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Los Alamos National Laboratory, Los Alamos, New Mexico (USA),
Department of Botany and Plant Pathology, Colorado State University,
Fort Collins, Colorado (USA),
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With 9 figures and 4 tables

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Abstract

If tissue culture is to be useful in plant breeding, methods for obtaining long-term, high-frequency plant regeneration from easily obtainable starting material (like seeds) are needed. Callus cultures of wheat (*Triticum aestivum* L.) initiated from germinating seeds or immature embryos produced two cell types. Embryogenic (E)*) callus consisted of small isodiametric cells and was compact in nature. Non-embryogenic (NE) callus consisted of long tubular cells and was friable in nature. Plant regeneration from embryogenic regions was of high frequency. Non-embryogenic (NE) callus infrequently produced regenerated plants. So long as calli produced regions of embryogenic cells, regeneration continued if the regions were placed on a defined regeneration medium.

Immature embryos produced a nodular, "rough" E callus and NE callus from the scutellum if this organ faced away from the medium. When immature embryos were placed with scutella in contact with the medium, the scutella did not usually form calli. Instead, the shoot apical region produced a "smooth" E callus, and the root apical region produced NE callus. When immature embryos were dissected into scutella, shoot apical regions, and root apical regions, they produced nodular E, smooth E and NE callus, respectively. Mature embryos (seeds) produced smooth E callus from the shoot apical region, NE callus from the root apical region, and no

*) Abbreviation list: embryogenic, E; non-embryogenic, NE; 2,4-dichlorophenoxyacetic acid, 2,4-D; 2,4,5-trichlorophenoxyacetic acid, 2,4,5-T; indoleacetic acid, IAA; kinetin, KIN; tryptophan, TRP; benzyladenine, BA.

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callus from the scutellum. The relative amounts of E and NE callus in mature and immature embryos could be significantly altered by different 2,4-D concentrations in the medium.

Key words: *Triticum aestivum* — callus formation — plant regeneration — somatic embryogenesis — tissue culture

If cereal tissue culture is to make a contribution to plant breeding, reliable, repeatable regeneration methods must be obtained. A number of workers have reported the regeneration of wheat plants from callus cultures derived from various plant parts (YURKOVA et al. 1981). The frequency and duration of plant regeneration has usually been low (CONGER 1981). SEARS and DECKARD (1982), using one defined series of media, achieved high rates from immature, embryo-derived callus of some cultivars and found that both the rate and occurrence of regeneration was quite cultivar-specific.

In recent years, close visual observation of tissue in calli in a number of cereals has revealed that a white, compact tissue — usually termed "embryogenic callus" is the source of most if not all plant regeneration (NABORS et al. 1983, OZIAS-AKINS and VASIL 1982). The frequently low rates of plant regeneration in cereal tissue cultures, particularly those derived from mature embryos, are explained by the fact that embryogenic callus typically makes up a small fraction of the callus. Also, most media which select for rapidly growing callus usually favor growth of larger non-embryogenic cells which form the friable, sometimes crystalline-appearing callus masses typical of cereal tissue cultures (NABORS et al. 1983).

For rice (HEYSER et al. 1982), oats (HEYSER and NABORS 1982a), and proso millet (HEYSER and NABORS 1982b) we have shown selection of embryogenic callus leads to long-term, high-frequency plant regeneration. We now report similar results for wheat and also show that the frequency and types of embryogenic callus can be manipulated by changes in medium composition

Materials and Methods

Calli were obtained from germinating seeds and immature embryos of the spring wheat cultivars 'Chris' (obtained from the Colorado State University Department of Agronomy), 'Glennson-81' and 'Pavon-76' (obtained from CIMMYT).

Mature and immature seeds were surface sterilized for ten seconds in 90% ethanol and 30 minutes in 2.6% sodium hypochlorite as appropriately diluted commercial bleach. Callus was initiated on LINSMAIER and SKOOG's (1965) basal medium, plus concentrations of hormones, which was solidified with 1% agar.

Calli were cultured in glass vials 25 mm in diameter and 70 mm in depth with plastic screw caps, and containing 10 ml medium or in multi-well tissue culture plates with 1 ml medium per well. They were grown 25 cm from two 40 w, wide-spectram fluorescent bulbs in continuous light at 28°C.

