

## Germination and *In-Vitro* Regeneration in 'Egusi' Melon, *Citrullus lanatus* (Thunb.) Matsum. and Nakai

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### Abstract

This study was carried out in order to determine the germination and *in-vitro* regeneration of five accessions of "egusi" melon. Seeds from de-coated melon were used for germination and *in-vitro* regeneration was carried out on excised pre-germinated cotyledons in MS medium (4.43g of MS, 30g of sucrose, water of pH 5.8, 1M NaOH and 3.5g Gelzan). The highest germination percentages after five days were observed for the accessions DD98/4 and A22. Regenerated cotyledon explants 14 days after plating in MS medium showed accession A22 producing the highest regeneration frequency. The study revealed that DD98/4 and A22 had the highest germination and best regeneration frequency in the Murashige and Skooge (MS) medium, thus making them useful materials for genetic transformation.

**Key words:** Regeneration, 'egusi' melon, cotyledon

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### Introduction

"Egusi" melon (*Colocynthis citrullus* L.) is among the 300 species of melon found in tropical Africa. It is cultivated for its seeds, which are rich in oil (53%), protein (28%) (Bankole *et al.*, 2005), vitamins (A, B1, B2 and C) and a good source of minerals such as S, K, P, Ca, Mg, Fe and Zn, which are known to eliminate tape worm and to serve as a purifier of internal organs. The seeds are not only edible but also used to produce fuel (An Ku, 2007). The fruit is not edible as its white flesh is dry and bitter enough to be repulsive (Purseglove, 1991; Ntui and Uyoh, 2005). In Nigeria, "Egusi" is cultivated over an area of 320,800 ha with a production figure of 0.98 t/ha (Federal Ministry of Agriculture, 1993). However, the crop is susceptible to climatic stresses and diseases such as cucumber mosaic virus, melon mosaic virus, Fusarium wilt, etc. Conventional breeding methods using wild rustic melon species in order to transfer desirable agronomic genes into the genus are limited because of interspecific hybridization barriers (Rhimi *et al.*, 2006). Recent developments in biotechnology has opened up several ways for cucurbit breeding using genetic transformation, in which heterologous genes can be introduced into existing cultivars (Sarowar *et al.*, 2003). In many instances, however, the lack of efficient regeneration system causes limitations on the use of gene transfer technologies for these crops. An efficient plant regeneration system is therefore necessary for transformation and propagation. Genetic engineering can be used to produce desirable agronomic characteristics quickly and efficiently, and it has already been used to improve fruit quality and introduce disease resistance and environmental tolerance in melons (Bordas *et al.*, 1997, Ayub *et al.*, 1996 and Yoshioka *et al.*, 1993). Unfortunately, in melon species, the transformation frequency is very low due to the

production of “escapes” (Guis *et al.*, 1998). In previous studies, transgenic plants were generated using adventitious shoot organogenesis. To reduce the problem of “escapes,” an alternative regeneration system that can enable transformation is needed. Several groups have reported the production of somatic embryos from melon cell suspension cultures (Oridate and Oosawa, 1986). Published protocols for melon genetic engineering use the process of organogenesis (Dong *et al.*, 1991 and Guis *et al.*, 2000). Although this sometimes leads to problems, such as abnormal embryos and hyper-hydricity, the liquid culture system is considered very useful for the efficient selection of transformed tissues, as whole explants absorb antibiotics more easily when suspended in liquid media than when cultured on solidified media. Embryogenesis is also a useful regeneration system for transgenic research because none of the transgenic plants are chimeric. The efficiency of embryogenesis in melons is closely related to genotype (Oridate *et al.*, 1992). In this study, we attempted to determine the germination and regeneration of cotyledon explants of various accessions of ‘egusi’ melon.

## Materials and Methods

Five accessions of ‘egusi’ melon with contrasting qualities were selected and used for this study (Table 1). Seeds were washed in water for 5 – 10 minutes, and then de-coated. The seeds were disinfected to prevent the growth of microorganisms like bacterial and fungi by adding 74% (V/V) alcohol for 30 seconds, then 4% sodium hypochlorite (NaClO) for 8 minutes. Seed were then rinsed in sterilized water and allowed to drain off. Murashige and Skoog medium (MS) was prepared by dissolving 4.43g of MS and 30 g of sucrose in 1 litre of water and heated in a microwave oven. Water of pH 5.8 (prepared by adding 1M NaOH to water and stirred with a magnetic stirrer) was added to Gelzan (3.5g) as a solidifying agent. The MS medium was sterilized by autoclaving under high pressure steam at 121°C for 15 to 20 minutes to make it free of microorganisms. Seeds were plated in the MS medium under sterile conditions and the plates were sealed with a parafilm tape and grown in dark (germination chamber at 25.5°C for 3 days). Growing points of the cotyledon were cut off and taken as the explants for regeneration. The explants were then plated in MS medium supplemented with growth hormones (abscisic acid (1mg/l) and indole-3-*butyric acid* (2mg/l)).

