development and juvenility are some of the responses associated with GA (George et *al*., 2008).

The completely differentiated, more compacted, and robust plantlets observed in CM5 and CM10 could therefore be attributed to a synergistic effect of AC, GA_3 and Morel vitamin. Apart from the external factors; CM composition and photoperiod, that affected rate of germination and organogenesis in tissue culture, the origin of the explants is also important. Significant variations in germination rate and organogenesis were noticed in the different oil palm cultivars. Similar results had been reported by Obisesan & Fatunla (1983) who attributed such differences to genotype x environment interaction. Given that all the seeds produced in La Dibamba have the same broad back ground; derived from Deli x La Mé crosses, the variation in MZE germination rate observed could be attributed to the differences in the environment factor which in this case is composition of culture medium as well as genotype. From the genetic base and diversity points of view, Deli and La Mé alongside Yangambi, AVROS, Binga, Calabar constitute Breeding Populations of Restricted Origins (BPROs) (Rosenquist, 1986). Within each BPRO, there are many origins and within the same origin there are many progenies. Though all the seed cultivars produced at La Dibamba are derived from crosses between two BPROs (Deli x La Mé), there are many origins. The difference in response of MZE used in this study could therefore be attributed to differences in genotype of D x P parents.

It was observed that MZE from the same FFB cultured within the same Petri dish presented variations in the level of organogenesis, while others did not even germinate. This observation might be attributed to differences in the level of physiological maturity of seeds attached at different positions of the spikelet at the time of harvest.

III.2.4.2. Optimization of rhizogenesis in *vitro* oil palm plantlets for better acclimatization

One of the major obstacles concerning the practical application of plant tissue culture to mass propagation has been the difficulty of successful transfer of plantlets from *in vitro* conditions to a soil medium. Losses from 50 to 90 % of *in vitro* propagated plantlets of many species have been encountered at the time of transfer to soil (Zaid & Hughes, 1989).
Acclimatization is an important phase in tissue culture given that it marks the beginning of autonomous plant growth. It was observed in this study that low rate of rhizogenesis is a major factor that hinders acclimatization of micro shoots derived from *in vitro* germination of oil palm MZE. Root formation is an important step in plant vegetative propagation and is mainly controlled by genetic, physiological, physical and chemical factors (De Klerk et *al.*, 1999). It is well known that exogenously applied natural or synthetic auxins favour rooting, and there is evidence that this hormone is the most effective inducer of the process (Ling et *al.*, 2013).

In the present study it was observed that type and concentration of auxin strongly influenced the quality of rhizogenesis (Fig. 45) within the studied period. The results showed that IBA was the most effective of the three auxin types used in terms of root initiation and elongation and root hair proliferation efficiency followed by NAA and lastly by IAA (Table XI). This result agrees with the finding of Han et *al*. (2009) who reported that the application of auxin, particularly indole-3-butyric acid (IBA), is one of the most common and effective means to enhance rooting of cultures. Superiority of IBA over NAA and IAA in root induction, profuse rooting and root development has been reported in several other plants, both woody and non woody species (Mialoundama et *al*., 2002; Thawaro & Te-Chato 2010; Mohammed et *al*., 2013).

The superior effects of IBA on root elongation as compared to NAA might be due to several factors such as; its preferential uptake, transport, metabolization and subsequent gene activation (Ludwig-Müller, 2000). Furthermore, more energy may be needed by the explants to convert the absorbed synthetic NAA from the medium to a natural form of IAA before being used by the explants. Consequently, additional energy would be used up and might eventually lead to insufficient energy needed for cell growth and development. This suggestion ties with the work of Zolman et *al*. (2008) who demonstrated that energy is needed in converting NAA to IAA, hence, displaying reduced responses of NAA in root elongation. This condition could likely explain the low efficiency in root induction on explants placed in NAA medium. Concerning the superiority of IBA over IAA, it has been explained that the rooting efficacy of IBA is due to the fact that IBA is more stable than IAA under various light and temperature conditions, both in solution and *in vivo* (Nissen & Sutter, 1990). More so, differences in transport, uptake, or metabolism might also contribute to its superior activity (Epstein & Ludwig-Müller, 1993).