Callus cultures were transferred to fresh medium every four-six weeks. At each transfer, all embryogenic and some similar sized non-embryonic regions were removed and

placed on a medium designed to encourage plant regeneration. The bulk of NE callus was placed in a vial of fresh callus-initiation medium.

Regenerated plants were removed from vials when the leaves reached 2.5 cm in length. They were placed with roots in distilled water for one week to encourage root growth, then planted in 10 cm pots in commercial potting soil.

Root-tip squashes were prepared by a modified method of MUJEEB et al. (1978). Root tips were removed from pot-grown plants and pretreated for 3 h in a solution of 0.02 M 8-hydroxyquinoline plus 0.01 M colchicine. Twenty ml of the solution contained four drops of dimethyl sulfoxide. When the solution was removed, 2% aceto orcein was added, and the tips were stored in the refrigerator until use. The root tip was then removed from stain and boiled in 45% acetic acid. A 1 mm root-tip section was trimmed and macerated on a slide. The cover slip was added; the slide was gently heated and squashed. Slides were made permanent by the dry-ice method.

For thin sections, tissues were killed and fixed in Craf III fixative, dehydrated through an alcohol/xylene series, embedded in Tissuemat, sectioned at 8 μm, and stained with safranin/fast green.

Results and Discussion

Characterization of E and NE callus

Cereal E callus consists of small, isodiametric cells which average 31 μm in diameter in wheat (*Fig. 1*). NE callus consists of long, tubular cells which average 52 μm in diameter and 355 μm in length. Visually, E callus appears as compact, dense regions, while NE callus is loose and friable in appearance (*Figs. 2—4*). E callus is more difficult to distinguish in wheat than in oats, pearl millet, proso millet, or rice (NABORS et al. 1983).

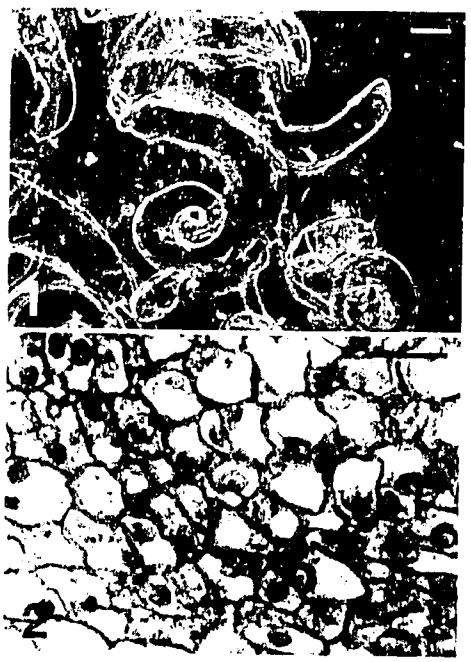

Fig. 1 Typical NE (1) and E (2) cells in wheat callus. Bar is 50 μm

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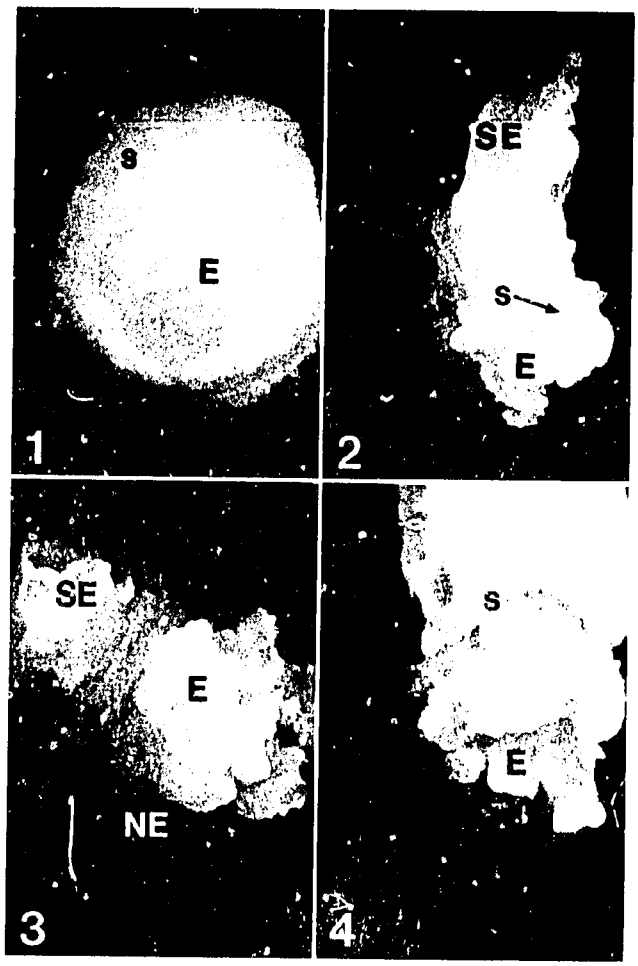

