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GA_3 and ABA mediated effect on maturation and germination of zygotic embryos in coconut (*Cocos nucifera* L.)

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INTRODUCTION

Mature nuts (12 months after fertilization) of coconut are being used conventionally for seed nut purpose. This restricts the size of collection in a germplasm expedition. Embryos (8 months old onwards) could be extracted and retrieved *in vitro* (Karun *et al.*, 11). The retrieval was observed to be very low in immature embryos compared to matured ones. The retrieval chances can be increased if immature embryos are matured *in vitro*. Immediate transportation of field-collected embryos may not be possible when collecting sites are located in far off places. Short-term storage (Karun and Sajini 10; Karun *et al.*, 12) of these collected embryos for maturation is necessary for further germination and development. The growth inhibitor ABA plays an important role in embryo maturation and inhibition of precocious germination (Pilet and Saugy, 18; Zeevarrt and Creelman, 23; Mayer and Poljakoff-Mayer, 17; Ackerson, 1), controlling morphogenesis (Quatrano 19), synthesis of storage proteins (Finkelstein *et al.*, 6) and desiccation tolerance (Kermode and Bewley 14). Gibberellic acid often counteracts ABA for morphogenesis like root elongation. Root growth was stimulated by gibberellic acid in *Zea mays* (Emons *et al.*, 5). The present study ws designed to find out the response of ABA and gibberellic acid on maturation and germination, and morphological changes in coconut zygotic embryos.

MATERIAL AND METHODS

The zygotic embryos of West Coast Tall coconut 9 months after fertilization (immature) and 11 months after fertilization (mature) were subjected to hormonal treatments. Extraction and surface sterilization of embryos were done as described by Karun *et al.* (9, 11). Four levels of GA_3 (filter sterilized), viz. 1.0 µM, 0.5 µM, 0.1 µM and 0.05 µM were tried. Three levels of ABA (filter sterilized), viz. 10 µM, 20 µM and 30 µM were used. A control treatment consisting of no hormones was also included. Eeuwen's Y3 (Eeuwens, 4) was used as the basal medium (without any hormone supplements). The experiments were replicated thrice and 20 embryos /treatment were inoculated in each replication. To study the effect of growth bormones on maturation, the embryos were incubated for 90 days in dark without sub-culturing. After 90 days, the embryos were sub-cultured in the retrieval medium (Karun *et al.*, 11) and transferred to the illuminated rooms with 27±2°C temperature and RH of 85% with 16 hr photoperiod for normal plantlet development. The data were subjected to multivariate analysis of variance and log linear analysis using SPSS.

RESULTS AND DISCUSSION

The effect of growth hormones (GA_3 and ABA) on maturation of embryos were observed by means of their ability for normal germination and growth. First phase of the experiment (90 days duration), per cent germination and growth parameters like shoot length and root length 45 and 70 days after culturing were recorded. It was observed that embryos were germinated in all the levels of GA_3 irrespective of their maturity (Table 1), indicating that the supplementation of GA_3 may not help in maturation of immature embryos with levels as tried in the present study.

Analysis of variance revealed that the growth of immature embryos was significantly more. However, the relative growth of mature and immature embryos in different levels of GA_3 was not uniform by 70th day and showed significant maturity by GA_3 interaction (Table 2). The immature embryos showed maximum growth in medium supplemented with highest level of GA_3 (Fig. 1a). The growth of mature embryos was relatively less (Fig. 1b) and on a par between 0.5 and 1.0 µM (Table 1).

Growth of coconut zygotic embryos in the medium supplemented with GA_3 was faster than that in the medium without GA_3 (Mashud and Idroes 15). Verdeil (21) noticed that higher levels, of GA_3 (10^{-4} M and 0.5×10^{-4} M) promoted shoot growth 4 months after planting, i.e. 18 cm ± 4 in 10^{-4} M and 15 ± 5 at 0.5×10^{-4} M and 9 ± 2 cm without GA_3 (control). Shoots developed with the highest GA_3 level look like etiolated (long with narrow leaves with 5 mm wide). Weerakoon (22) reported that GA_3 had no significant difference in embryo germination of mature embryos of Sri Lanka Tall varieties. However, in Dikiri (soft endosperm type) mature embryos GA_3 resulted in embryo germination. Filter sterilized GA_3 at lower concentration gave considerable increase in embryo germination than the autoclaved GA_3.

The cultures were transferred to retrieval medium 90 days after incubation in GA_3. The

Table 1. Effect of GA_3 on germination, shoot length and root length of mature (M) and immature (IM) embryos after 45 and 70 days in culture.

