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Studies of callus tissue derived from the scutellum of maize

C. E. Green and R. L. Phillips have previously reported on their studies of maize tissue culture (Crop Sci. 14:54-58 and 14:827-830, 1974) and on their success with plant regeneration (Crop Sci. 15:417-427, 1975). The significance of such research is apparent: cell cultures provide the opportunity for *in vitro* study of a higher organism and regeneration provides a link between *in vitro* culture and classical genetic and breeding procedures.

The material employed in this study was inbred A188, obtained from C. E. Green; a genetic stock obtained from M. G. Neuffer; and their reciprocal hybrids. The genetic stock represents the F_1 of M14/W23 by a p r Y strain derived from a synthetic involving Longfellow Flint, inbred Kys, and Argentine Pop. Over a thousand immature embryos have been excised following the procedures outlined by Green and Phillips (1975). Excision was performed 12 to 24 days after pollination. The embryos, with their attached scutellum intact, were placed on a solid culture medium with the flat embryonic axis in contact with the medium.

The culture medium used was that of T. Murashige and F. Skoog (Physiol. Plant. 15:473-497, 1962), modified according to Green and Phillips (1975) by replacing the MS organic components with the following: 7.7 mg glycine, 1.98 g L-asparagine, 1.3 mg niacin, 0.25 mg thiamin-HCl, 0.25 mg pyridoxine-HCl and 0.25 mg Ca pantothenate per liter. Sucrose and agar were added at 20 g and 8 g per liter respectively. The plant hormone 2,4-dichlorophenoxyacetic acid (2,4-D) was added at 2 to 4 mg/l. The medium was adjusted to pH 6.0 with NaOH and autoclaved for 20 minutes at 20 psi.

Cultures were incubated at 28 to 30 C with a 16/8 hour photoperiod. Lighting was provided by cool-white fluorescent lamps in combinations to provide intensities ranging from 150 to 15,000 lux. Subculturing was performed every 3 to 4 weeks.

After 24 to 48 hours of incubation, the dome-shaped scutellum showed a characteristic swelling. Within 72 hours of excision, there was evidence of embryonic growth. Except for 6 instances, the embryo flipped over and callus began to form at the first node of the shoot within 2 weeks after excision. The 6 noted exceptions showed a swelling of the scutellum, no embryonic development, and the development of what appeared to be a brown-colored callus on the dome of the scutellum within the same time period. No further growth was apparent in these cases even after subculture.

The sequence of events described above was the same for 14, 16, 18, 20, 22, and 24 day old embryos placed on media containing 2, 3, or 4 mg/l 2,4-D. Twelve-day embryos placed on media containing 2 mg/l 2,4-D showed little or no shoot development. Callus formed only after the scutellum flipped over due to root growth from the area of the embryonic axis.

A compact yellow callus proliferates once the remains of the embryo are removed after the first subculture. A few roots are produced which rapidly increase in number if the callus is left on the same medium without subculture for more than 4 weeks. This is attributed to a rapidly decreasing concentration of 2,4-D.

Quite often areas of the callus begin to green and structures apparently lacking any organization appear in association with these green areas. If these cultures are subcultured to media containing the same concentration of 2,4-D, these structures persist and the callus continues to proliferate. If the cultures are transferred to media containing 0.25 mg/l 2,4-D, the green areas may or may not persist, and roots will begin to differentiate. This response is continued if the culture is then transferred to media lacking any hormone, with a concomitant increase in the number of roots. If cultures containing a plethora of roots are broken up and returned to media containing 2 mg/l 2,4-D, callus may again proliferate but at a much slower rate. Table 1 provides data from the most recent set of excisions.

The only regeneration was from a 24-day embryo from a self of Neuffer's genetic

Table 1. Ability of immature embryos to form callus.

Genotype	Embryo age	Embryo size[1]	Number isolated	Nodal callus[2]	Scutellar callus[3]
(Light intensity 15,000 lux; 2 mg/l 2,4-D)					
A188/"ABPHYLL"	18	6-7	20	20	0
A188/genetic stock	18	6-7	30	30	0
A188/genetic stock	16	5-6	20	20	0
genetic stock/"ABPHYLL"	16	4-5	10	10	0
A188	12	2-3	10	4	0
A188/genetic stock	12	2-3	20	8	0
genetic stock/"ABPHYLL"	12	3	1	0	0
genetic stock	12	1-3	5	2	0
(Light intensity 7500 lux; 2 mg/l 2,4-D)					
genetic stock	18	5-6	20	20	0
A188	18	5-6	10	10	0
A188/"ABPHYLL"	18	5-6	20	20	0
genetic stock/"ABPHYLL"	18	5-6	10	10	0
"ABPHYLL"	18	3-4	5	5	0
(Light intensity 1400-3400 lux; 2 mg/l 2,4-D)					
A188	16	4	2	2	0
"ABPHYLL"	18	3-4	5	5	0
(Light intensity 150 lux; 2 mg/l 2,4-D)					
A188/genetic stock	18	5-6	5	5	0
A188	16	4	2	2	0
"ABPHYLL"	18	3-4	5	5	0