The concentration of auxin needed to optimize root induction, varies with plant species and explants. It was observed in the present study that at different concentrations of IBA, different results in rooting efficiency were obtained. It showed that a specific concentration (1.5 mg L^{-1}) was vital to obtain maximum rooting. Bertoni (2011) reported that application of supra optimal levels of exogenous IAA or IBA to germinating wild-type *Arabidopsis thaliana* seedlings inhibits hypocotyl elongation. This result corroborates with Evans et *al*. (2003) who noted that a higher IBA concentration of up to 3 mg/L supplemented in MS medium demonstrated a better response with the highest percentage of rooting as well as the longest root formation on leaf explants as compared to the other concentrations of IBA tested. The same author however noticed that, when the concentration of IBA was further raised from 3 to

7 mg/L, lower rooting efficiency from the leaf explants was observed. This could be explained by the fact that auxins at high concentration may possess herbicidal properties which inhibit the adventitious root induction from leaf explants (Evans et *al*., 2003). The fact that under acclimatized conditions, *vitro* plantlets had developed 3 leaves could be an indication that growth was normal when compared to growth of seedlings derived from the conventional method.

CHAPTER IV. CONCLUSION, RECOMMENDATION AND PRESPECTIVE

IV.1 Conclusion

The productivity of an oil palm plantation depends on many factors, including the critical starting point of the quality and quantity of the germinated seeds derived from cross pollination of selected parent palms used for planting. The production of high quality germinated oil palm seeds is also very much dependent on good management practices at the seed production unit. The supply of improved oil palm planting material by CEREPAH is hampered by a low and slow germination rate. It is within this framework that this study was carried out first to identify the causes of low and variation in germination between cultivars and secondly to identify approaches that could scale up germination rate so as to bridge the gap between demand and supply.

To identify possible causes of low germination rate, position-dependent effect and impact of harvesting date on germination were studied. The results indicate that apical fruits have a higher germination rate than basal fruits of spikelets. The general low germination/FFB is caused by low germination rate of basal seeds which have a high numerical strength than the apical fruits. FFBs harvested 7 months after assisted pollination registered a higher germination rate compared to FFB harvested after 6 months. This presumably indicates that, the harvesting date is one of the plant-linked causes of low germination of oil palm seeds in CEREPAH. Seeds from the same FFB however, show variation in germination rate hence variation in level of dormancy within the same FFB. Given that dormancy is a strategy for survival of the species, it will be very difficult if not impossible to have all seeds from same FFB germinate at the same time.

In an attempt to improve germination rate, three main approaches were studied. The first approach was the association of GPCs to DHT. The results obtained showed that out of the three GPCs and their three concentrations, 1 % H_2O_2 and 0.5 % GA_3 improved rate of germination and shortened time of germination than the control while CH_2N_2 gave germination rate lower than the control. This result had the same trend for 7 out of 10 oil palms cultivars that showed a positive response, with the overall best germination scored by C2001 II for all the treatments. This implies that treatment of pre scarified oil palm seeds with H_2O_2 and GA_3 at concentrations of 1 % and 0.5 % respectively promote germination while CH_2N_2 has an inhibitory effect on the process of germination. In accordance with results obtained in this study therefore, submerging oil palm seeds initially exposed to DHT (40 ^oC for 80 days), in 1

% H_2O_2 solution for 48 hours is a cost effective method to enhance germination capacity and reduce germination time of improved oil palm seeds.

In the second approach which consisted of substituting dry heat scarification with pure sulphuric acid and hot water scarification, it was observd that the latter cannot be used as alternatives to the DHT as 0.0% germination was obtained for both approaches. This seemingly implies that dormancy in oil palm is not only physical and DHT is inevitable in breaking it.

The third approach was focused on using embryo rescue to produce *vitro* oil palm plants. It was observed that CEREPAH material has a high germination potential for regeneration of *vitro* oil palm plantlets through embryo rescue. However, low rhizogenesis remains a key problem, even though treatment with 1.5 mg L^{-1} IBA, increased rhizogenesis and survival up to 70 % during acclimatization. Nonetheless, the *vitro* oil palms regenerated and transplanted in the field showed normal growth with 10 % of them commencing inflorescence production 15 months after.

IV.2. Recommendations and perspectives

To scale up germination rate of improved oil palm planting material the following recommendations are made to CEREPAH:

Harvesting of FFB predestined for seed production should be done at least 7 months after pollination to enhance embryo maturity of many seeds. Within these 7 months the pollination bag should be maintained on the FFB to avoid loss of FFs.

Seeds scarified by DHT (40 °C + 80 days) should further be submerged in 1 % H_2O_2 for 48 hours followed by 3 days soaking in pipe borne water; before allowed to germinate.

Cultivar C2001 should be engaged in seed production given that it has high chances of possessing more than one endosperm / seed, out of which >70 % possessed viable embryos.

