ABSTRACT

Investigations have been conducted on factors affecting the viability and mitotic activity in mesophyll protoplasts isolated from *Sorghum bicolor*. This was part of an investigation ultimately aimed at producing callus and plant regeneration from Sorghum protoplast.

A number of factors were shown to affect protoplast viability over prolonged periods of incubation including seedling age at time of leaf excision and light conditions under which plants were grown (Section 1 - Results). It was not possible to isolate viable protoplasts from leaves of very young seedlings (less than 6 days old). Leaves from 6-9-day old seedlings grown under normal light regimes (20,000 lux, 16 h photoperiod) yielded about 1.6×10^6 protoplasts per gram leaf material but these protoplasts had lost viability within 24 hours. Pre-treatment of excised leaves with senescence retarding agents such as arginine, kinetin and lysin not only reduced the yield but also accelerated the rate of lysis of protoplasts. Viability of protoplasts was enhanced slightly when isolated from plants grown under low light intensity. However, plants grown in complete darkness throughout the growth period gave very low protoplast yields (1×10^4 protoplasts per gram leaf material). High yields of viable protoplasts were obtained when seedlings germinated under low light intensity (5000 lux) were incubated in continuous darkness for a period of 2-4 days prior to protoplast isolation. The yield of protoplasts doubled (3.5×10^6 protoplasts per gram leaf material) when isolated from these seedlings and the protoplasts remained viable during extended periods of incubation. However, no significant division was observed in these protoplasts when cultured in a defined medium.

High viability (over 90% of the population after one day incubation) and mitotic activity (5.2% dividing nuclei and 0.6% bicells) were observed when protoplasts isolated from dark pre-treated seedlings were cultured in a medium conditioned by prior growth of Sorghum shoot callus (Section 2 -

Results). Protoplasts derived from seedlings grown under normal light regimes and incubated in the conditioned medium underwent complete lysis within 24 hours of incubation.

Mitotic activity of protoplasts isolated from dark pre-treated seedlings and cultured in the conditioned medium was not enhanced by modifications to the culture medium or to the incubation conditions of the protoplasts (Section 3 - Results).

A series of investigations was also carried out to study the conditioning process and to characterize the active principle(s) of the conditioned medium (Section 4 - Results). The cell suspension culture medium acquired growth promoting activity towards protoplasts 6-10 days after incubation. It was observed that the growth promoting activity of the conditioned medium was determined by the type and concentration of the hormones incorporated into the medium during the process of conditioning. Activity of the conditioned medium was irreversibly destroyed at elevated temperatures (80°C and above). Viability and mitotic activity of protoplasts cultured in the diluted conditioned media were deleteriously affected by both the type of diluent and the extent of dilution.

Growth promoting properties of the conditioned medium were associated with two major components in the medium. Active components were detected in both the dialysate and the dialysable fraction of the medium. Two active fractions both absorbing at 280 nm and having molecular weights of approximately 12,000 and 500 were detected after elution of the conditioned medium placed on a sephadex G-75 column. Viability of protoplasts deteriorated rapidly when cultured in the high molecular weight fraction concentrated (3-fold) by freeze drying or by ultrafiltration. Although mitotic activity was detected in protoplasts at lower concentrations (2-fold and below), no enhancement was observed compared to the untreated conditioned medium.

It is concluded that dark pre-treatment of seedlings prior to protoplast isolation and the use of conditioned medium are necessary for high viability and nuclear division in Sorghum protoplasts. Two major components of the conditioned medium, a high molecular weight fraction (probably proteinaceous in nature) and a low molecular weight fraction [probably an amino acid(s) and/or a hormone(s)] released into the medium during the process of conditioning appear to impart protoplast division stimulating properties to the medium.

Publications arising from this dissertation:

Karunaratne, S.M. and Scott, K.J. (1981). Mitotic activity in protoplasts isolated from *Sorghum bicolor* leaves. *Plant Sci. Lett.* 23: 11-16.