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Short communication

The use of embryo culture for the recovery of plants from cassava (*Manihot esculenta* Crantz) seeds

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Abstract. Cassava fertility and seed viability are frequently low, which can be a disadvantage in a breeding programme. An embryo culture method is described whereby embryonic axes are excised from mature seeds and placed on a culture medium containing 1.23 μM indolebutyric acid (IBA) at 30°C under continuous light. The number of plants recovered by embryo culture was much greater than the number recovered from conventional seed germination procedures.

Introduction

Cassava (*Manihot esculenta* Crantz) is an important crop of the lowland tropics and is a major source of calories to nearly 500 million people throughout the world. Cassava root products can also be used as a source of carbohydrate in animal feed concentrates, as an industrial source of starch and as a feedstock for fermentation industries.

As interest in cassava intensifies, emphasis is being placed on the development of improved cultivars. In Australia, breeding for such traits as increased yield, herbicide resistance and chilling tolerance are important. Breeding and selection programmes can be limited by fertility problems and low seed production [3], as well as low rates of germination in certain cultivars and hybrids [1, 5]. Problems of seed dormancy and low seed viability have been overcome in numerous plants through embryo culture [2]. Embryo culture has been used to recover plants from wild cassava species known to have poor seed germination, but there have been no published reports of embryo culture from cultivated cassava [8].

A technique is presented for the culture of embryos excised from mature seeds of cultivated cassava. A much higher percentage of plants was recovered through embryo culture than by conventional germination procedures.

Materials and methods

Source of seed. Seed was harvested during July and August, 1984, from plots planted in December, 1982, on the Australian Cassava Products Pty. Ltd.

plantation near Torbanlea, Queensland. Following open pollination, seed was available from the cultivars; M Aus 2, M Aus 4, M Aus 7, M Aus 10 and CMC 39 (M Col 1467). Seed pods were collected by hand when the exocarp had dehydrated and started to separate from the endocarp and prior to dehiscence. The seed pods were air dried in trays and under mosquito netting until dehiscence occurred and the seed was stored in ventilated jars at ambient temperature.

Seed germination. Seeds were placed in water and those which floated were discarded. The remaining seeds were sown in vermiculite, watered, and placed in a growth cabinet at 32°C. Germination was protracted and occurred over a period of 4–20 days.

Embryo culture. Seeds were washed for ten minutes in 30% Dairychlor ($100\,g\,l^{-1}$ available chlorine) containing a drop of detergent (Skippa, Campbell Brothers Limited, Brisbane, Australia), and then rinsed three times in sterile water. The seeds were immersed in sterile water in the dark for 2–3 days to soften the seed coat.

After imbibition, the seeds were washed in Dairychlor and water as described above. Seeds awaiting dissection were placed in sterile water to prevent desiccation. Under aseptic conditions, the seeds were split along the raphe (Figure 1A), with the aid of forceps and scapel, and embryonic axes (1–2 mm) were removed. Care was taken to avoid damaging the embryonic axis, especially at the shoot end, and any attached cotyledon was not removed from the embryonic axis (Figure 1C).

Embryonic axes were placed radicle down on medium containing half strength Murashige and Skoog's [6] (MS) salts and vitamins with inositol and glycine omitted, $20\,g\,l^{-1}$ sucrose and $1.23\,\mu M$ IBA with pH adjusted to 5.7. Difco Bacto agar at 0.7% was added to the medium which was autoclaved at 120°C for 15 minutes. Medium was dispensed in petri dishes or screwtop tubes.

Cultures were incubated at 30°C under continuous illumination from a 40 W fluorescent light ($35\,\mu E\,m^{-2}sec^{-1}$). Plants grown in vitro could be successfully established in soil in the greenhouse, if care was taken to ensure plants did not dry out by provision of high humidity under plastic sheets.

