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Responses of Different Explants of Sweet Potato on Modified MS and LS Based Nutrient Media *in vitro*

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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Short Research Article

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ABSTRACT

A good number of reports on sweet potato (*Ipomoea batatas* L.) tissue culture using different tissues of various cultivars with varying level of efficiency and reproducibility are available but callus induction and plantlet regeneration are recalcitrant and limited to a few genotypes in response to different treatments *in vitro*. Reported in this paper is a procedure in which several explants of three high yielding and drought tolerant sweet potato cultivars (SK010, WHCH005 and PRAP496), mostly grown and cultivated in the highlands of Papua New Guinea (PNG) were used to induce embryogenic callus and regenerate plantlets. To achieve a reliable and an efficient system for inducing embryogenic callus on stem, petiole and leaf disc explants, a modified form of Murashige and Skoog (MS) and Linsmaier and Skoog (LS) media, supplemented with 1 gL^{-1} picloram and 8 gL^{-1} agar was used. This procedure resulted in large amount of embryogenic calli that were potentially capable of regenerating whole plantlets on all the different types of explants tested. Further attempts made in plantlet regeneration failed, however ways in which improvement of the tested regeneration media can be made for plant regeneration in similar studies were discussed.

Keywords: Plant regeneration; sweet potato cultivars; embryogenic callus; in vitro; MS media.

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1. INTRODUCTION

A good number of reports on sweet potato (*Ipomoea batatas* L.) tissue culture using different tissues of various cultivars with varying levels of efficiency and reproducibility are available, but there are severe limitations of genotypes for regeneration, moreover efficiency is still low [1]. Several other investigators have also shown that sweet potato somatic embryogenesis is initiated from leaf disc, petiole fragment, nodal segment, internodal fragment and root portions [2]. Many of these groups reported *in vitro* stock cultures as the ideal source of explants for inducing callus and direct shoot regeneration [3]. Additionally, callus was induced from auxenic leaf explants containing 4-5 pairs of leaf primodia, stem segments, internodal section directly behind the apex and freshly harvested roots on basal MS [4] based-medium supplemented with 60 gL^{-1} sucrose and 0.5-4 mgL^{-1} 2, 4-dichlorophenoxy acetic acid (2, 4-D) and solidified with 7 gL^{-1} agar [2]. In this study, pale to dull-yellow, compact and organised callus emerged from the explants 3 to 8 wks later after incubation. Further, young leaves and leaf primodia of the shoot tip explants readily produced callus than older leaves.

Research has shown that the use of stem and root explants to induce callus on LS [5] and based medium [6]. The results obtained showed that the younger explants readily produced callus than older ones. These workers [5,6] reported developing an efficient and variety independent method for the production of embryogenic calli from meristem tissues using LS based-medium supplemented with 1 mgL^{-1} of picloram. Despite the numerous progresses made by different workers [e.g. 7], it is generally reported that sweet potato regeneration is recalcitrant and limited to a few genotypes because each cultivar shows different responses to *in vitro* treatments [8]. Comparison of several protocols on regeneration of sweet potato [6,8] also showed that every cultivar vary widely in their responses to issue culture and plant regeneration [9]. Hence, genotype is one of the most important factors affecting the evolution of *in vitro* culture and regeneration [10]. Reported in this paper is an approach in which different explants of three sweet potato cultivars were used to induce embryogenic callus and attempts made to regenerate plantlets *in vitro*.

