Abstract

Seeds of cacao which were surfaced sterilized in a solution of 30% Clorox for 30 minutes gave the highest percentage of noncontamination and viability. Sterile seeds were cultured on MSG 2 medium without plant growth regulator. Segments of cacao seedlings were excised and cultured on MSG 1, MSG 2 and MSG 3 media. It was found that yellowish friable callus was initiated from epicotyl cultured on MSG 3 medium supplemented with 0.0125 mg/l 2,4-D and 0.5 mg/l BA.

Epicotyl-derived callus was transferred to MSG 2 liquid medium containing 0.5 mg/l 2,4-D to establish cell suspension culture. Cell suspension was subcultured every 10 days and filtered several times until the desired uniform size was met. Protoplasts were isolated from 6 day old cells after each subculture. A sample of cells, of 0.25 g fresh weight, was incubated in a 20 ml solution of 0.5 M sorbitol, 10 mM $CaCl_2.2H_2O$, 1 mM MES and Driselase 2% (w/v) at pH 5.0 for 3 hours. The yield of isolated protoplasts was 4.5×10^5 protoplasts/ml and the protoplast viability as monitored by fluorescein diacetate was 95%. The protoplasts were cultured in the dark at a density of 4×10^5 protoplasts/ml in the same liquid medium as cell suspension, except 0.5 M sucrose was added to maintain osmolarity of the protoplasts. After 8 days in culture, wall formation was evident and cell division of cultured protoplasts appeared within 4 weeks. The small aggregate cells obtained were multiplied by culturing in the same liquid medium with a reduction

of sucrose to 0.09 M (3%)

Embryoids were regenerated from callus derived from cacao cotyledons and protoplasts cultured on MSG 1 medium supplemented with 100 ml/l coconut water and 1.5 mg/l NAA for 4 weeks and then subsequently transferred to the same medium without coconut water and NAA. By subculture onto a similar medium for several times, the embryoid devloped into complete plantets. Histological analysis revealed that the embryoid showed anatomical structures similar to those of the zygotic embryo, including leaf primordium, shoot apex, vascular strand and root apex.