Spinach Tissue Culture Improved with Coconut Water

Jameel M. Al-Khayri, Feng H. Huang, Teddy E. Morelock, and Tahani A. Busharar

Department of Horticulture and Forestry, University of Arkansas, Fayetteville, AR 72701

Abstract. A preliminary study has shown that the addition of 15% (v/v) coconut water (CW) to the culture medium significantly improved callus growth, shoot-regenerative capacity, and shoot growth in leaf disk cultures of spinach (Spinacia oleracea L.). Subsequently, the influence of a range of CW concentrations, 0%, 5%, 10%, 15%, or 20% (v/v), was examined. Callus weight obtained after 5 weeks showed direct relationship to the concentration of CW. This stimulator action was observed in both cultivars tested in this study, 'High Pack' and 'Baker'. On CW-containing medium, shoot regeneration was expedited to 4 to 5 weeks compared with 8 to 12 weeks on a CW-free medium. Callus of 'Baker' induced on a CW-free medium exhibited a significant increase in shoot regeneration frequency when transferred to a regeneration medium enriched with CW, suggesting that the addition of CW to the regeneration medium only is sufficient to achieve improved regeneration.

Recent studies established a tissue-culture system for the plant regeneration of spinach from leaf-derived callus (Al-Khayri et al., 1991a, 1991b). Improvement in the percentage of spinach calli forming shoots had been desired because of the low regeneration frequency. Our objectives were to 1) examine the influence of various concentrations of coconut water (CW) on callus induction and subsequent shoot regeneration, and 2) determine whether limiting the enrichment of CW only to the regeneration medium would improve shoot formation.

The influence of CW on callus regeneration frequency was observed in both cultivars tested in this study, 'High Pack' and 'Baker'. On CW-containing medium, shoot regeneration was expedited to 4 to 5 weeks compared with 8 to 12 weeks on a CW-free medium. Callus of 'Baker' induced on a CW-free medium exhibited a significant increase in shoot regeneration frequency when transferred to a regeneration medium enriched with CW, suggesting that the addition of CW to the regeneration medium only is sufficient to achieve improved regeneration.

Fig. 1. The response of two spinach cultivars to coconut water (CW) at 0%, 5%, 10%, 15%, or 20%: (A) Callus fresh weight; (B) percentage of calli exhibiting shoot regeneration.
rate of increase in callus weight diminished. Therefore, 10% to 15% CW is recommended for spinach medium. Increasing the CW level to 20% gave only a slight increase, particularly with ‘High Pack’.

Shoot regeneration. In the absence of CW, calli derived from ‘High Pack’ regenerated at a frequency significantly higher than that exhibited by ‘Baker’. This relationship also held true in the presence of CW. The percentage of calli, which regenerated shoots from ‘High Pack’ on CW-free medium, was not significantly different from the percentage of regeneration from ‘Baker’ on CW-enriched medium. A significant interaction of genotype and coconut water was also observed in a study with wheat (Triticum aestivum L., em. thell) callus (Mathias and Simpson, 1986). They found that shoot development was promoted by 10% (v/v) CW in some lines but was inhibited in others. In our study, however, shoot formation in both genotypes of spinach was promoted by CW addition, but the degree of increase in regeneration frequency was cultivar dependent.

The percentage of calli that regenerated shoots increased with concentration of CW up to 15% (Fig. 1B). With concentrations > 15%, however, shoot regeneration was inhibited in both cultivars tested.

Growth of regenerated shoots of both cultivars was significantly improved as a result of CW enrichment (data not shown). Shoot length of cultivars was unaffected by CW.

Regeneration was substantially expedited with CW enrichment. In the absence of CW, up to 12 weeks of culture was required before shoot formation became apparent. With CW, however, the time required was reduced to 4 to 5 weeks. There was no definite pattern of the onset of regeneration in relation to increasing concentration of CW.

Limiting CW addition to the regeneration stage. Augmenting only the regeneration medium with CW was sufficient to provide a significant improvement in shoot regeneration of spinach. Calli induced on a CW-free medium exhibited a significant increase in regenerative capacity upon transfer to CW-enriched regeneration medium. Regenerative capacity of calli induced on a CW-free medium (33%), and callus induced on a CW-enriched medium (41%) were similar when these calli were transferred onto a CW-enriched regeneration medium. In contrast, calli grown and induced to differentiate on a CW-free medium exhibited significantly less shoot regeneration (15%).

Although CW addition to callus induction medium is not required to achieve high regenerative capacity, when callus multiplication or rapid callus growth is desired, callus induction and continuous subculture of callus on CW-enriched medium are recommended. Regenerating calli should be maintained on CW-enriched regeneration medium if a shoot multiplication phase is used to increase the number of the regenerated shoots. A multiplication step consists of transferring the regenerating calli onto fresh regeneration medium in GA-7 Magenta ves-

sels (Magenta, Chicago) that provide ample space for shoot multiplication.

Stimulation of callus growth and shoot regeneration by CW in two cultivars of spinach has been shown in this study. Enrichment of the culture medium with CW during the regeneration phase of the system would provide the same effect as enriching the media at the callus induction and shoot regeneration phases. The increase in shoot regeneration capacity of callus, the expedited callus proliferation and the shoot differentiation associated with CW addition would maximize potential use of this tissue culture system as a method for the rapid propagation of selected spinach plants.

Literature Cited