Identification of P x D crosses that gives high germination capacity should be prioritized, as this would enable the establishment of a germination potential based crossing plan.

Stable electricity supply is inevitable during dry heat scarification.

CEREPAH should invest in oil palm seedling production through tissue culture.

As far as perspectives are concerned, further studies on germination promoting role of H_2O_2 could be carried out at the molecular level. Besides, studies to develop a root culture medium that improves rhizogenesis of *vitro* plantlets could be envisaged.

Prospective studies to identify new materials of sufficient genetic variability for valuable agronomic characteristics like breeding (P x D) for hybrids with higher germination capacity and lower mean germination time could be interesting.

Comparison studies on growth parameters of *vitro* plants derived from embryo rescue and seedlings produced by the classical DHT could also be carried out

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Appendix

Appendix1. Types of dormancy, causes and conditions to break

Dormancy type	Cause	Condition to break
1. **Exogenous dormancy**		
• Physical	Impermeable seed coat	Scarification
• Chemical	Inhibitors in seed coverings	Removal of seed coverings. Leaching seeds
• Mechanical	Seed coverings restrict radicle growth	Cold stratification Removal of seed covering
2. Endogenous dormancy		
2.1Morphological	The embryo is not fully developed at the time the seed is shed from the plant	Cold stratification and potassium nitrate Warm
• Rudimentary	Small undifferentiated embryo	Warm or cold stratification
• Undeveloped	Small differentiated embryo less than ½ size of seed	stratification and GA
2.2 Physiological	**Factors within embryo inhibits germination**	
• Non deep	Positively photodormant Negatively photodormant	Red light Darkness
	After-ripening	Short period of dry storage
• Intermediate	Embryo germinates if separated from the seed coat	Moderate periods (up to 8 weeks) of cold stratification
• Deep	Embryo does not germinate when removed from seed coat or will form a physiological dwarf	Long periods (> 8 weeks) of cold stratification
3. **Combinational dormancy**	Combinations of different dormancy conditions that must be satisfied sequentially	
• Morphophysiological	Combination of underdeveloped embryo and physiological dormancy	Cycles of warm and cold stratification
• Epicotyl	Radicle is non-dormant and growth begins when temperature and water permit, but epicotyl is dormant	Warm followed by cold stratification
• Epicotyl and radicle	Radicle is dormant and growth begins after chilling stratification treatment, but epicotyl is dormant	Cold stratification followed by warm followed by a second cold stratification
• Exo-endodormancy	Combinations of exogenous and endogenous dormancy conditions. Example: physical (hard seed coat) plus intermediate physiological dormancy. Sequential combinations of dormancy releasing treatments.	scarification followed by cold stratification
Secondary dormancy		
• Thermodormancy	After primary dormancy is relieved, high temperature induces dormancy	Growth regulators or cold stratification
• Conditional dormancy	Change in ability to germinate related to time of the year	Chilling stratification

Appendix 2**.** Composition of common culture media in tissue culture (IAEA, 2002)

Composition (mg /l)	Culture media							
	MS	**G5**	**W**	**LM**	**VW**	**Km**	**M**	**NN**
1.macronutrients								
$Ca_3(PO_4)_2$	1650.0				200.0			
NH_4NO_3	1900.0			400.0				720.0
KNO_3	440.0	2500.0	80.0		525.0	180.0	180.0	950.0
$CaCl_2.2H_2O$	370.0	150.0		96.0				166.0
$MgSO_4.7H_2O$	170.0	250.0	720.0	370.0	250.0	250.0	250.0	185.0
KH_2PO4				170.0	250.0	150.0	150.0	68.0
$(NH_4)_2SO_4$		134.0			500.0	100.0	100.0	
$NaH_2PO_4.H_2O$		150.0	16.5					
$CaNO3.4H_2O$			300.0	556.0		200.0	200.0	
Na_2SO_4			200.0					
KCl			65.0					
K_2SO_4				990.0				
2.micronutrients								
KI	0.83	0.75	0.75			80.0	0.03	
H_3BO_3	6.20	3.0	1.5	602		6.2	0.6	10.0
$MnSO_4.4H_2O$	22.30		7.0		0.75	0.075		25.0
$MnSO_4.H_2O$		10.0		29.43				
$ZnSO_4.7H_2O$	8.60	2.10	2.6	8.6			0.05	10.0
$Na_2MoO_4.2H_2O$	0.25	0.25		0.25		0.25	0.05	0.25
$CuSO_4.5H_2O$	0.025	0.025		0.25		0.025		0.025
CoCl2.6H2O	0.025	0.025				0.025		
$Co(NO_3)_2.6H_2O$							0.05	
Na_2EDTA	37.3	37.3		37.3		74.6	37.3	37.3
$FeSO_4.7H_2O$	27.8	27.8		27.8		25.0	27.8	27.8
$MnCl_2$						3.9	0.4	
$Fe(C_4H_4O_6)_3.2H_2O$					28.0			
3.Vitamins and other supplements								
Inositol	100.0	100.0		100.0				100.0
Glycine	2.0	2.0	3.0	2.0				2.0
Thiamine HCl	0.1	10.0	0.1	0.1		0.3	0.3	0.5
Pyridoxine HCl	0.5		0.1	0.5		0.3	0.3	0.5
Nicotinic acid	0.5		0.5	0.5			1.25	5.0
Ca-panthothenate			1.0					
Cysteine HCl			1.0					
Riboflavin							0.5	
Biotin						0.3	0.5	0.05
Folic acid							0.3	0.5