Fig. 2 Nodular E callus (E) forms on the scutellar surface of immature embryos when it is placed away from the medium. (1) Face view of scutellum (s) showing early stages of nodular E callus formation. (2) An immature embryo in which the scutellum has produced nodular E callus and the shoot apical region has produced smooth embryogenic (SE) callus. S indicates the scutellar-axial interface. (3) An immature embryo similar to that shown in (2) except that the scutellum has also produced NE callus. (4) Well-developed nodular E callus on a scutellum

In wheat, the two callus types are both straw-colored whereas in the other cereals NE callus is straw-colored to brown while E callus is white. We inspect cultures carefully under a dissecting microscope to insure accurate determination. Callus squashes are used to confirm identification in questionable cases.

Localization of E and NE callus production

Many workers have reported that regenerative callus in cereals arises from the scutellum of immature embryos when they are placed scutellum-up on the surface of solid medium (Green and Phillips 1975, Sears and Deckard 1982). For wheat, we find that E callus can arise either from the scutellum or from the shoot region of the axis (*Figs. 2—4*). With immature embryos a nodular E callus forms on the scutellum when the embryo is placed with the scutellum away from the medium (*Fig. 2*). The nodules appear in many cases to be somatic embryos (*Fig. 3*). If the axis is placed away from the medium,

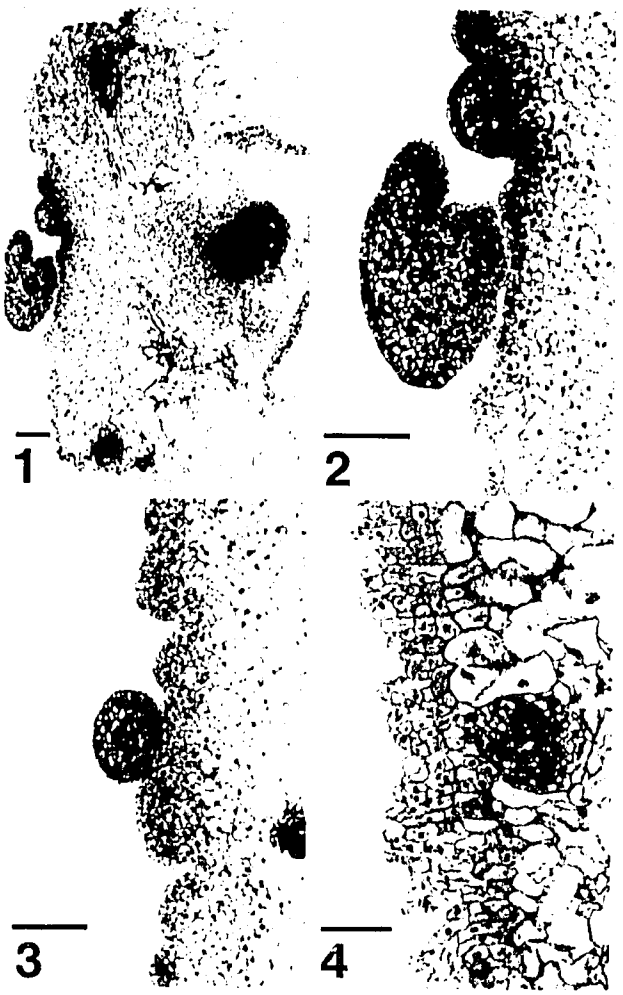

Fig. 3 Longitudinal sections through scutella of immature embryos showing embryoids (e) forming on the abaxial surface (1, 2, 3) and internally (4). Bar is 70 μm