GA_3 (µM)	Germination (%)		Shoot length (cm)				Root length (cm)			
			45th day		70th day		45th day		70th day	
	M	IM	M	IM	M	IM	M	IM	M	IM
1.0	70.0	73.3	0.19	0.15	0.40	0.62	0.11	0.14	0.33	0.61
0.5	51.7	61.0	0.20	0.16	0.35	0.54	0.15	0.23	0.30	0.38
0.1	83.3	75.8	0.23	0.35	0.27	0.53	0.14	0.27	0.15	0.24
0.05	62.5	58.1	0.15	0.19	0.26	0.35	0.08	0.11	0.07	0.07

Table 2. Summary of ANOVA showing the levels of significance for the various parameters.

Source of variation	Shoot length		Root length	
	45th day	70th day	45th day	70th day
Maturity	0.04	0.04	0.00	0.00
GA_3 levels	0.07	0.01	0.38	0.06
Maturity* GA_3	0.23	0.02	0.46	0.04

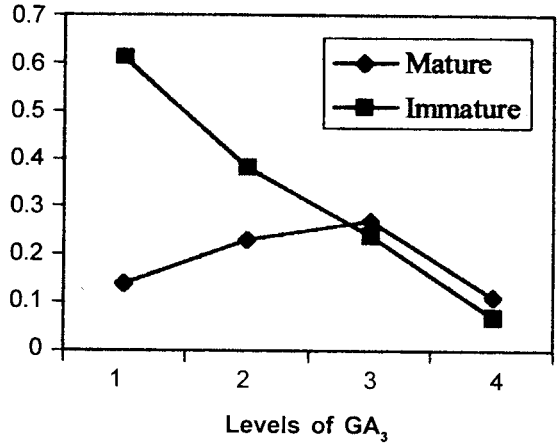

Fig.1a. Maturity by GA_3 interaction for root length at 70th day

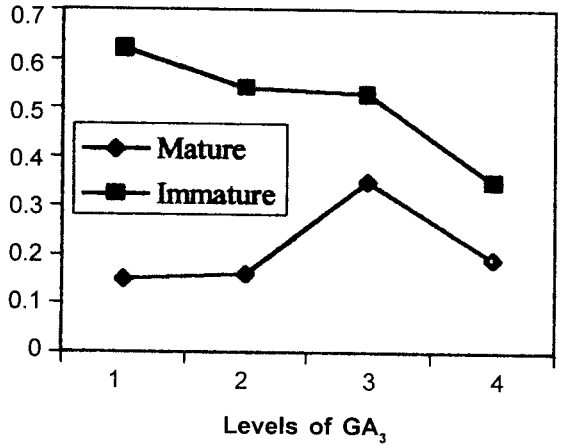

Fig.1b. Maturity by GA_3 interaction for shoot length at 70th day

weight gained by the cultures was recorded at the time of transfer. The length of shoot and root at 120th and 150th day was also recorded. The levels of GA_3, age of embryo and their interaction were significant with regard to weight of embryo. The weight gain for immature embryos was on a par among different levels of GA_3 except the lowest level. In mature embryos, maximum weight gain was associated with 0.1 µM. There was no significant difference for the growth characters of retrieved embryos that were first inoculated in different levels of GA_3. Comparisons were also made by removing the effect of initial growth (analysis of covariance by considering length of shoot and root at 70th day as covariates). Again no significant difference was observed among the treatments.

The overall growth of cultures during the first 90 days in medium supplemented with different levels of ABA was observed to be less compared to GA_3. Since the root growth was very less in the initial phase of the experiment, only the length of shoot at 60th day was recorded. The growth in the retrieval medium was measured in terms of length of shoot and root at 40th day following transfer to the retrieval medium. The log-linear analysis of per cent germination of embryos revealed significant effect of levels of ABA (Fig. 2). The ABA (10-20 µM) improved germination of immature embros contrary to the expectation of inhibition of germination.

The length of shoot in medium containing different levels of ABA was significant 60 days after inoculation (Table 3). All the 3 levels of ABA were significantly superior to the control. Significant interaction between maturity of embryos and levels of ABA was also observed. Irrespective of maturity of embryos, supplementation of ABA contributed for gaining weight. The third level of ABA (30µM) recorded lower values than other 2 levels (Table 3). Growth of immature embryos treated with 10 µM was better than the rest. The poor growth and germination of embryos at higher level of ABA indicate that for dormancy, higher levels of ABA may be tried.

Comparisons of treatments after removing the effect of initial growth (analysis of covariance by considering length of shoot and root at 60th day as covariate) showed non-significant maturity-ABA interaction for length of shoot at 130th day (Table 4). This indicates that supplementation of ABA in the initial cultures has no adverse effect on growth and development of plants in retrieval medium.

Table 3. Effect of ABA concentration on embryo shoot length (cm) and weight gain (g) after 60 days in culture and on shoot and root length after 130 days in culture (M: mature: IM: immature).