MS= Murashige and Skoog, **G5**= Gamborg *et al*., **W**= White, **LM**= Lloyd and McCown, **VW**= Vacin and Went, **Km**=Kudson modified, **M**= Mitra *et al*. and **NN**= Nitsch and Nitsch media.

Appendix 3.Various parameters used to measure germination

Germination parameter	Symbol	unit	Formula for Calculation	Description of formula	Notes
Final germination percentage	FGP	%	No. of seeds germinated in a lot x 100	-	The higher the FGP value the greater the germination of a seed lot
Mean germination time (Orchard, 1977)	MGT	Day	MGT= $\frac{\sum f.x}{\sum f}$	f= seeds germinated on day x	The lower the MGT, the faster a seed lot has germinated
First day of germination (Esechie, 1994)	FDG	Day	FDG= day on which the first germination event occurred	-	Lower FDG value indicates a faster initiation of germination
Last day of germination (Esechie, 1994)	LDG	Day	LDG= day on which the first germination event occurred	-	Lower LDG value indicates a faster ending of germination
Coefficient of velocity of germination (Jones and Sanders, 1987)	CVG	-	CVG= $100\frac{N_1+N_2+\cdots+N_x}{N_1T_1+N_2T_2+\cdots+N_xT_x}$	N= No o seeds germinated each day, no. of days from seedling corresponding to N	CVG gives an indication on the rapidity of germination
Germination rate index (Esechie, 1994)	GRI	% or day	GRI=$\frac{G_1}{1}+\frac{G_2}{2}+\ldots\frac{G_x}{x}$	G_1= germination percentage x 100 at the first count after sowing, G_2= germination percentage x 100 at the second count after sowing etc	GRI reflects the germination percentage of each day of the germination period
Germination index (Arnold et *al*., 1991)	GI	-	GI=$(10+n1)+(9+n2)+\cdots(1+n10)$	$n1, n2, n10 =$ No. of germinated seeds on 1st, 2nd and subsequent days ill the 10th day. 10, 9, 1 are the weights given to the number of germinated seeds on the 1st 2nd and subsequent days	In the GI, maximum weight is given to the seeds that germinate on the first day while less weight is given to the subsequent days. The lowest will be given to seeds that germinated on the 10th day. Therefore, GI emphasizes on both the percentage of germination and its speed. A higher GI value denotes a higher percentage and speed of germination
Time spread of germination	TGS	day	TGS= Time in days between the first and the last germination event in the seed lot	-	The higher the TSG value, the greater the difference in germination seeds between the fastest and the slowest germination member in the seed lot

