EFFECT OF DIFFERENT BRANDS OF ACTIVATED CARBON ON GROWTH AND DEVELOPMENT OF COCONUT (Cocos Nucifera L) EMBRYOS IN VITRO

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ABSTRACT

The effect of different brands of activated charcoal [Merck GR (Art. 2186), Sigma acid washed (C-4386), Sigma neutralized (C-3790), and Duchefa neutralized (C-1302)] on growth and development of coconut zygotic embryos in vitro was evaluated. Analysis of data noted after one month revealed that there was no significant difference on percentage germination, shoot length, and number of primary root of cv. Laguna Tall embryos cultured in Y3 liquid medium supplemented with 2.5g/l AC of different brands. Length of primary root of embryos cultured in Duchefa neutralized AC was significantly different from those in Sigma neutralized AC only after one month from initial culture. For the succeeding periods (2-4 months), no significant difference was observed among the treatments in terms of increment in plant height, number of scale and true leaves and length of primary root. Statistical analysis revealed that percentage of seedlings with primary, secondary and tertiary roots did not differ significantly among the treatments 1-4 months from initial culture. Results suggest that any brand (even alternately) can be used satisfactorily in the in vitro culture of coconut embryos.

INTRODUCTION

Activated carbon (AC) is prepared by the controlled carbonization of wood in steam or air (George, 1993). At present different brands of AC are being manufactured which according to Pierik (1987) could be obtained either from an animal or vegetable sources. However, not all brands of AC are the same and their properties differ according to the method they are prepared (George, 1993). A specific brand of AC maybe identified beneficial and/or promotory to growth and development of coconut embryos in vitro, hence this study.

REVIEW LITERATURE

Owing to its strong adsorptive properties, activated carbon (AC) is being used in many tissue culture laboratories. Like sugars, its addition to the culture medium had been considered indispensable as it is associated with better growth response of cultured tissues (Anagnostakis, 1974; Fridborg & Eriksson, 1975; Horner et al., 1977; Johansson & Eriksson, 1977; Weatherhead et al., 1978; Peck & Cuming, 1986; Bon et al.; 1988; Venketeswaran et al.; 1988, Zaghmout & Torello, 1988). It has the properties of adsorbing growth inhibitors (Fridborg et al., 1978; Weatherhead et al., 1978; 1979; Compton & Preece, 1986; Pierik 1987; Nairn, 1988) as well as growth regulators, organic nutrients and inorganic ions (Fridborg & Eriksson, 1975; Weatherhead et al., 1979; Nissen & Sutter, 1988; 1990; Ebert and Taylor, 1990; 1993).

The presence of the AC in the medium. sometimes leads to further adjusting the growth regulators used to a much higher concentration. Paranjothy & Rohani (1982) had to use auxin 10 times higher in concentration to initiate embryogenic callus of oil palm. Nwanko & Krikorian (1983) had to increase the concentration of NAA or 2,4-D from 5-10 mg/l to 10-70 mg/l in culture medium

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with 0.5 g/l AC. To Krikorian (1983) had to increase the concentration of NAA or 2,4-D from 5-10 mg/l to 10-70 mg/l in culture medium with 0.5 g/l AC. To induce embryogenesis in cultures of palms, Tisserat (1979; 1984) added 0.15-0.50 mM 2,4-D which is accordingly 5-20 times of what is normally required. If high concentrations of AC are used, Nissen & Sutter (1990) reported that 10-100 times more auxin should be added to a medium.

The levels of AC used in tissue culture media vary from 0.2% to 3.0% (Pierik, 1987). Tyagi et al (1980) reported that effective concentrations of charcoal for promoting embryogenesis from Datura pollen, varied according to the type of agar used. Addition of 3 g/l AC was promotory for root and shoot growth of date palm embryos (Rebechault et al., 1976; Reynolds & Murashige, 1979). In embryo culture of coconuts, the use of 2.5 g/l AC has proven beneficial in either Y3 or MS media (Rillo & Paloma, 1990).