scutellar callus does not normally develop, but the shoot axis region forms a smooth type of E callus which consists internally of organized regions of E cells and some less organized NE regions (*Fig. 4*). Some immature embryos produce both smooth and nodular E callus from the shoot axis and scutellum, respectively (*Fig. 2*). OZIAS-AKINS and VASIL (1983) noted similar tissue developing from axes of excised mature wheat embryos. NE callus can also form from the scutellum when it is away from the medium and from the root apical region when the scutellum is next to the medium (*Figs. 2* and *4*). These developmental potentials are summarized in *Table 1*. If immature embryos are dissected into scutellar, shoot axial and root axial portions, they form nodular E, smooth E, and NE callus, respectively (*Fig. 5*).

With mature embryos, only the smooth type or very rarely the nodular type of axial E callus forms when the embryo is attached to the seed. The formation of this callus is more common and extensive for seeds placed on

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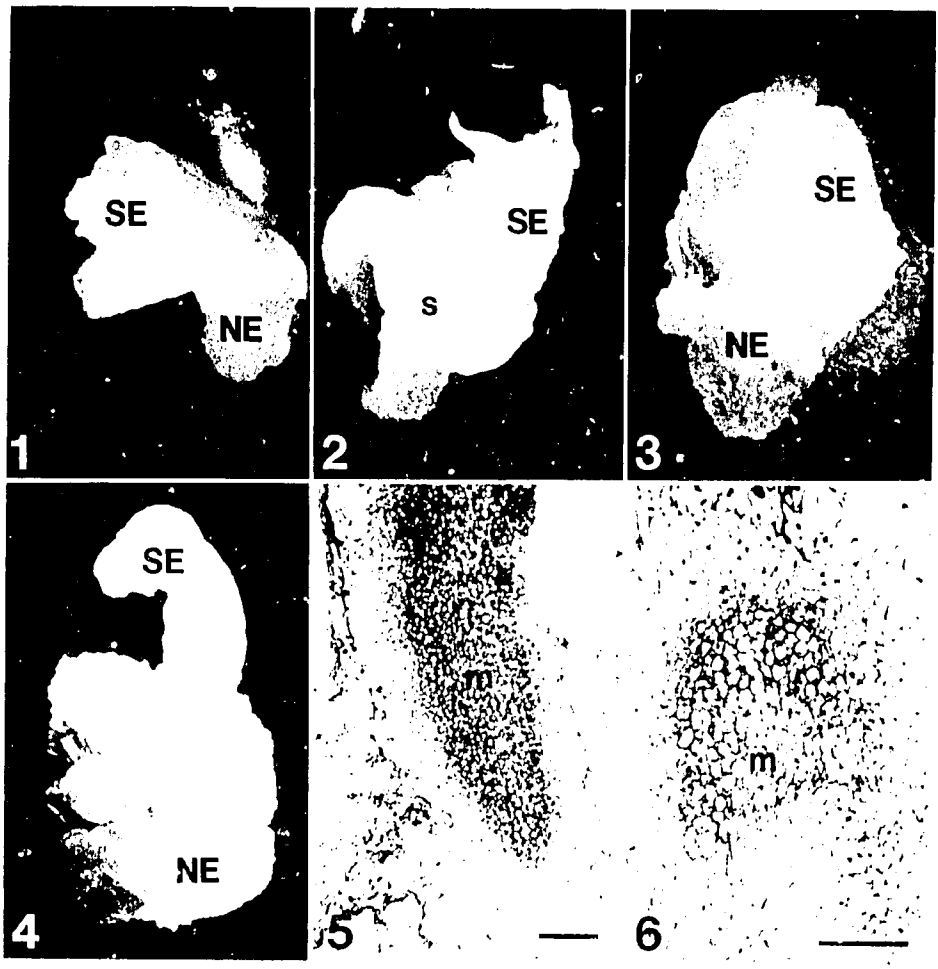

Fig. 4. Smooth E callus (SE) forms from the shoot axial region of immature embryos placed with the scutellum [illegible] and the medium and from the shoot axial region of seeds. NE callus forms from the root axial region in both cases. (1) SE and NE from a mature seed. (2) SE callus on shoot axial region of immature embryo. (3) and (4) SE and NE callus on immature seed. (5) and (6) Meristematic regions seen in sections of SE callus. Bar is 222 μm.

media containing higher levels of 2,4-D (e.g. 20 mg/l), in which normal development of the shoot apex into coleoptile and leaves does not occur. If the shoot apex does develop, smooth E callus is normally initially found only as a ring of tissue surrounding the leaf-base meristematic region (*Fig. 4*).

