Induced Regeneration of Plants in Tissue Cultures of *Brassica oleracea*

Viable plants can regenerate in tissue cultures, if both roots and buds are differentiated. Regenerated plants may develop normally, but they may be polyploid or otherwise changed. Such plants may have, if produced in sufficient amount, besides theoretical importance, also a definite practical value.

Regeneration and development of autotroph plants of Brassicaceae from callus cultures has not yet been reported, so far as is known to us, despite botanical and economical importance of this family. It is known, however, that both cuttings and tissue cultures of *Brassica oleracea* form roots readily[1,2]. Recently, MARGARA[3,4] described bud and root differentiation in primary explants of cauliflower induced by benzylaminopurine and naphtaleneacetic acid.

Callus cultures described here were established to investigate their organ-forming capacity in relation to their metabolism and to employ them, if possible, for breeding purposes.

Materials and methods. Roots, hypocotyls and cotyledons excised from seedlings of marrow stem kale (*Brassica oleracea* L., convar. acephala (DC.) ALEF., var. *medulosa*, cv. Krasa) and discs of mature leaves and of stem pith, ovaries, fertilized ovules and stamina of field-grown plants were cultured on agar medium containing: MURASHIGE and SKOOGS' macro- and microelements[5], sucrose 3%, thiamine 0.4, inositol 80, pantothenate 5.0, triptic casein hydrolysate 100–500, 2,4-dichlorophenoxyacetic acid (2,4-D) 0.2–1.0, and kinetin 0.5–3.0 ppm. Optimal concentrations of the last 3 compounds differed according to

[1] A. T. JAGENDORF and D. M. BONNER, Pl. Physiol. *28*, 415 (1953).
[2] B. P. STROGONOV, E. I. KOMIZERKO and R. G. BUTENKO, Pl. Physiol., Moscow *15*, 203 (1968).
[3] J. MARGARA, C. r. Acad. Sci., Paris *268 D*, 686 (1969).
[4] J. MARGARA, C. r. Acad. Sci., Paris *268 D*, 803 (1969).
[5] T. MURASHIGE and F. SKOOG, Physiologia Pl. *15*, 473 (1962).

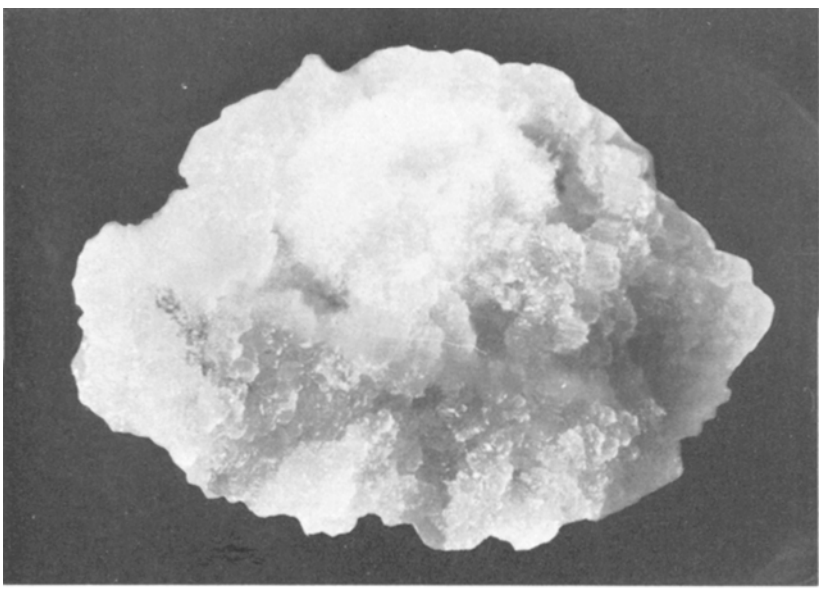

Fig. 1. Mass of callus formed on the disc of stem pith. ×4.2.

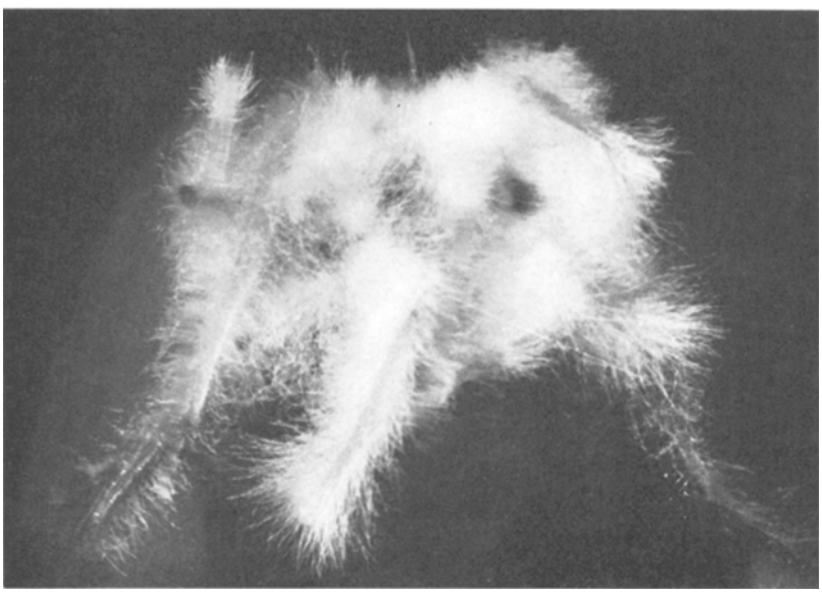

Fig. 2. Roots and root hairs differentiating on the medium in which 2,4-D was omitted. ×4.2.

