SHORT COMMUNICATION

PLANT REGENERATION FROM THE CALLUS INDUCED
FROM WHEAT EMBRYO¹⁾

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Efficient production of wheat plants from cultured tissues is valuable, if realized, for breeding and genetical works in wheat. Unfortunately, however, a very small number of plants have been obtained only by chance from wheat calluses derived from various organs (Shimada *et al.* 1969; Dudits *et al.* 1975; Ogura and Shimada 1978). In maize, Green and Phillips (1975) succeeded in induction of the callus from embryo scutellum and in subsequent regeneration of complete plants. The author tested the regeneration of wheat plants from the callus originated of the embryo, the results being reported here.

Triticum aestivum cv. Chinese Spring and cv. Salmon (2n=42) were used. Wheat seeds aseptically excised on 4 to 15 days after self-pollination were placed on the RM-1964 agar medium (Linsmaier and Skoog 1965) containing 2.0 mg/l of 2,4-D. Each excision consisted of the complete embryo, some part of seed coat and a piece of endosperm. When the excisions were incubated about 10 days under continuous fluorescent illumination, calluses were induced from the embryo. The results of callus induction after about one month incubation are shown in Table 1. Frequencies of callus formation from 13 and 15 days old embryos of cv. Chinese Spring were 32.1%

Table 1. Callus induction from the embryos of various ages in two cultivars of common wheat; cultured on RM-1964 basal medium supplemented with 2.0 mg/l of 2,4-D

Material	Embryo's age (days)	No. embryos inoculated	No. calluses induced (%)	No. calluses with green spot or shoot (%)
Chinese Spring	4	18	1	—*
	5	28	3	—*
	13	28	9 (32.1)	5 (51.3)
	15	32	18 (56.3)	5 (27.8)
Salmon	14	111	41 (36.9)	6 (14.6)

* not proliferated

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and 56.3%, respectively, while that of the 14 days old embryos of cv. Salmon was 36.9%. Few calluses were formed from immature embryos (4 to 5 days after pollination), which did not proliferate. About 15 to 50% of the calluses from mature embryos (13-15 days old) exhibited localized chlorophyll development (green spots) with occasional formation of many green shoots from the green spots (Fig. 1). These calluses with green spots and/or shoots, which were called the totipotent callus by Green and Phillips (1975), were transferred to 2,4-D-free, RM-1964 medium. After two weeks many curled leaves and roots were formed (Fig. 2).

Fig. 1. Calluses induced from the embryos of two cultivars of common wheat; cultured on RM-1964 medium containing 2.0 mg/l of 2,4-D, and having green spots.

Fig. 2. Shoot formation from the calluses subcultured on 2,4-D-free medium: Curled leaves and roots are developed.

Calluses without green spot and shoot were subsequently subcultured on RM-1964 medium containing 2.0 mg/l of 2,4-D every month. The results are shown in Table 2. 13.6% to 38.5% of the calluses were found to be totipotent, which grew to seedlings after transferring to the 2,4-D-free medium. When grown on the 2,4-D-free medium, the calluses without green spot and/or shoot produced roots, often extensively, but none of them gave shoots.

When differentiated plants of both cultivars, Chinese Spring and Salmon were transplanted to soil, most of them grew normally in the greenhouse (Fig. 3), in spite of the fact that they possessed abnormal leaves during plantlet differentiation. In total, about 40 complete plants were restored. Four differentiated plants of cv. Salmon

Table 2. The ability of embryo-derived calluses to form green spots or shoots on RM-1964 medium supplemented with 2.0 mg/l of 2,4-D

Material	Duration of culture (months)	No. calluses inoculated	No. calluses with green spot or shoot (%)
Chinese Spring*	1	26	10 (38.5)
	2	44	6 (13.6)
	3	29	8 (27.6)
Salmon	1	41	6 (14.6)
	2	25	9 (36.0)
	3	37	6 (16.2)
	4	11	4 (36.4)

* Calluses derived from 13 or 15 days old embryos.

Fig. 3. Plants ($2n=42$) restored from one callus of cv. Salmon; three months after transplanting to pots.

had 42 chromosomes in their root tip cells. Meiosis of those plants has not yet been tested.

To summarize, wheat calluses were obtained from the young embryo of *T. aestivum* cv. Chinese Spring and cv. Salmon, and were maintained by subculture every month on RM-1964 medium containing 2.0 mg/l of 2,4-D. The calluses remained capable of differentiation for four months and more, and normal complete plants are easily restored from them.

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