After four weeks the callus types have proliferated to such an extent that their origin is difficult to determine. At the end of this first passage, calli are sometimes exclusively NE or E but usually a complex of E and NE regions. NE callus from which all visible E regions were removed continued to produce E regions although this production declined over time.

Tab. 1 Developmental potential of embryos as a function of orientation of scutellum to medium

	immature		*mature*
	scutellum away from medium	scutellum toward medium	random placement of seed
scutellum	nodular E and NE	no callus (small volume of nodular E)	no callus
root apical region	no callus (small volume of NE	NE	NE
shoot apical region	no callus (small volume of smooth E)	smooth E	smooth E

The ability of cereal embryos to produce two types of E callus has been observed in wheat and triticale (unpublished data), but not in rice, in which mature or immature embryos rapidly proliferate only the nodular, scutellar E callus regardless of orientation to medium.

It is also of interest that mature wheat scutella do not form callus of any type under our cultural conditions. In rice the scutellum is smaller and more easily detachable from the endosperm than in wheat. We thought this condition might somehow inhibit E callus formation on the wheat scutellum of intact seeds. However, isolated, mature wheat embryos do not produce scutellar nodular E callus when medium producing this callus in immature embryos is utilized. In rice, mature scutella produce nodular E callus less frequently than immature scutella. The distinction is even more severe in wheat, in which

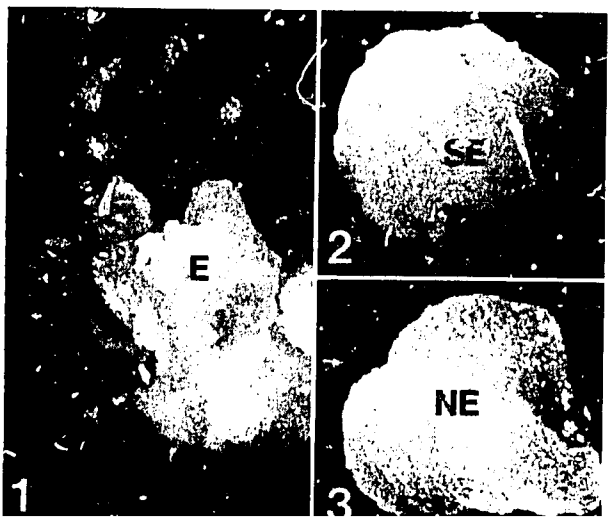

Fig. 5 Immature embryos dissected into scutellum (1), shoot axis (2), and root axis (3) produce nodular E, SE and NE callus, respectively. Medium contained 2 mg/l

Tab. 2a Milligrams of total E and NE callus produced from mature embryos (seeds) as a function of 2,4–D or 2,4,5–T concentration in medium

mg/l auxin plus 0, 20, 50, 70, or 100 mg/l TRP	'GLENNSON' 2,4–D E	2,4–D NE	2,4,5–T E	2,4,5–T N	E/NEx100
2.0	56.3	296.2[b]	28.0	449.0[c]	12.7
2.0 + 0.5 ppm KIN	50.3	239.0[b]	39.1	390.1[c]	
5.0	114.8[a]	327.5[d]	104.9[a]	460.9[e,f]	25.8
5.0 + 0.5 ppm KIN	89.4[a]	358.3[d]	110.5[a]	479.8[e,f]	
10.0	51.2	213.4[f]	48.6	208.4[f]	23.4
10.0 + 0.5 ppm KIN	52.5	157.3[f]	34.8	220.3[f]	
20.0	96.3	98.9[f]	77.0	109.5[f]	64.1
20.0 + 0.5 ppm KIN	86.9[a]	133.9[f]	63.2[a]	162.4[f]	

a = significantly different (at .05 level) from 2 ppm auxin experiments, E values
b = significantly different (at .10 level) from c
d = significantly different (at .05 level) from e
f = significantly different (at .05 level) from pooled 2mg/l auxin experiments, NE values

mature scutella produce no E callus. We are investigating the possibility that an inhibitor, such as abscisic acid, increases in concentration in scutella during seed maturation and causes the observed result.