Appendix 4. Project design matrix of the thesis

Topic: SCALING UP OIL PALM PLANTING MATERIAL PRODUCTION BY MODIFIED DRY HEAT SCARIFFICATION PART I

General objective	Specific objectives	materials	methodology	Rational	Expected results	Chronogram
Alternative method to boost up germination of improved oil palm seeds in IRAD La Dibamba	1. measure average shell thickness and weight of the of NF and TF	• 50 seeds x 10 categories • Callipers • Electronic balance	• Seeds of relatively same size selected • Seeds are cracked and thickness of shell and endosperm measured using callipers • Average weight of shell and endosperm assessed using the balance	Verify if germination variation b/w TF and NF is linked to difference in shell and endosperm thickness	No variation in average shell thickness of TF and NF	10/04/14 To 13/04/14
	2. Access the effect of seed position on spikelet on germination	• Open and controlled pollinated FFB • Callipers • Electronic balance •	• Prone FFB immediately • Separate apex (deep red seeds) from basal seeds (orange coloured) • Depulped seeds, measure biometric parameters • Determine germination rate via dry heat treatment	check if seed position on spikelet contribute to the general low rate of germination	apex seeds show higher germination than basal seeds in each apex	12/04/14 To 17/04/14
	3. Compare moisture content before and after storage in both categories	• Electrical balance • Soaking dishes • Scarified and unscarified seeds	• Before each scarification method, DW is taken • After soaking FW is measured	Identify the scarification method that optimizes up take of water	Highly Significant disparity with method of breaking dormancy	
	4. Effect of traditional thermal treatment on seeds germination (control)	• 200 seeds/category • germinationplasticbags • thermometer • H_2O	• 100 unscarified seeds • Soak in H_2O for 7 d • heat at 40 ^oC + 80d • Soak in water measuring daily till FW is 22%	Control experiment	Significant difference in T_{50} between traditional and H_2O_2 supplement	09/04/14 To 16/08/14
		• H_2O_2	• 100 seed same condition above + 3% H_2O_2	• study influence of H_2O_2 addition on traditional method of dormancy breakage		
	5. Effect of chemical scarification on seed germination	• 100 seeds/category • H_2SO_4concentration • Distilled water	• Lot 1: (25 unclarified seeds soaked in 10 % H_2SO_4 + 10 min, +30 min, +60 min • Rinse seeds 3 times with tap water	• Compare the present method of breaking dormancy (dry heat) and acid scarification	Higher rate of germination and T_{50} than	14/04/14 To 20/05/14

		• GA_3 • Tetrazolium	• Soak in water to raise MC to 22% note number of days • Air dry in shade and put in plastic and evaluate germination for 5 weeks	• Determine H_2O_4 concentration and soaking duration that optimize suppression of dormancy	traditional method difference	
			• Lot 2: (25 unscarified seeds soaked in 50 % H_2SO_4 + 10 min, +30 min, +60 min • Other points are same		Variation with H_2SO_4 concentration and time of soaking	
			• Lot 3: (25 unscarified seeds soaked in 70 % H_2SO_4 + 10 min, +30 min, +60 min • Other points are same			
	6. outcome of mechanical scarification on seeds	• 300 seeds /category with operculum tegument removed • Knives • 90% alcohol • GA_3 • H_2O_2 • Tetrazolium	• Lot 1: weight 100 seeds (control) • Soak in 90 % alcohol for 3min and rinse trice with tap water • Immense in water to raise MC to 22 % • Air dry in shade and put in plastic and evaluate germination for 5 weeks	compare effect of removal of operculum tegument with other methods of scarification	Highly significant variation between mare operculum tegument removal and GA_3 and H_2O_2 added-on	17/04/14 To 30/05/14
			• Lot 2: weight 100 seeds • Soak in 90 % alcohol for 3min and rinse trice with tap water • Immense in 3g/L GA_3 for 2 d • Soak in water to raise MC to 22 % and measuring FW daily • Air dry in shade and put in plastic and evaluate germination for 5 weeks	Evaluate synergistic impact of GA_3 + removal of operculum tegument		
			• Lot 3: measure DW of 100 seeds • Soak in 90 % alcohol for 3min and rinse trice with tap water • Immense in 3g/L GA_3 +3% H2O2 for 2d • Soak in water to raise MC to 22 % and measuring FW daily • Air dry in shade and put in plastic and evaluate germination for 5 weeks	Evaluate synergistic impact of GA_3 + H_2O_2 + removal of operculum tegument		
	7. Impact of hot water scarification	• Electrical heater • Thermometer • Water bath	• Boil water to 90 ^oC • Immense 50 seeds for 48 h • Soak in water until MC = 22 %. • NB: Water is changed daily • Air dry in shade and put in plastic and evaluate germination for 5 weeks		Significant difference with other methods of scarification	10/04/14 To 27/05/14

Part II Protocol

Regeneration of seedlings from mature zygotic embryo (MZE) of commercial oil palm hybrids seeds produced at CEREPAH

<table>
<tr><th>General objective</th><th>Specific objectives</th><th>Materials</th><th>Methodology (Munier et al. (2008), Thawaro and Te-chato (2010) and Suranthran et al. (2011) mordified.</th><th>Expected results</th></tr>
<tr><td></td><td rowspan="2">1.Identify oil palm seed category with the highest MZE morphogenesis pace