2. MATERIALS AND METHODS

2.1 Plant Materials

Sterile, virus-free plantlets of three high yielding, drought tolerant and widely cultivated sweet potato cultivar; SK010, WHCH005 and PRAP496, from the PNG National Agriculture Research Institutes (NARI) were supplied as axenic shoot cultures by the University of Technology Biotechnology Centre (UBC) were used for the study. Shoot cultures with four plants of each cultivar were maintained in 11 cm honey jars containing 40 ml of MS medium supplemented with 3% (w/v) sucrose, and solidified with 0.8% (w/v) agar. The pH was adjusted to 5.8 and autoclaved at 15 psi (121°C) for 15 minutes (hereafter MS0.8 medium). The screw-caps of the jars containing the medium were securely fastened after cooling at room temperature (28±2°C) and kept in a metallic tray prior to use. The shoot cultures were grown at 26±2°C under a 16 h photoperiod of 30 µmol m^{-2} s^{-2} photosynthetic photon flux using cool white fluorescent tubes. The stock plants (20 honey jars containing 4 plants each) were subcultured monthly into 40 ml fresh MS0.8 medium by culturing the nodal segments (1 cm in length) and maintained throughout the study period to provide source of explants.

2.2 Induction of Embryogenic Callus and Shoot Regeneration

For callus induction, internodes between the first and the second most youngest leaves of *in vitro* axenic stock cultures of the three cultivars (14-21 d old) were excised (~5 mm in length). They were cut transversely into sections and again in half along the axis and used as explants. Additionally, leaf discs (~1 cm^2) and petiole fragments (~5 mm in length) of the same age were prepared and wounded throughout and cultured on semi-solid LS based-medium supplemented with 0.1% (w/v) picloram and 0.8% (w/v) agar (hereafter LSP.8 medium) (see Table 1). Additionally, a modified MS medium supplemented with same amount of picloram and 0.8% (w/v) agar (hereafter MSP.8 medium) were also formulated to assess the response of explants to *in vitro* treatment and callus induction.

Embryogenic calli for shoot regeneration were transferred onto fresh LS0.8 and MS0.8 medium (without plant growth regulators) as reported

Table 1. The different media and the explant types

Medium	Volume	Explant types	Replication	Total cultured
MS-based	30 ml	1, 2, 3	4	48 (16+16+16)
LS-based	30 ml	1, 2, 3	4	48 (16+16+16)

1 = nodal segment, 2 = petiole portion and 3 = leaf disc. A culture contained 4 explants and replicated 4 times, giving a total of 16 explants. The MS-based media were MS0.8 and MSP.8 and the LS-based were LS0.8 and LSP.8, respectively

previously by other workers [5]. Five Petri dishes containing 30 ml of LSP.8 and MSP.8 medium with four explants cultured in each dish were set and replicated 4 times. The dishes were sealed with Nescofilm and kept at 24±2°C in the dark for 4-6 wks for callus induction. Explants were subcultured monthly onto fresh media until sufficient amount of calli were induced. Additionally, proliferating calli were isolated and transferred onto fresh medium (40 ml) of each formulation and allowed to multiply. After 2-4 wks of multiplication, the primary calli were transferred to jars containing the same amount of LS and MS medium but without plant growth regulators for plantlet regeneration [6]. These cultures were maintained in a growth chamber at 24±2°C under a 16 h photoperiod of 30 µmol m^{-2} s^{-2}.

3. RESULTS AND DISCUSSION

3.1 Induction of Embryogenic Callus

The three sweet potato cultivars responded differently on LSP.8 medium in terms of callus induction (Fig. 1). More than 80% (38/48) of intermodal fragments, 75% of the leaf discs (36/48) and 55% (26/48) of petiole explants produced roots instead of callus (Table 2). A few of each explant that responded (formed calli) died out without forming adequate calli. Root initiation began after 14 d of culture on explants of all the cultivars and continued to produce white and hairy roots. Initially, individual roots were produced which produced secondary, tertiary and quaternary roots throughout the culture period.

Root initiation and development on the internode and petiole explants were confined to the older portions of the explants, especially towards the cut edges and occasionally, petiole explants rooted from or near wounds, similar to the reports of [11]. The leaf discs on the other hand, rooted mainly from the older regions, near the remaining portion of the petioles (Fig. 1D). Compared to all the explants cultured on LSP.8 medium, younger nodal segments were the only explants that induced callus. These calli were hard and non-embryogenic (Fig. 1E). There was no indication of callus induction on leaf discs and petiole fragments cultured on LSP.8 medium, as previously reported [6].