ABA concentration	Length of shoot (60th day)		Weight gained	Length of shoot (130th day)		Length of root (130th day)	
(μM)	M	IM	(90th day)	M	IM	M	IM
Control	0.37	0.13	0.39	1.27	0.34	1.50	*
10	0.46	0.88	0.42	1.03	1.60	0.17	0.93
20	0.42	0.62	0.42	1.10	1.35	1.02	0.49
30	0.34	0.45	0.31	1.03	0.95	0.57	0.43

* In control, no root growth was noticed.

Table 4. Summary of ANOVA showing levels of significance for various morphological characters.

Source of variation	Shoot length (60th day)	Weight gain (90th day)	Root length (130th day)	Shoot length (130th day)
Maturity	0.599	0.597	0.708	0.785
ABA levels	0.087	0.054	0.030	0.264
Maturity * ABA	0.045	0.244	0.012	0.086
ems	0.06102	0.005749	0.07274	0.192
edf	14	14	11	14
cv	33.83	19.44	42.81	39.12

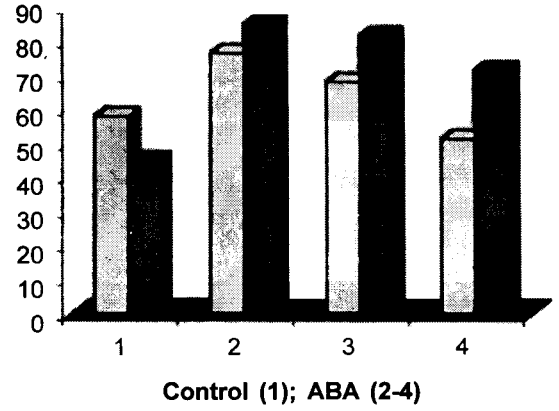

Fig.2. Per cent germination of embryos in different levels of ABA

Mashud (16) reported that GA_3 and ABA promoted the germination of 9 months old embryos of coconut. However, Damasco (2) observed that ABA was not effective in reducing growth in coconut. The ABA in solid medium was found to be more effective in enhancing coconut embryo maturation than the liquid medium (Weerakoon, 22). The ability of ABA to promote the accumulation of storage proteins in zygotic embryos is well established. The ABA has been identified as an important media component for maturation of somatic embryos of Norway spruce (Hakman *et al.*, 8; Durzan and Gupta 3). The embryo maturation process was also affected by ABA concentrations, the somatic embryos of spruce developed on 10-20 μM ABA germinated precociously but as the levels of ABA were increased, premature germination was inhibited. Further, they also reported that optimal level of ABA for the production of mature somatic embryos

was 40 µM and these embryos contained approximately 50% more storage protein than those developed on 20 µM. Redenbaugh *et al.* (20) recognized a relationship between degree of maturation of embryos, and vigour and higher levels of storage proteins of resulting plantlets.

Thus, it was concluded that the supplementation of either GA_3 or ABA did not inhbit the germination of coconut zygotic embryos. Compared to GA_3, higher concentrations of ABA significantly reduced the germination (irrespective of maturity). The poor growth and germination of embryos at higher levels of ABA suggest that, higher levels of ABA may be tried (more than 30 µM).

SUMMARY

The effects of growth hormones, viz. GA_3 and ABA were studied on mature (11 months after fertilization) and immature (9 months after fertilization) zygotic embryos of coconut cv. West Coast Tall for maturation, germination and plant development. The GA_3 levels tried were 1.0 µM, 0.5 µM, 0.05 µM and 0.01 µM, and of ABA 10 µM, 20 µM and 30 µM. The germination percentage, weight gain (g), shoot length (cm) and root length (cm) were recorded both at incubation period (90 days) as well as in retrieval medium. Embryos germinated in all the levels of GA_3 irrespective of their maturity. The immature embryos showed maximum growth in medium supplemented with highest level of GA_3. The growth of mature embryos was relatively less and on a par between 0.5 and 1.0 µM. The weight gained for immature embryos was on a par among different levels of GA_3 except the lowest level. There was no significant difference for growth characters of retrieved embryos that were first inoculated in different levels of GA_3.

Significant effect of ABA on germination per cent was noted. The ABA (10 µM - 20 µM) improved germination of immature embryos. All the 3 levels were significantly superior to the control. Significant interaction between maturity of embryos and levels of ABA for initial shoot length (60 days) and root length (130 days) was observed. Interaction between maturity of embryos and levels of ABA was significant with regard to length of shoot and root in the retrieval medium. Irrespective of the maturity of embryos, supplementation of ABA contributed for gaining weight. The poor growth and germination of embryos at higher levels of ABA indicate that, for dormancy, higher levels of ABA may be tried.

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