2.Determine the CM that optimizes seedling growth of MZE of the ten oil palm categories in vitro</td><td>Plant material
• 240 oil palm seeds /category (240X10=2400)</td><td>AT CEREPAH
• random selection of 240 fruits /category
• Stripping and fermentation of fruits (5d)
• depulping using a depulping machine
• finishing and treatment with fungicide (penncozeb)
• air dried in air condition room for 20 d</td><td rowspan="2">1.Variation in speed of seedling regeneration from MZE of different oil palm categories

2.Better seedling development in ½ MS supplemented with AC and Vitamins</td></tr>
<tr><td>Set a base for oil palm tissue culture in Cameroon in ground works for future micro propagation research</td><td>Reagents
• 100 g MS powder
• Vitamins
• Activated charcoal
• sucrose
Sterilisation reagents
• sterile water
• 70 % alcohol
• Sodium hypochloride
• Clorox
• Tween
Lab materials
• Autoclave
• Laminar flow cabinet
• 30 Caped pyrex test tubes
• Forceps
• 30 petri dishes</td><td>AT CARBAP
1. preparation of culture media
• full strength MS stock
• ½ strength MS stock
2. Sterilisation of explants
• cracking using a hammer
• soaking of kernel in 70 % alcohol for 1 min followed by Clorox for 20 min
embryo excised aseptically from kernel in a laminar flow cupboard
3. sowing of explants
<table><tr><th>Medium no</th><th>Composition + 3 % saccharose</th></tr><tr><td>1.</td><td>Full strength MS salt</td></tr><tr><td>2.</td><td>½ strength MS salt</td></tr><tr><td>3.</td><td>Full strength MS salt + 0.2 g /l AC</td></tr><tr><td>4.</td><td>½ strength MS salt + 0.2 g /l AC</td></tr><tr><td>5.</td><td>Full strength MS salt + vitamin</td></tr><tr><td>6.</td><td>½ strength MS salt + vitamin</td></tr></table></td></tr>
</table>

			7. Full strength MS salt + 0.2 g /l AC + vitamin 8. ½ strength MS salt + 0.2 g /l AC + vitamin • 10 explants sown in 8 culture medium (CM) in triplicates as follows Observations will be collected on weekly basis for 30 d	
	3.Quantify the degree of media and embryo discoloration		Based on visual observations, the degree of media and embryo discoloration (entire explants and at the media contact point) will be rated on a scale of 1-5 (1 = No discoloration and 5 = Extreme discoloration), modified from the rating scale given by Ziv and Halevy (1983).	**3**.High discoloration in embryo and media that do not contain AC
	4.Evaluate vigour of *vitro* plantlets in the nursery	- *Vitro* plants - Polyethylene bags - Kieserite and urea fertilizer - Vermiculite	• Acclimatization of *vitro* plant • Sterilisation of soil • Filling of polyethylene bags • Application of Kieserite and urea • Transplanting and data collection on growth parameters	**4**.Variation in vigour with CM and seed category
	5.Compare seedling production with the traditional germination method	Data on germination from experiments at CERAPAH		

Detailed chronogram of activities

ACTIVITY	Feb 2014	April	May	June	July	Aug	Sep	Oct	Nov	Dec	Jan - Feb 2015
Control pollination in the seed garden											
Development of protocol											
Harvesting and preparation of seeds											
Transportation of seeds to CARBAP											
In vitro culture											
Acclimatization and nursery growth											
Data collection											
Analysis and presentation of results											
Seminar and submission of manuscript											

Appendi x 5: Publications originated from this thesis

1. Tabi-Mbi K., Ntsomboh-Ntsefong G, Ngando-Ebongue G.F, Anjambang W.N & Youmbi E., 2016. Position dependent effect of oil palm (Elaeis guineensis Jacq.) seeds on germination aptitude. International journal of Agronomy and Agricultural research, 8: 44-53.

2. Tabi Mbi K., Tonfack L.B, Ntsomboh Ntsefong G., Bilal A. M., Ngando Ebongue G. F., Ngaha D., Njembele C., Kato S.N, & Youmbi E., 2016. Mature zygotic embryo rescue improves in vitro germination andseedling production in high value oil palm (Elaeis guineensis Jacq.) cultivars. Industrial crops and products 94: 445-453.

3. Tabi K.M., Ngando Ebongue G.F., Ntsomboh G.N. & Youmbi E., 2017. Effect of dry heat treatment along with some dormancy breaking chemicals on oil palm seed germination. South African Journal of Botany, 112: 489-493.