HALPERIN (1967) and STREFT (1979) proposed, with respect to dicots, that E and NE cells constituted two non-interconvertible populations established during culture initiation. Specific media were believed to encourage the growth and division of one or of both cell types. Our data are consistent with their view. However, we believe that E and NE cells may be interconvertible. The most obvious differences between E and NE cells are their size and their potential for morphogenesis. In the whole plant, small, meristematic cells with morphogenetic potential regularly elongate and lose this potential. Also, large, elongated cells are known on occasion to give rise to small meristematic cells by unequal cell division. This occurs as carrot embryos formed from isolated single cells (RAGHAVAN 1976); in normal embryogenesis of many plants (MAHESHWARI 1950), in stamen-hair initiation in TRADESCANTIA (MERICLE and HAZARD 1980); and in root-hair development (SALISBURY and ROSS 1978). Visually, E callus frequently appears to give rise to NE callus and *vice versa*. Of course, without exhaustive cytological work it cannot be demonstrated that the observed changes are not caused by growth and division of unnoticed cells of the opposite type.

Tab. 2b Milligrams of total E and NE callus produced from mature embryos (seeds) as a function of 2,4-D or 2,4,5-T concentration in medium

mg/l auxin plus 0, 20, 50, 70, or 100 mg/l TRP	'CHRIS' 2,4-D E	2,4-D NE	2,4,5-T E	2,4,5-T NE	E/NEx100
2.0	47.7	240.6[b]	47.7	383.0[c]	14.2
2.0 + 0.5 ppm KIN	58.5	317.9[b]	49.5	489.4[c]	
5.0	67.5	244.3[e]	52.9	253.5[e]	20.8
5.0 + 0.5 ppm KIN	52.5	281.5[e]	41.3	250.2[e]	
10.0	55.5	154.1[d,e]	52.0	167.9[d,e]	29.7
10.0 + 0.5 ppm KIN	46.9	142.1[d,e]	40.9	192.7[d,e]	
20.0	56.3	92.0[d,e]	48.2	112.7[d,e]	61.9
20.0 + 0.5 ppm KIN	86.4[a]	105.8[d,e]	68.8[a]	109.0[d,e]	

a = significantly different (at .05 level) from rest of values in E column
b = significantly different (at .05 level) from c
d = significantly different (at .05 level) from b
e = significantly different (at .05 level) from c

Maximization of E and NE callus production

1. Mature embryos (seeds)

Media containing low concentrations (1 to 5 mg/l) of 2,4-D or 2,4,5-T as the only hormonal additives are typically used in cereal tissue culture and favor the production of NE callus from mature embryos (seeds). This is the reason plant regeneration from cereals has historically been of low frequency and of short duration (CONGER 1981). *Table 2* shows that although media containing 2, 10, or 20 mg/l 2,4-D or 2,4,5-T all produced mostly NE callus from mature embryos, NE callus growth was significantly (5% level) favored at low levels and smooth E callus growth at high levels. Thus, in the case of 'Glennson' seeds on medium containing 2,4-D, a callus at 2.0 mg/l plus 0 to 100 mg/l TRP consists of only 16% E callus, whereas at 20 mg/l plus 0 to 100 mg/l TRP, it contains 39% E cells.

In rice E callus production from seeds is not increased by media containing 20 mg/l 2,4-D but can be increased by media containing low concentrations of 2,4-D (0.5, 1.0 or 2.0 mg/l) plus 1—100 mg/l TRP or IAA and 0.1 to 0.5 mg/l KIN or BA (SIRIWARDANA and NABORS 1983, RAGHAVARAM and NABORS 1984). Cultivars vary considerably in their response to these variables, and the E callus is nodular and scutellar. We tested the effect of 20, 50, 70, and 100 mg/l TRP and of 0.5 mg/l KIN separately and together on smooth

E and NE callus production in 'Glennson' and 'Chris' wheat (Table 2). Overall, significant differences at the 5% level were not found, although TRP or TRP and KIN significantly increase E callus production in medium containing 2 mg/l 2,4-D in some single treatments (data not shown and NABORS et al. 1983). Since wheat seeds do not produce nodular E callus derived from the scutellum, they do not represent a system readily comparable to rice seeds, in which smooth E callus production from the shoot apical region does not occur.