*Statistical Analysis:* Data collected on germinated de-coated seeds and explants generated were computed as percentages. Standard error was calculated and Duncan multiple range test (DMRT) was used to separate the means, using SAS/PC version 9.1 (SAS, 1999).

## Results

Germinating ‘egusi’ melon seeds cultured in MS medium is shown in Plates 1 and 2, after 3 and 5 days, respectively, while plate 3 shows the cotyledonary explants 12 days after plating in MS medium supplemented with growth hormones, abscisic acid (1mg/l) and Indole-3-*butyric acid* (2mg/l) (mention the supplementary growth hormones). Germination percentage and regeneration frequencies from cotyledonary explants of five seed types of ‘egusi’ melon accessions is presented in Table 2. Number of germinated seeds ranged from 27 (A20) to 46 (A22 and DD99/75). The highest germination percentage (92%) was observed also for accessions A22 and DD99/75. Cotyledons resulting into explants ranged from 44 - 97 for accessions NG/TO/APR/09/029 and A22, respectively. A22 also recorded the highest regeneration frequency. The regeneration frequencies of NG/TO/APR/09/029 and A20 were comparatively lower than that of all others, with frequencies of 44 % and 47 %, respectively. Cotyledonary explants from the ‘egusi’ melon accessions expanded about three times of their initial sizes after 14 days of culture in MS medium (Plate 4). Leaf explants from NG/TO/APR/09/029 had the highest survival rate, 14 days after culture in MS medium supplemented with growth hormones, abscisic acid (1mg/l) and indole-3-*butyric acid* (2mg/l) (which of them?), closely followed by A20 and the lowest for accessions DD98/550 and DD99/75 (Table 2).

## Discussion

Several factors affect the germination of seeds, such as moisture, temperature, thickness and permeability of seed coats and seed quality amongst others. The higher germination percentage recorded for accessions DD/99/75 and A22 compared to the other accessions could

probably be as a result of permeability and / thickness of seed coat. *In-vitro* regeneration of plants from cotyledon of mature seeds have received considerable attention in recent years and this is most likely because of easy accessibility, quick response and high ability for shoot organogenesis (Tabei *et al.*, 1991). The use of mature cotyledon explants was justified with earlier transformation experiments in melon (Gaba *et al.*, 1995). Germination and regeneration of the different 'egusi'



Plate 1. Germinating decoated 'egusi' melon seeds 3 days after plating in MS medium supplemented with sucrose.



Plate 2. Germinating decoated 'egusi' melon seeds 5 days after plating in MS medium supplemented with sucrose.



Plate 3. Cotyledon explants 12 days after plating in MS medium supplemented with abscisic acid (1mg/l) and indole-3-butyric acid (2mg/l)



Plate 4. Cotyledon explants 14 days after plating in MS medium supplemented with abscisic acid (1mg/l) and indole-3-butyric acid (2mg/l)

seed types on Murashige and Skooge (MS) medium with antibiotic differed significantly from one accession to the other. DD98/4 and A22 had the highest germination percentage. The highest number of explants generated and survived on MS medium was also observed for A22. These findings are similar to those reported by Oridate *et al.* (1992) while working on different varieties of melon. Moreno *et al.* (1985) and Lee *et al.* 2003 also reported that the type of explant plays an important factor in determining the morphogenic response. The high survival rate of A22 in the selection medium will make it a useful material for regeneration and for subsequent transformation. Germination and regeneration of selected accessions on MS medium with *Agrobacterium* and antibiotic revealed DD98/4 and A22 having the highest germination

percentage. The high survival rate of A22 makes it a useful material for regeneration and subsequent transformation.

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Table 1. Accessions of 'egusi' melon used in this study.

S/N	Accessions	Seed physical quality	Source
1.	DD98/550	Black tips	NIHORT, Ibadan
2.	DD99/75	Black edges	NIHORT, Ibadan
3.	NG/TO/APR/09/029	Large seeded	NACGRAB, Ibadan
4.	A20	White edges	Saki, Oyo
5.	A22	Smooth seeds	'Serewe' Abeokuta

Table 2. Germination percentage and regeneration frequencies from cotyledon explants of five seed types of 'egusi' melon accessions

Accessions	Seed physical quality	Number of seeds plated	Germinated seeds	Germination percentage	Cotyledons cultured in MS medium	Explants generated	Regeneration frequency (%)	Survival of explants after culture 14 days in MS medium + (abscisic acid + butyric acid)
DD98/550	Black tips	50	30b	60b	100	59b	59b	16d
DD99/75	Black edges	50	46a	92a	100	54c	54c	20d
NG/TO/APR/09/029	Large seeded	50	29b	58b	100	44d	44d	41b
A20	White edges	50	27b	53b	100	47d	47d	36c
A22	Smooth seeds	50	46a	92a	100	97a	97a	82a
(±)Std. Error			1.41	2.81		1.45	1.45	1.68

\*Means with the same case along columns letters are not significantly different from one another at 5% probability level.