[1]length in millimeters

[2]scutellum flipped over due to embryonic growth, callus formed from first node of epicotyl

[3]scutellum did not flip over, embryonic growth aborted, callus formed from dome of scutellum

stock. The culture was initiated and maintained on 4 mg/l 2,4-D and incubated under a combination of fluorescent and incandescent lamps with a combined intensity in excess of 15,000 lux. The temperature was 28 to 30 C and the photoperiod 16/8 hours. It should be noted that this culture was in a group of cultures that were regularly subcultured late, that is, every 5 to 6 weeks. Consequently, it had been nearly 6 months since the initial excision.

Approximately 5 weeks after the third subculture, the callus had a dull yellow color with several roots and a few white-colored, unorganized structures along with a small, leaf-like structure. The culture was divided in half and the half with the leaf-like structure was placed in media lacking any hormone. The other half was placed in media containing 4 mg/l 2,4-D. The latter exhibited no further organization.

Within 4 weeks, a definite shoot had developed and the culture was transferred to a medium used for the in vitro culture of mature embryos. It contained 0.6 g NH_4NO_3, 0.4 g $MgSO_4 \cdot 7H_2O$, 0.4 g $Ca(H_2PO_4)_2 \cdot H_2O$, 0.4 g KH_2PO_4, 0.160 g K_2HPO_4, 6 mg ferric citrate, 10 g sucrose, and 7 g agar per liter. The plantlet was placed in a 4 liter flask containing a liter of this medium and was incubated at 28 to 30 C with a combination of fluorescent and incandescent lamps closely approximating daylight. The photoperiod was 16/8 hours. Growth continued, and after 3½ weeks

the plant was potted in sterilized soil in a 10" pot and returned to the growth chamber. After 3 weeks it was placed in the greenhouse. A month later it was evident that the regenerated plant had a decussate leaf arrangement closely resembling the ABPHYLL syndrome reported by R. I. Greyson and D. B. Walden (Am. J. Bot. 59:466-472, 1972). The plant produced two ears, at opposite nodes, and had fully fertile pollen. There were 27 leaves on the plant compared to 12 to 14 leaves on other plants of the same background. Leaf width ranged from 3.5 to 4.5 cm and leaf length from 20 cm (the short, upper leaves) to 67 cm (the long, lower leaves). The plant, in a 10" pot, was 50 cm tall. It was selfed and also outcrossed to the genetic stock and to A188. The F_1 seed from these crosses produced plants with a distichous leaf arrangement and 12 to 14 leaves. These plants were selfed and the F_2 will be analyzed with a winter greenhouse crop. Chromosome counts of root tips prepared from seedlings grown from the seeds from the self of the regenerated plant revealed no chromosome aberrations or any deviation in ploidy.

Unfortunately, attempts for further successes at regeneration by mimicking the conditions that produced the "ABPHYLL" plant have been in vain. No plants have been regenerated by using the same procedures outlined by Green and Phillips. Experiments are being continued in this area.

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Pollen suspending media: A retraction regarding cyclohexane

In 1973 (MNL 47:144) we reported experiments in which cyclohexane appeared to be a promising medium for suspension of pollen if the solvent was volatilized before pollination. Experiments in three subsequent seasons have failed completely; no reasons for the difference between the original and subsequent tests have become apparent, and we have discontinued attempts to exploit this solvent. If anyone has succeeded with cyclohexane (or any other) in obtaining good seed sets following suspension and volatilization, please compare notes.

E. H. Coe, Jr.

Symbolization: Need for a generic form

The common practice in the past of using a to represent generically any or all of a (i.e., a1, a2, etc.,) is precluded by the current rules, since the subscript 1 is no longer used. Ambiguity arises in expressing this generic meaning with a symbol rather than with words or a phrase (e.g., white seedling). A simple solution would be to use the asterisk, already stipulated for new isolates, without the isolation number: a*, w*, etc. In developing symbol listings and indexes, I plan to follow this practice, and would appreciate reactions from cooperators.

E. H. Coe, Jr.

Dating pollinating bags

To date bags rapidly, we have been using in our laboratory a simple binary-number system marked onto the bags with a soft graphite carpenter's crayon. Bags in groups of 50 can be fanned out uniformly in the lengthwise direction with a little practice, then marked in strokes along the stepped upper segments. Identifying five positions across the top of the bag as 16, 8, 4, 2, 1 (left edge, center, center right, right edge), markings in these positions identify 31 dates in typical binary notation: 00001, 00010, 00011, 00100, 00101, etc. The markings are unmistakeable and remain until harvest if applied carefully and firmly.

E. H. Coe, Jr.