MORPHOGENIC RESPONSE OF *IN VITRO* EXPLANTS EXCISED FROM *EX VITRO* SEEDLINGS OF CASHEW (*ANACARDIUM OCCIDENTALE* L.)

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Introduction

Cashew (*Anacardium occidentale* L.) is a perennial crop which is cultivated commercially for its kernels that have great demand internationally. Among the tree nuts, cashew nuts lead in world production and ranked third in international trade with 20% of the market (Mardel, 2007). It is being an important cash crop for farmers and there is a potential to increase production in Sri Lanka. However, the cashew production in Sri Lanka has been limited by the inadequate planting material of superior varieties, cultural practices and other factors. Seeds are mostly used as planting materials in Sri Lanka even though cashew is highly cross pollinated crop and also cashew nuts has high demand in local and international market. Conventional methods of propagations are not efficient enough to provide high yielding planting materials (Shivantham *et al,* 1990). Thus, *in vitro* culture technique is a vital role for propagation and improvement of cashew. Sumita *et al* (2004) reported that cashew nut was found difficult to propagate *in vitro* from mature plant tissues. Aliyu *el al.* (2005) stated that explants from seedlings germinated and raised seedlings under *in vitro* are most suitable for micropropagation of elite cashew. However, there is limited information on *in vitro* propagation of cashew therefore this experiment was done to study the morphogenic response of *in vitro* explants excised from *ex vitro* seedlings of cashew.

Methodology

This experiment was done to select the most suitable plant parts of young seedlings for *in vitro* culture establishment. Cashew nuts were collected from Kiran Cashew Cooperation of Sri Lanka to raise the young seedlings. They were sown in sandy soil for germination under the laboratory conditions in order to isolate various types of explants for *in vitro* inoculation. After four weeks of sowing, seedlings were uprooted and washed in running tap water for 1 hr and various plant parts were separately excised from them. Subsequently they were thoroughly washed with distilled water and surfaced sterilized by using 30% Clorox^{TM} for 20 min. They were then rinsed in sterile distilled water before being inoculated. The different types of sterilized plant parts namely shoot tip, first leaf and petiole, stems from both tip and basal portions, hypocotyl and cotyledon were isolated as explants and each plant part was excised (10 mm long) aseptically and inoculated in MS medium supplemented with 10 mg/L BAP as culture medium based on the previous study and they were incubated at 25±2 °C under white fluorescent light with 16 hrs photoperiod. Cultures were observed daily and data were recorded. This experiment had 20 replicates for each treatment and repeated twice.

Discussion and Conclusion

Survival of the cultured explants: The results showed that the survival percentage was more than 50% in all types of explants at the first week of culture. Hypocotyl and petiole segments exhibited high percentage (80%) of survival followed by first leaves and stem near basal portion (70%) while comparatively low percentage (50%) was recorded on shoot tips. The survival of shoot tip decreased to 20% at the third week and remained same

thereafter. Although the survival percentage was 60% in cotyledon at the initial stage, it continuously decreased and at the fourth week only 30% of explants survived. The first leaf showed high survival (60%) at the fourth week meanwhile stem near tip portion and petiole recorded 40% survival. No remarkable changes were observed thereafter.

Browning of the cultured explants: The degree of browning varies with different explants types and medium around shoot tip, stem near basal stem portion and hypocotyl showed pronounced browning and some media completely turned dark brown. Similar observation was made by Sumita *et al.* (2004). First leaf and petiole from first leaf showed low extend of browning whereas shoot tip showed high extend of browning. No remarkable changes were observed after fourth week of culture. Browning occurs due to the phenolic compounds presented in the explants and subsequently it leaches into medium mostly from the cut end of the explants. The amount of phenolic compound may vary with the plant parts it may be the reason for low extent of browning compared to other types.

Nature of the cultured explants: It was observed that most of the shoot tips cultured turned brown and died at the fourth week. Although the first leaf cultured showed less contamination, it remained same without any morphological response in cultured medium. Petiole from first leaf also showed similar response to medium containing 10 mg/L BAP. Stem near the tip portion started to initiate small white protuberance (Figure 1A) on the cut surface at the second week and no change was observed with time. Pale white friable structures were observed on the surface of stem basal portion (Figure 1B). Stem near basal showed more *in vitro* response than the stem near tip portion. Compact greenish white structures and shinny globules (Figure 1 C&D) were mainly concentrated on the surface of cut edges of stem near basal portion.

Cotyledon tissues initiated to produce greenish white shinny globular structures at the second week of culture. These structures were mainly concentrated on the central portion of the adaxial side of the cotyledon explants (Figure 1E) and subsequently somatic embryoids were developed which were confirmed by the cytological studies. Wachira and Ogada, (1995) reported that cotyledon explants of *C. sinensis* produced numerous somatic embryos directly developed from the epidermal portion of the cotyledons within three weeks of culture in MS medium. Further it was noted that the cultured hypocotyl segments ruptured and formed small adventitious shoot buds at the second week of culture which were light green and shinny in nature (Figure 1F). The number of adventitious shoot buds formed ranged from 8-10 at the six weeks of culture.

The result exhibited that the most responsive plant part for the initiation of somatic embryoids was the stem segment obtained from basal portion and the for adventitious buds it was the hypocotyl segments cultured in MS medium containing 10 mg/L BAP under *in vivo* conditions among the various types of explants excised from the young seedlings.

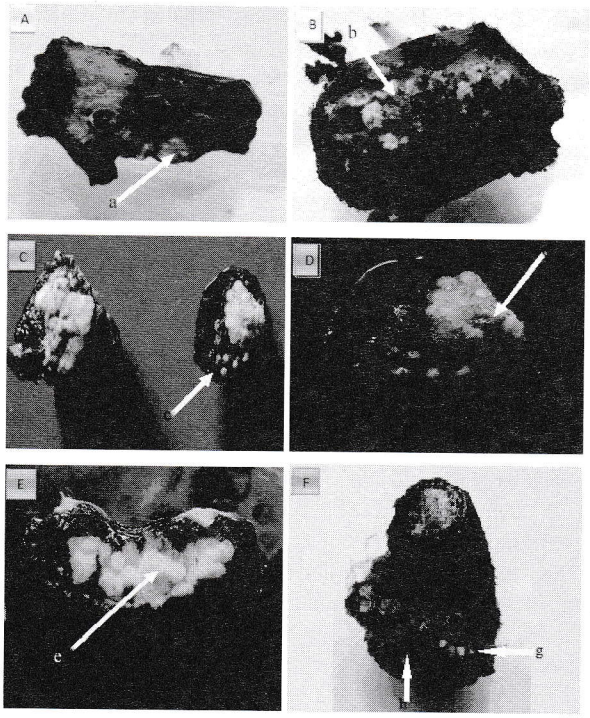

Figure 1: *In vitro* response of various types of the cultured explants at the fourth week [A: stem piece near tip portion; B-D: Stem piece near basal portion; E: cotyledon segment; F: hypocotyl segment; arrows indicated symbol a: small white protuberance on the surface; b:pale white friable structures on the surface; c: globular structures on the cut surface; d: greenish white structures on the cut surface; e:shiny white structures on the surface of cotyledon explants; g: small green adventitious shoot buds; h: rupture on the hypocotyl segments]

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