EFFECTS OF CULTURE MEDIA AND VITRIFICATION ON SURVIVAL OF DURIAN (*DURIO ZIBETHINUS* MURRAY) SHOOT TIPS

Y.L Hor

Faculty of Agriculture
Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

Keywords: cultural studies, vitrification, durian, durio zibethinus, shoot tips.

Introduction

Durian (*Durio zibethinus* Murray) belongs to the family Bombacaceae and is an important fruit crop throughout South-East Asia. As such germplasm conservation is crucial for future crop improvement. The present study was carried out in two phases. The first phase focuses on screening for suitable culture media that support normal growth of durian shoot tips whereas the second phase evaluates the potential of vitrification (Sakai et al. 1990) for cryopreservation of durian shoot tips. The effects of different loading solutions, exposure times and glycerol concentrations sin the vitrification solution were evaluated.

Materials and Methods

Durian shoot tips were excised aseptically and surface sterilized with 0.1% mercury chloride before use. Three media tested were Murashige and Skoog's (MS), Woody Plant Medium (WPM) and Gambong's B5 (B5) medium. Different strength of MS medium (full, ¾, ½ and ¼ strength) were also evaluated with or without hormones namely. ∝–napthalene acetic acid (NAA), benzylaminopurine (BAP) and gibberellic acid (GA3) of 1 mg/l. In the second study, the vitrification procedure described by Sakai et al. (1990) was evaluated. Firstly, five loading solutions were compared: (a) 2M glycerol + 0.4M sucrose (b) 1M glycerol + 0.4M sucrose, (c) 20% Plant Vitrification Solution 2 (PVS2), (d) 0.5M glycerol + 10% dimethyl sulfoxide (DMSO) + 0.3M sucrose (e) 1.5M glycerol + 50% DMSO + 0.4M sucrose. Further studies were carried out using PVS2 especially in terms of duration of exposure and concentration of glycerol (30%, 20% and 10%). Plantlets were considered as viable when they remained green or showed shoot development after 2 weeks culture. All cultures were maintained in a 28°C culture room with 12 hours photoperiod of approximately 3,000 lux intensity provided by fluorescent tubes.

Results and Discussion

The results showed that the MS medium was better for durian shoot tip culture than B5 and WPM with or without hormone. Cultures on MS medium with and without hormone gave 90% and 63% normal developing shoots respectively whereas B5 and WPM showed very low shoot development (less than 16%), even though the shoot tips remained green. Further evaluation of the MS medium shows that with hormone supplement, there was no significant effect of medium strength on normal development of the shoot tips (shoot multiplication or shoot elongation >2cm). Eighty-five percent or above of the shoot tips gave normal development. For MS medium without hormone supplement, regeneration was lower although full and ¾ strength MS medium gave significantly higher regeneration of 66% compared to only 26% for ¼ strength medium. As a result, full strength MS medium supplemented with 1mg/l of NAA, BAP and GA3 was selected as a standard for durian shoot tip culture. For investigations on different loading solutions, it was observed that the percentage viability of non-cryopreserved shoot tips drop drastically from 80% (after preculture) to 25-54% (after loading). Although loading was reported to induce dehydration-tolerance in many cells and meristem, this was not so for durian shoot tips which are very sensitive and fragile. Exposure to PVS2 further reduced the percentage viability (10-30%). All shoot tips were dead after freezing. Loading solution consists of 2M glycerol and 0.4M sucrose with highest viability was used for further experiments. For different duration of exposure to PVS2, the treated control gave 100% viability. This is probably due to the shorter time of exposure to loading solutions. The percentage viability of non-frozen shoot tips decreased as time of exposure increased. The durian shoot tips retained 70% viability after 15 minutes exposure time, but were further reduced to only 40% after 30 minutes due to phytotoxic effects of PVS2 with longer exposure. No viability was observed in frozen shoot tips. Glycerol which is a component of PVS2 was reported to be relatively toxic to some embryos. With different concentrations of glycerol in PVS2, there was no significant effect the percentage viability of encapsulated durian shoot tips ranges from 78% to 95% irrespective of exposure time (10-60 minutes). At 30 minutes exposure time, the percentage viability of encapsulated shoot tips (80%) was much higher than naked shoot tips.

Conclusions

MS medium supplemented with 1 mg/l of NAA, BAP and GA3 was best for normal developing of durian shoot tips irrespective of different strength of MS. All loading solutions evaluated were highly toxic to the durian shoot tips. The PVS2 solution was also toxic and viability decreased with increasing time of exposure even as low as 15 minutes. There was no significant effect of glycerol concentration irrespective of the exposure time. Encapsulation gave better protection to the shoot tips compared with the naked tissues.

References

Sakai, A., Kobayashi, S. and Oiyama, I. 1990. Cryopreservation of nucellar cells of navel orange (*Citrus sinensis* Osb. Var. *brasiliensis* Tanaka) by vitrification. *Plant Cell Rep.* 9: 30-33.

Supported by IRPA Grant 01-02-04-0007