BRIEF COMMUNICATION

Increase in Dry Weight and Protein Content in *Brassica oleracea* and *Nicotiana tabacum* Pith Explants During Short-term Cultivation on Simple Media

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Received March 22, 1972

Abstract. The stem pith discs of kale and tobacco were cultured on simple sugar-mineral media. The dry weight of the explants rose almost linearly for 20 days and was doubled in 10 to 12 days. Neither protein accumulation nor the increase in cell number are responsible for this relatively high increase in dry weight of discs. The accumulation of sugars or polysaccharides is thought to be the cause of this increase.

Developing defined media for the cultivation of marrow-stem-kale callus tissue derived from stem pith, we observed an unexpected increase in the dry weight of explants on simple media. The increase in dry weight of explants starts soon after planting and no lag-phase was observed. It was interesting to determine which constituent of the media is essential for this increase. In order to know if this phenomenon also exists in other pith explants, tobacco stem pith, was also employed in our experiments.

Kale plants (*Brassica oleracea* L. var. *medullosa* Thellung cv. Krasa) were grown under field conditions. At the end of the first growing season in October they were transferred into a special store-room where they were kept for three months at 3 to 10 °C. The stems of plants were about 60 cm high and their diameter in the thickest region was approximately 10 cm. Tobacco plants (*Nicotiana tabacum* L. cv. Wisconsin 38) were grown in a greenhouse and harvested before flowering when they were about 70 cm high.

Cylinders of a standard diameter were excised from the pith parenchyma by means of a cork-borer and divided into equal parts with the multiple-blade-cutter. The kale and tobacco discs were 3 and 4 mm high and 9 and 6 mm in diameter, respectively. The explants were distributed on the different media according to a random sampling pattern in order to eliminate the effect of their original position in the stem on their medium-response. The discs were cultured on a 0.8% agar medium without additions and on media containing sugar (glucose 3% and sucrose 3%, for the kale and tobacco, respectively) with the addition of mineral salts (microelements of Heller 1953). All these media were solidified with the addition of 0.8% of agar and twice steam-sterilized in an

Arnold apparatus at 100 °C for 20 min. The explants were cultured in 100 ml Erlenmeyer flasks. Each flask contained 40 ml media and four explants. The cultivation was carried out in darkness at 26 °C.

The protein nitrogen was determined by the Kjeldahl method in TCA-insoluble fractions of explant homogenate.

Figures 1 and 2 show that the increase in the dry weight of discs was almost linear during 20 and 15 days of cultivation, respectively. The dry weight of discs was doubled in ten to twelve days. This increase was dependent entirely on the presence of sugars in the medium. Elimination of Heller microelements or addition of Knop mineral salts (1/4, GAUTHERET

Fig. 1. Changes in the dry weight of marrow-stem-kale pith tissue during cultivation on 0.8% agar medium without additions (A) and with the addition of 3% glucose and microelements of HELLER (1953). B. Abscissa: cultivation period [d], ordinate: dry weight of discs [mg]

Fig. 2. Changes in the dry weight of excised tobacco pith discs during cultivation on 0.8% agar medium without additions (A) and with the addition of 3% sucrose and microelements of HELLER (1953). B. Abscissa: cultivation period [d], ordinate: dry weight of discs [mg]

1959) to the sugar medium, however, reduced the dry weight of explants by 10 to 30%. The addition of inositol, thiamin, riboflavin, pyridoxin, ascorbic acid and cobalamin to the media in concentrations of 80, 1, 1, 5, 5 and 1 p.p.m. respectively had no effect on disc growth. Gibberellic acid at 5 p.p.m. decreased the dry matter accumulation by 10%.

The increase in the dry weight of discs after 10 days of cultivation was 10 mg and 3.5 mg for kale and tobacco, respectively. On the other hand the increase in the amount of protein, as calculated on the basis of TCA-insoluble nitrogen (× 6.25) was only 0.79 and 0.50 mg per disc. This shows that only 10% of the dry weight increase is caused by the accumulation of protein. It may be supposed that an accumulation of sugars and/or polysaccharides is responsible for the dry weight increase. The formation and accumulation of polysaccharides has been frequently observed in different plant tissue cul-