When calli inductions on explants were compared among the cultivars, SK010 produced more calli than PRAP496 and PRAP496 produced more than WHCH005, indicating a strong different varietal response to *in vitro* treatment as pointed out by other researchers [7,8] in sweet potato. As was seen in direct shoot regeneration on different explants, sufficient amount of calli were induced on younger compared to the older explants. Leaf explants that were 14-21 days old responded to MSP.8 medium more quickly than explants of 21-28 days old. Contrarily, [3] reported callus induction and somatic embryogenesis initiation on leaf discs and stems on MS based-medium containing 2, 4-D.

Preliminary study carried out indicated that calli induction on modified MS medium containing 2, 4-D on similar explants as reported by [3] under the culture conditions was difficult, if not impossible, with the PNG cultivars tested. In addition, [6] reported that embryogenic calli was induced on LS-based medium supplemented with 1 mgL^{-1} picloram and more than 50% of the embryogenic calli of 11 genotypes cultured produced shoots. Ninety percent (55/60) of embryogenic calli, however, were only produced in two of the genotypes (Kokei 14 and Beniazuma) only. Only one of the cultivars (Beniazuma) produced bright yellow embryogenic calli on stem explants within 28 d [6]. In the present study, LSP.8 medium was not suitable for callus induction (Table 2), at least for the sweet potato cultivars, used under culture conditions reported by [6].

The MS-based callus induction medium (MSP.8) which was formulated for this study was more suitable for inducing calli from younger explants that yielded large amounts of calli (Table 2) that were compact, white and fast-growing. On the other hand, intact and unwounded explants

never produced callus. Callus proliferation was profound only on wounded than unwounded explants (Fig. 1A). Callus obtained from leaf explants were smaller compared to those obtained from internodal fragments. The white, friable and easily broken calli were mostly produced on younger explants while yellow and hard calli that were somewhat rounder in shape were produced on the older explants (Fig. 1B). The calli produced on older explants were not compact, when cut open for subculturing and transferred to fresh MSP.8 medium for multiplication.

When calli were subcultured at 2 wks interval for 28 d on MSP.8 medium, the growth of the calli were rapid and large masses of embryogenic calli, both yellow and white in colour were produced. Continuous subculture of the calli resulted in dehydration, reduction in growth and loss of regenerative potential and subsequent death. Similar results were observed in sugar cane (*Saccharum* hybrid spp.) tissue culture where callus growth was negative after more than 9 wks of subcultures [9]. [1] further reported that small and fast growing calli obtained from lateral buds rapidly turned brown and died within few wks, which were cultivar dependent. Certain other cultivar (Duclos 11, 90), previously reported to regenerate plants from protoplast derived calli did not give rise to embryogenic calli [1], indicating a strong varietal response, similar to the results of this study.