This paper reports the effect of four brands of AC on the growth and development of coconut embryos in vitro.

**METHODOLOGY**

Ten to eleven month-old cv. Laguna Tall embryos were extracted, sterilized and initially cultured onto Y3 liquid medium following the protocol described by Rillo and Paloma (1992). At 2.5 g/l each, the following brands of activated charcoal were used as the treatments:

- TI - Merck, GR (Art. 2186)
- T2 - Sigma, acid-washed (C-4386)
- T3 - Sigma, neutralized (C-3790)
- T4 - Duchefa, neutralized (C-1302)

For the initial culture, 40 g/l of table grade sugar was used. The Data on the length of plumule (shoot length) length of the radicle (root length) and germination rate were noted four weeks after initial culture. Embryos were considered germinated when their shoots were about 1 mm long (de Guzman & del Rosario, 1964).

Germinated embryos were transferred onto fresh Y3 liquid medium with 45 g/l sugar. Transfer interval was monthly. Plant height, leaf and root formation were noted before each subculture.

Treatments were replicated three times in a Completely Randomized Design. Data were analyzed using Analysis of Variance (ANOVA) and treatment means were compared using Duncan's Multiple Range Test.

**RESULTS**

Analysis of data noted after one month revealed that there was no significant difference on percentage gemination, shoot length and number of primary root among embryos cultured in different AC treatments (Table 1). In terms of average primary root length, significant difference was observed between the neutralized brands of AC. Primary root length of embryos cultured in Duchefa neutralized AC was significantly different from those in Sigma neutralized AC. This trend, however, was not observed during the succeeding periods (2-4 months -Table 2). Both AC were comparable from the other treatments (Merck and Sigma acid washed).
Also there was no significant difference among the treatments in terms of average increment in plant height and primary root length 2-4 months after initial culture (Table 2). Average primary root formation in embryos cultured in Duchefa neutralized AC was significantly different from Sigma acid washed AC. This trend, too, was not observed on the following 3-4 months. Both were comparable to Merck and Sigma neutralized brands of AC.

Analysis showed that there was no significant difference in terms of formation and average increment in scale and true leaves among the embryos 2-4 months after initial culture (Table 3).

Almost the same percentages in terms of seedling with primary root/s was noted among the treatments 4 months after initial culture (Table 4). Secondary and tertiary roots of embryo-cultural seedlings started to form during the second month of the culture period and onwards. When analyzed statistically, percentage of seedlings with primary, secondary and tertiary roots did not differ significantly among the treatments.

### Table 1. Mean percentage germination, shoot and primary root lengths, primary root formation of germinated cv. Laguna Tall embryos cultured in Y3 Uquid medium supplemented with different brands of AC one month after initial culture.

| AC Brand               | Germination (%) | Shoot Length (mm)* | Root Length (mm)* | Primary root formation* |
|------------------------|-----------------|--------------------|-------------------|-------------------------|
| Merck (M)              | 65.57<sup>a</sup> | 7.42<sup>a</sup>   | 13.59<sup>ab</sup> | 0.98<sup>a</sup>       |
| Sigma, acid washed (SAW) | 58.57<sup>a</sup> | 6.99<sup>a</sup>   | 13.34<sup>ab</sup> | 0.99<sup>a</sup>       |
| Sigma neutralized (SN) | 56.84<sup>a</sup> | 7.23<sup>a</sup>   | 11.68<sup>b</sup>  | 0.99<sup>a</sup>       |
| Duchefa, neutralized (DN) | 62.56<sup>a</sup> | 7.43<sup>a</sup>   | 14.54<sup>a</sup>  | 0.99<sup>a</sup>       |