Results and discussion

Embryonic axes could be removed from seeds with less than 1% incurring damage that would affect subsequent development. Embryonic axes developed roots after 2–3 days in culture and the shoots elongated and became green within 5–7 days (Figure 1D). Of those embryonic axes which failed to produce plants, development was arrested at various stages with 12–19% producing normal roots but no shoot growth. Two weeks after the

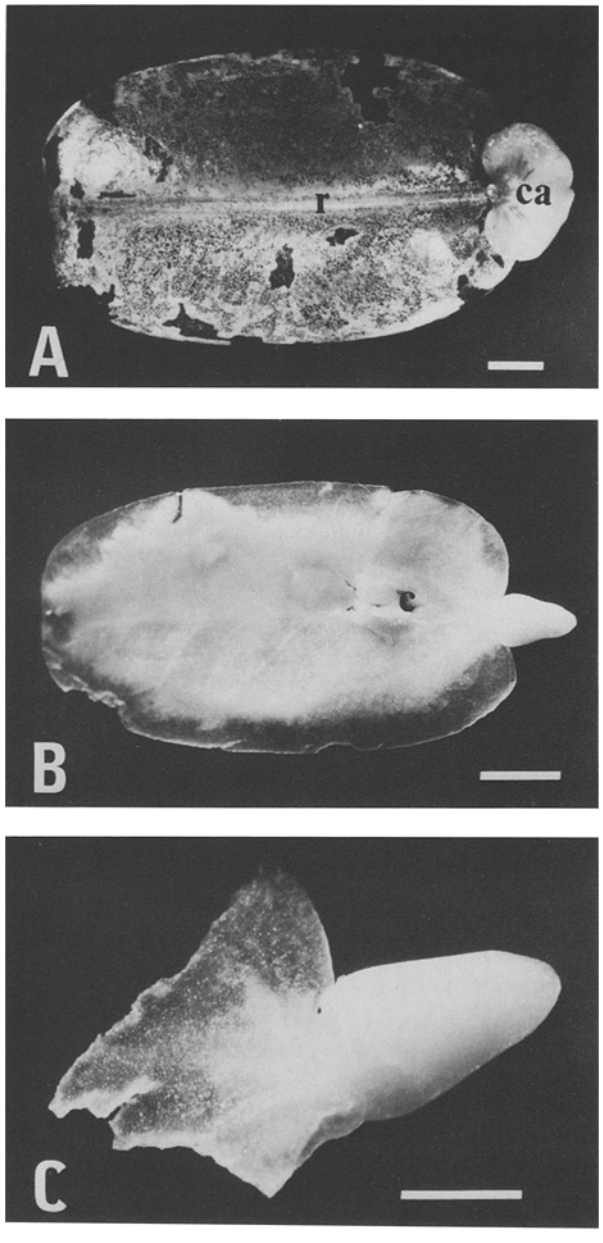

Figure 1. Stages in the recovery of plants from embryos dissected from mature seeds of cassava cultivars. (A) Whole seed of M Aus 4 (*ca* caruncle, *r* raphe). (B) Embryo (cotyledons and embryonic axis) removed from M Aus 4 seed (*c* cotyledon). (C) Embryonic axis excised from M Aus 4 seed, ready for placing on culture medium. (D) M Aus 4 plantlet with elongating root and shoot axis after one week. (E) M Aus 7 plantlet after two weeks. Bar represents 1mm.