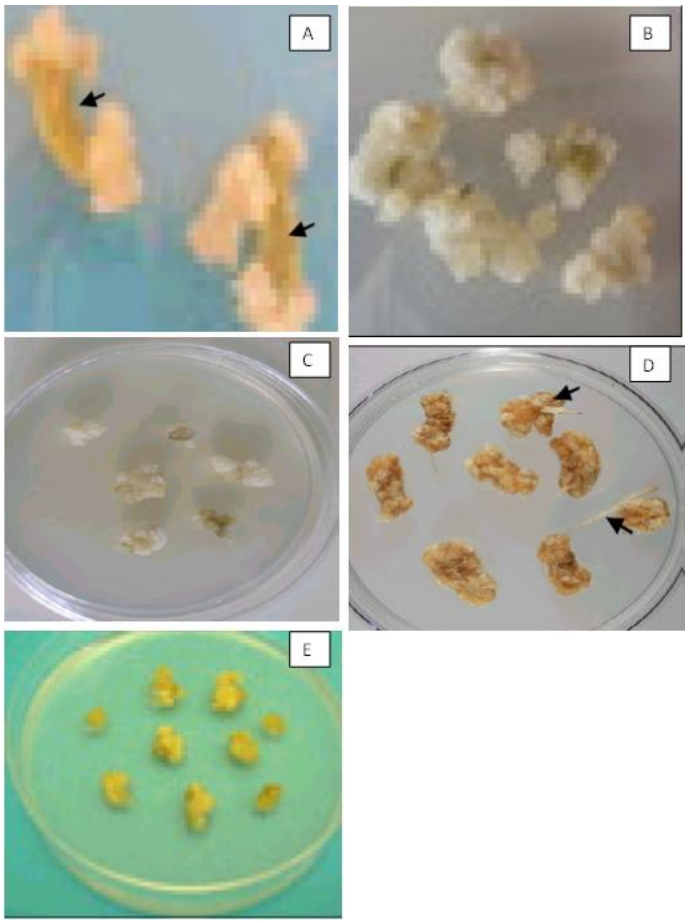

Fig. 1. Callus induction using different explants on MSP.8 medium. A, Callus developing on internode fragments after 28 d of culture. B, Calli isolated and transferred onto fresh MSP.8 medium for multiplication. C, Development of white embryogenic calli of cultivar SK010. D, Formation of non-embryogenic, brown callus on leaf explants after 21 d of culture. E, Hard calli developing on petiole fragments on MSP.8 medium 21 d after transfer. The arrows show intact, unwounded internodal fragments without callus (B) and root development on leaf explants (D).

Table 2. Response of explant on modified MS and LS based callus induction media

Medium	Volume (ml)	Total responses (%)		
MSP.8	30	99[1]	98[2]	98[3]
LSP.8	30	80[1]	75[2]	55[3]

Superscripts are 1 = nodal segment, 2 = petiole portion and 3 = leaf disc. The response total (%) was calculated based on the total number of explants cultured (Table 1)

3.3 Plantlet Regeneration Potential of Calli

When 28 d old calli were transferred to MS0.8 medium for shoot regeneration, the embryogenic calli turned brown and died within 21 d of culture. Similar observations were made on MS0.8 medium supplemented with indole-3-acetic acid (IAA) and kinetin. In sweet potato, induction of somatic embryogenesis and formation of embryos, as well as maturation occurred in the presence of high levels of auxin. The subculture of globular embryos on MS- based medium containing 2, 4-D and kinetin significantly improved development into shoots upon transfer to hormone free medium [1]. When younger (21 d old) calli were transferred to MS0.8 medium for plantlet regeneration, the calli started producing roots instead of shoots. The bright white and yellow calli became watery within 14 d of culture and after 28 d; the calli deteriorated and lost its regeneration potential (Fig. 2C). This result suggests that the regeneration medium (MS0.8) had different effects on the regeneration potentials of the calli and should have been modified, with other growth factors, as reported previously by [1], which is an ongoing work in our laboratory. Subulturing of the calli onto fresh LS0.8 medium also gave similar results (dehydration, loss of viability and deaths within 14-21 d after transfer).

Reports of similar research by others [3,6,10] showed that sweet potato is recalcitrant to every *in vitro* culture treatment and occur at low frequencies for various explants. In fact, shoots can easily be regenerated from explants of stems, petioles, leaves and roots but callus induction and shoot regeneration is difficult, if not impossible [11]. Series of transfer of internodal- and leaf disc- derived calli, both obtained from *in vitro* regenerated and glasshouse grown plants to media composition evaluated, for establishing an efficient system for callus induction and shoot regeneration, gave different results. The explants (nodal segments, internodal, and petiole fragments and leaf discs) of the three sweet potato cultivars evaluated on LS based-media for callus induction (LSP.8) failed to respond as against the responses of other sweet potato cultivars elsewhere [6].