Means with the same letter are not significantly different at 5% level

*-------represents the initial data

### Table 2. Average increment in plant height, root length and primary root formation 2-4 months From initial culture

| AC | Plant Height (mm) | Root Length (mm) | Primary Root Formation |
|----|-------------------|------------------|------------------------|
|    | 2 mo   | 3 mo   | 4 mo   | 2 mo   | 3 mo   | 4 mo   | 2 mo   | 3 mo   | 4 mo   |
| M  | 17.11<sup>a</sup> | 36.99<sup>a</sup> | 57.05<sup>a</sup> | 13.47<sup>a</sup> | 7.32<sup>a</sup> | 1.04<sup>a</sup> | 0.05<sup>ab</sup> | 0.18<sup>a</sup> | 0.17<sup>a</sup> |
| SAW | 14.56<sup>a</sup> | 37.37<sup>a</sup> | 68.38<sup>a</sup> | 9.16<sup>a</sup>  | 8.36<sup>a</sup> | 5.04<sup>a</sup>  | 0.02<sup>b</sup>  | 0.22<sup>a</sup> | 0.14<sup>a</sup> |
| SN  | 15.89<sup>a</sup> | 37.01<sup>a</sup> | 71.21<sup>a</sup> | 10.48<sup>a</sup> | 12.56<sup>a</sup> | 8.54<sup>a</sup> | 0.05<sup>ab</sup> | 0.28<sup>a</sup> | 0.19<sup>a</sup> |
| DN  | 16.62<sup>a</sup> | 42.36<sup>a</sup> | 49.50<sup>a</sup> | 9.38<sup>a</sup>  | 8.39<sup>a</sup> | 0.78<sup>a</sup> | 0.10<sup>a</sup> | 0.26<sup>a</sup> | 0.12<sup>a</sup> |

Means with the same letter are not significantly different at 5% level
Table 3. Initial formation and average increment of scale and true leaves 2 and 3-4 months after initial culture, respectively

| AC  | Scale Leaf Formation | True Leaf Formation |
|-----|----------------------|---------------------|
|     | 2 mo* | 3 mo** | 4 mo** | 2 mo* | 3 mo** | 4 mo** |
| M   | 2.02* | 0.80* | 0.29* | 0.05* | 0.48* | 0.36* |
| SAW | 1.87* | 0.89* | 0.48* | 0.04* | 0.45* | 0.47* |
| SN  | 1.88* | 1.08* | 0.54* | 0.05* | 0.46* | 0.52* |
| DN  | 2.04* | 0.80* | 0.16* | 0.07* | 0.45* | 0.46* |

Means with the same letter are not significantly different at 5% level

*------- initial data
**------ average increment

Table 4. Percentage of plantlets with primary, secondary and tertiary root formation 1-4 months after initial culture

| AC  | With Primary Roots | With Secondary Roots | With Tertiary Roots |
|-----|-------------------|---------------------|-------------------|
|     | 1 mo | 2 mo | 3 mo | 4 mo | 1 mo | 2 mo | 3 mo | 4 mo | 1 mo | 2 mo | 3 mo | 4 mo |
| M   | 97.93a | 99.35a | 100a | 100a | 0.00a | 48.18a | 53.25a | 61.12a | 0.00a | 1.93a | 16.20a | 31.23a |
| SAW | 97.70a | 99.19a | 99.19a | 99.19a | 0.00a | 36.48a | 50.67a | 61.71a | 0.00a | 4.35a | 12.33a | 30.96a |
| SN  | 98.83a | 100a | 100a | 100a | 0.00a | 35.63a | 52.24a | 65.83a | 0.00a | 1.62a | 21.43a | 36.28a |
| DN  | 98.66a | 100a | 100a | 100a | 0.00a | 36.39a | 54.97a | 57.74a | 0.00a | 1.75a | 17.75a | 19.64a |

Means with the same letter are not significantly different at 5% level

CONCLUSION AND RECOMMENDATION

The above results suggest that any of the four brands of activated charcoal, even alternately, can be used satisfactorily in the in vitro culture of coconut embryos.

To lower the production cost, AC of the least price can be used.

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