Based on our present data the best medium for E callus production from mature embryos in 'Glennson' and 'Chris' wheat contained 20 mg/l 2,4-D as the only added hormone. The best medium for production of NE callus contained 2 mg/l 2,4,5-T plus 50 mg/l TRP.

2. Immature embryos

Table 3 shows that when immature embryos are placed with the scutellum away from the medium, high amounts of scutellar nodular E and NE callus are produced as well as low amounts of smooth E. The nodular E and NE form on the scutellum, while the smooth E forms on the shoot apical region facing the medium. No clear correlations between formation of the three callus types and the concentration of 2,4-D concentration is found. The effects of KIN or TRP on scutellar callus production has not yet been tested.

Tab. 3 Milligrams of total smooth E, nodular E, and NE callus produced from immature embryo of 'Pavon' wheat as a function of 2,4-D concentration and orientation of the embryo to the medium

	Scutellum *up*			Scutellum *down*		
mg/l	nodular E	smooth E	NE	nodular E	smooth E	NE
0.5	9.73	3.75	8.56	0.85	5.23	12.32
1.0	1.76[a]	3.79	16.90	2.28[d]	6.57	6.56[e]
2.0	3.64[a]	1.02[b]	5.13	0.62	6.31	2.97[e]
10.0	6.85[a]	0.73[b]	10.50	0.31[d]	6.23	2.17[e]
mean	5.50	2.32	10.32	1.02	6.08	6.13

a = significantly different at .05 from 9.73
b = significantly different at .05 from 3.75
d = significantly different at .05 from 0.85
e = significantly different at .05 from 12.32

When immature embryos are placed with the scutellum facing the medium, very little development of nodular E or NE callus occurs on the scutellar surface. Instead, smooth E callus is produced by the shoot apical region and NE callus by the root apical region. As with mature embryos, root NE callus is reduced significantly in volume as the 2,4-D concentration in the medium increases. In contrast to mature embryos, no correlation between the volume of smooth E callus and the 2,4-D concentration is found.

Selection for salt-tolerant callus

Callus derived from mature embryos was selected for tolerance to medium containing 0.0, 3.0, 6.0 and 9.0 g/l NaCl. Plants were regenerated from the first three media and are currently being selfed to increase seed stock for greenhouse testing. NaCl concentrations below 15 g/l were found in a separate experiment to increase the proportion of smooth E callus (*Fig. 6*).

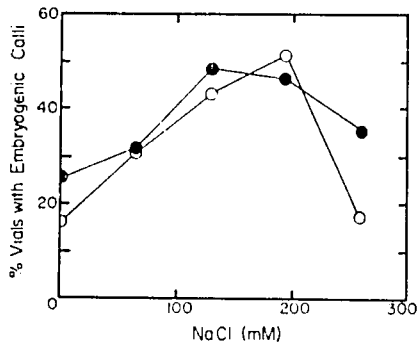

Fig. 6 Mature embryos were cultured for six weeks on medium containing 5 mg/l 2,4-D and 0.0 mg/l (O) or 10 mg/l KIN. Resulting SE callus was placed on a regeneration medium containing no added hormones for two four-week passages. NaCl was present at the indicated concentrations at all times

Plant regeneration from E and NE callus

To measure the potential of callus for plant regeneration, uniform pieces of approximately 150 mg were transferred to regeneration medium. Plant regeneration occurred on medium containing no added hormones or low concentrations of IAA and BA, but not on medium containing 2,4-D or 2,4,5-T (*Fig. 7*). Experiments to optimize regeneration media are still in progress.