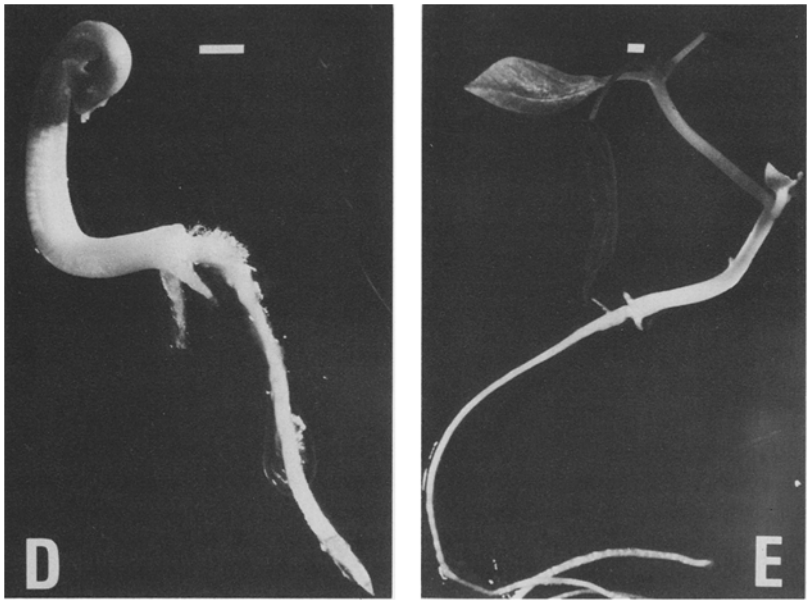

Figure 1. (D) and (E).

embryonic axes were placed on culture medium, the plantlets consisted of 2–3 nodes (Figure 1E). After a month, plantlets could survive transfer to soil.

The embryo culture technique was compared with conventional seed germination techniques to determine whether the number of plants recovered from cassava seed could be increased. Table 1 shows a markedly improved frequency of recovery using embryo culture for every cultivar tested. Plants also developed from embryonic axes excised from seed that floated in water, albeit at a low frequency. These seeds either did not contain developed embryos or there was poor endosperm development. Seeds that float in water do not germinate normally.

The germination frequencies, seen in Table 1, for the various cultivars were low but this is not unusual in cassava. Martin [5], concluded that germination of cassava seeds is poor and erratic. Capinpin and Bruce [1] state that poor recovery of plants from seed is one of the limiting factors in a breeding programme.

Percentages of germination higher than those in Table 1 have been observed in seeds of the Australian cultivars, but germination varies with time in storage. It is highest about three months after harvest and declines to negligible levels after 12 months (N. Harris, personal communication). Four months after harvest, seed of M Aus 7 which sank in water (83% of total) germinated with a frequency of 39%. Two months later the frequency had fallen to 9% and concomitantly there was a decrease in the number of seeds which sank in water to 54%.

Table 1. Comparison of the percentage recovery of cassava plants from an embryo culture technique and a conventional germination procedure

Cultivar (Months in storage)	Number of seeds used for embryo culture	% Recovery from embryo culture	Number of seeds used for germination	% Recovery from germination
M Aus 2 (4)	101	61	–	–
M Aus 4 (7)	120	82	120	1
M Aus 7 (9)	116	41	254	15
M Aus 10 (7)	122	79	120	4
CMC 39 (7)	82	68	88	2
M Aus 7 low density seeds (9)[a]	114	3	–	–
CMC 39 low density seeds (7)[a]	6	17	–	–

[a] Seeds which floated in water

Embryonic axes were excised from mature seeds and placed on medium containing 1.23 μM IBA at 30°C under continuous illumination (35 $\mu E\, m^{-2}\, s^{-1}$). For germination seeds were placed in moist vermiculite at 32°C. Seedlings derived from culture/germination were counted after two weeks

Germination of cassava seeds has been improved by various procedures designed to break dormancy. Nartey et al. [7] were able to increase germination to 85% by exposing seeds to red light (651 nm) for 24 hours prior to scarifying and sterilizing seeds, and germinating them in mineral solution at 29°C in the dark. Embryo culture is another simple technique for breaking seed dormancy and increasing the recovery of plants from cassava seeds.

Embryo culture will find application in a breeding programme for cultivar improvement principally in those cases where the number of seeds from important test crosses is low in quantity or lacking endosperm and/or cotyledon development, and where seed rapidly looses viability during storage. Embryo culture also allows for the preparation of aseptic seedling material which can be rapidly propagated in vitro prior to field trials and evaluation.

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