When comparing the responses of explants, MSP.8 medium was the most suitable for callus induction compared with LSP.8, either from intermodal fragments, leaf discs or petioles with high frequencies, ranging from 85-90%, 70-75% and 15-20%, respectively. In this study it was observed that, 60% of all the explants of SK010, 10-20% of PRAP496 and 6-10% of WHCH005 responded to the callus induction medium. However, these calli derived from leaf explants and petiole fragments were hard and non-embryogenic [15, 16]. Negative *in vitro* treatment responses were obtained from media compositions reported by [1] and [6] suitable for shoot regeneration from embryogenic callus previously with the PNG cultivars evaluated.

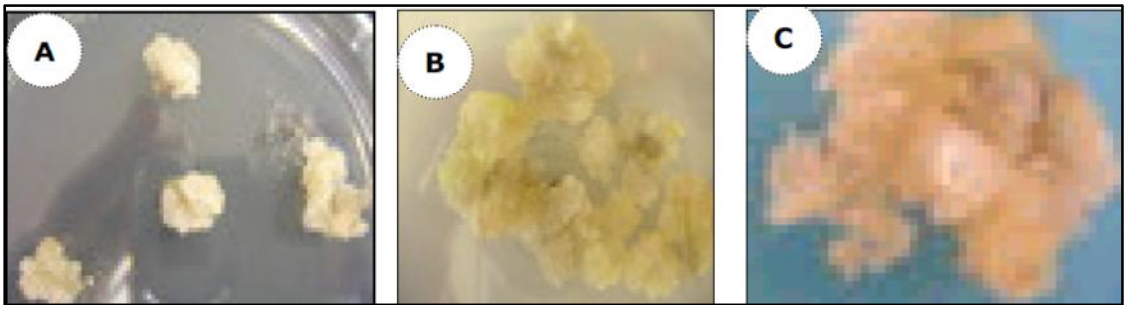

Fig. 2. Shoot regeneration potential of embryogenic calli on MS0.8 medium. A, Fresh embryogenic calli 14 d after transfer for shoot regeneration. B, Dehydrating embryogenic calli 21 d after culture on the same medium. C, Deteriorating calli 28 d after transfer (brownish yellow)

Regeneration of shoots from calli in any of the two media (MS0.8 and LS0.8) tested could not be achieved, especially from the white and compacted calli obtained, indicating a strong varietal response to *in vitro* treatment. Similar results were noted by other workers where white or brown compact surface calli failed to regenerate shoots [17,18,19].

4. CONCLUSION

Sweet potato is the staple of the PNG highlands and is a strategic crop for food and nutritional security. The importance and in the field management of the diversity is threatened by monoculture, loss of soil fertility, build-up of pests and diseases and unpredictable changes in weather patterns. In the light of these, tissue culture provides an important option to conserve and propagate sweet potato germplasm *in vitro* in large numbers that are free of pathogens and pests. An attempt to induce callus on different explants of three widely cultivated cultivars of sweet potato gave rise to varying results. Among the explants tested, younger explants of internode and leaf disc readily produced callus however plantlet regeneration from the calli were unsuccessful. The responses of the explants to the modified media were varietal specific, with SK010 producing more cali than the other two cultivars. The recalcitrance of these cultivars to regenerate plantlets is an on-going study at our laboratory.

CONFERENCE DISCLAIMER

Some part of this manuscript was previously presented and uploaded in the following conference: Proceedings of the TWAS Regional Young Scientist Conference. In 'Food, Health and Fuel: Plants for the Future in November 2009, At Selangor, Malaysia. Web Link of the proceeding:https://www.researchgate.net/profile/Patrick_Michael2/publication/274139439_Embryogenic_callus_induction_on_different_explants_of_sweet_potato/links/551736ae0cf29ab36bc0bf84/Embryogenic-callus-induction-on-different-explants-of-sweet-potato.pdf?origin=publication_detail

COMPETING INTERESTS

Author has declared that no competing interests exist.

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