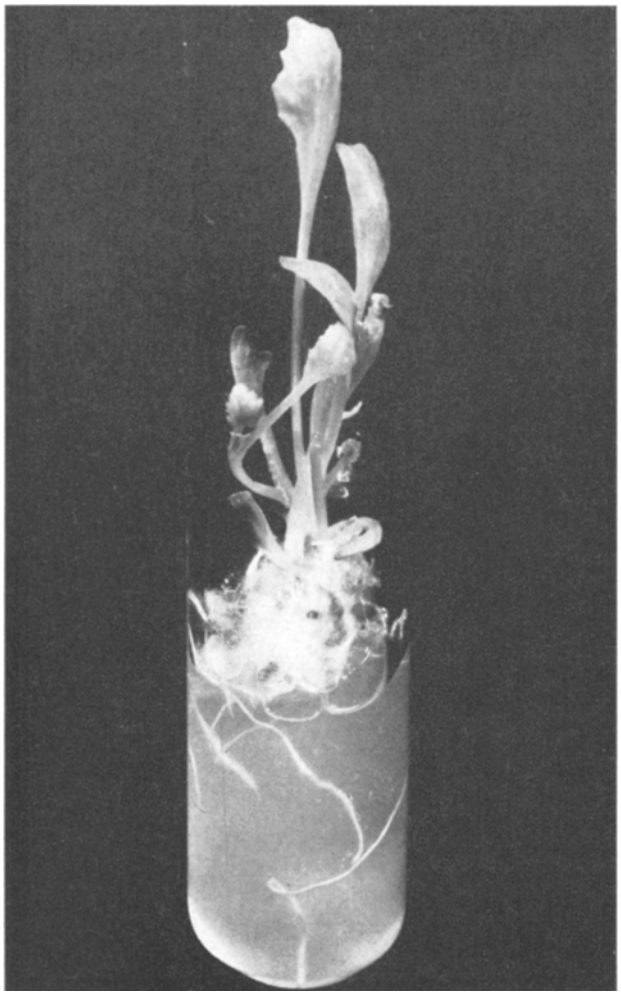

Fig. 3. Plant of marrow stem kale regenerating from the root callus in the fourth subculture. ×1.

the origin of the explants. In the case of roots and leaves they were 0.2–0.5–100 and 1.0–0.5–500 ppm respectively. In natural day-light at 20–24 °C, the explants produced in 10–30 days greyish yellow callus, which grew on the same or slightly modified media, in 1-month subcultures, for more than 1 year (Figure 1). Following 3 months of culturing, the concentration of 2,4-D was progressively lowered.

Results and discussion. Numerous roots and root trichoms were formed in 3–7 days, if the calli were transferred to the medium without 2,4-D (Figure 2). 10–30 days later, leaves and shoots appeared (Figure 3). Usually 40–70% of root calli, 10–30% of calli derived from ovaries and 5–20% of hypocotyls, cotyledons, leaves, stem pith, ovules and stamina calli could differentiate entire plants. Morphogenetic potentiality of callus tissues decreased with time. Shoot-forming ability disappeared in 6–8, and that of roots in 10 or 11 subcultures. Regenerated plants were transferred to soil in pots and then to the field where they developed normally.

Organ-forming capacities of callus tissues originating from different plant parts seem to be influenced by the degree of functional specialization of mother tissues, or by the proportion of the genome involved in its control.

Résumé. Dans les cultures de tissus repiqués de Chou moellier une néoformation de plantes entières se produit en conséquance de l'exclusion du 2,4-D du milien de culture.

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[6] We thank Miss A. ŠTEMBEROVÁ and Mrs. M. PALDUSOVÁ for valuable technical assistance.

Regeneration of Limbs in Adult *Rana ridibunda ridibunda* Pallas

Studies on limb regeneration in most of the salientians among Amphibia revealed that they lose, in general, their regenerative capacity either early or later during the metamorphic period. The few salientians that partially preserve the power of regeneration at the post-metamorphic, and even till the adult stages, form heteromorphic limb outgrowths that never attain the normal pattern of limb morphogenesis.

Briefly, the salientian limb regenerative capacity falls within 3 main categories, viz. 1. early loss before the onset of the metamorphic period as in *Rana sylvatica* and *R. pipiens*[1]; 2. conspicuous decline during the metamorphic period resulting in a large cartilaginous condylar mass capping the stump skeleton as in *Alytes obstetricans*[2] and *Bufo regularis*[3,4]; and 3. better partially preserved regenerative capacity up to the adult stage in members of the families Pipidae[5–10] and Discoglossidae[10]. Their limb regenerates are heteromorphic and range from simple, spike-like to spatulate outgrowths, sometimes with rudimentary digits. In general, these outgrowths mainly consist of cartilage and connective tissue fibres with hardly any bone or muscle fibres[5,8,10].

Normally, the ranids so far studied lose completely the regenerative capacity of their limbs when transected at the adult stage[11–13]. However, this capacity could be partially enhanced by the use of various stimulating procedures[14–18]. Even these experimentally induced limb outgrowths are also mainly supported with cartilage material, with the exception of *Rana clamitans*[18] which showed ossification distally. The present investigation aimed at reporting on the power of limb regeneration in the adult ranid, *Rana ridibunda ridibunda* Pallas and on the osteogenetic pattern within the stump skeleton at, and distal to, the level of amputation.

Individuals, with average head-trunk length of 7 cm, were collected from the fields of Hamman Al-Alil (south of Mosul). One fore-limb in each was transected through the distal level of the antebrachium. Out of a total of 82 cases, 56 were operated on the left-side limb, the remaining animals on the right side one. The series operated on the left side was reared for 5 months, from April till September, while the right side series was reared for 4 months during the same season. The rate of mortality reached 12% of the total number of cases. Soon after