NE callus rarely produced plants (*Table 4*). In early experiments, the regeneration of E callus was measured without making the distinction between

Tab. 4 Plant regeneration rates from smooth E, nodular E, and NE callus in various experiments

callus derived from	plants per gram of smooth E callus	plants per gram of nodular E callus	plants per gram of NE
'Pavon' immature embryos[1]	9.6	35.4	0.0
'Pavon' immature embryos[1]	1.3	79.9	0.0
'Chris' mature embryos (seeds)[1]	3.4		
'Chris' mature embryos (seeds)[1]	5.9		
'Chris' mature embryos (seeds)[2]	5.1		

[1] regeneration medium contained 1.0 mg/l IAA and 1.0 mg/l BA
[2] regeneration medium contained 2.0 mg/l IAA

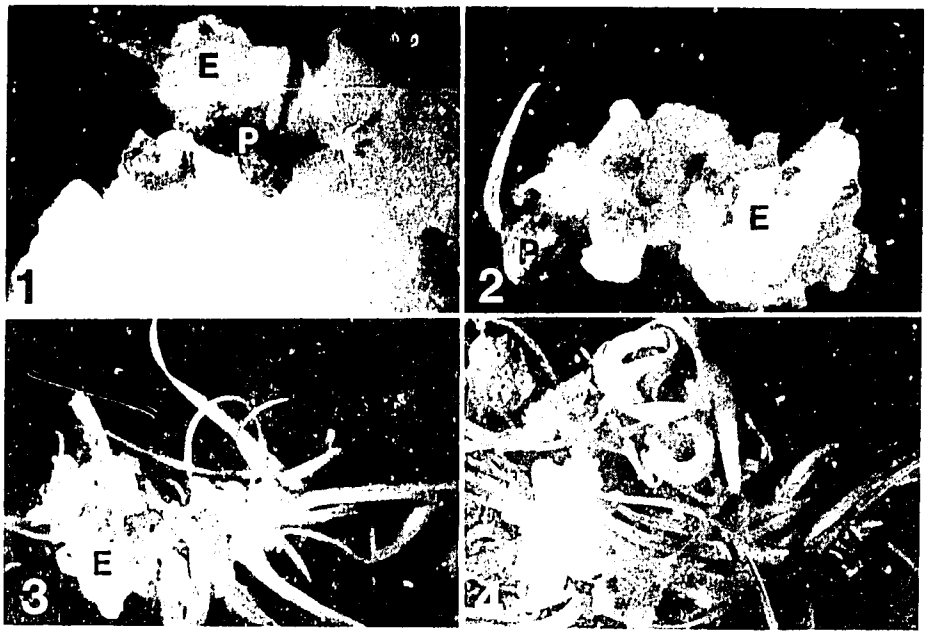

Fig. 7. Regeneration in wheat from nodular E callus. Small plantlets (p) form from so[illegible]

smooth and nodular types (NABORS et al. 1983). *Figure 8* shows results of experiments in which new mixed E callus was removed to one four-week passage on regeneration medium following various lengths of subculture. In general, E callus production declined with time. Thus, 152 mg pieces of E callus came increasingly to contain NE cells as well. This accounts for the decline in plant regeneration in *Figure 8*. Pieces of healthy E callus maintain first passage regeneration rates (plants/gm).

When a distinction is made between nodular E and smooth E callus, the former is found to have a much higher rate of plant regeneration than the latter (*Table 4*). Also, smooth E callus rarely produces regenerates during a second or third passage on regeneration medium whereas nodular E callus

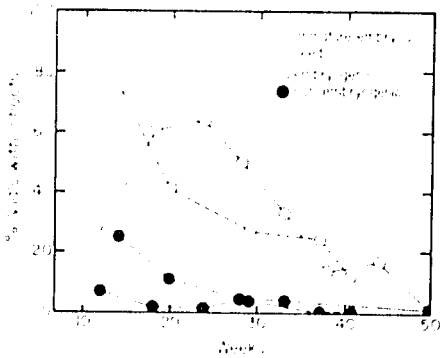

Fig. 8. 'Chris' wheat immature and mature embryos were cultured on various media. 152 mg pieces of mixed E, SE and nodular E or NE callus were removed at four- to six-week intervals and placed on regeneration medium containing no added hormones for four weeks. Remaining NE callus was placed on callus production medium. As total culture time increased, 152 mg E callus pieces became increasingly contaminated with NE cells.

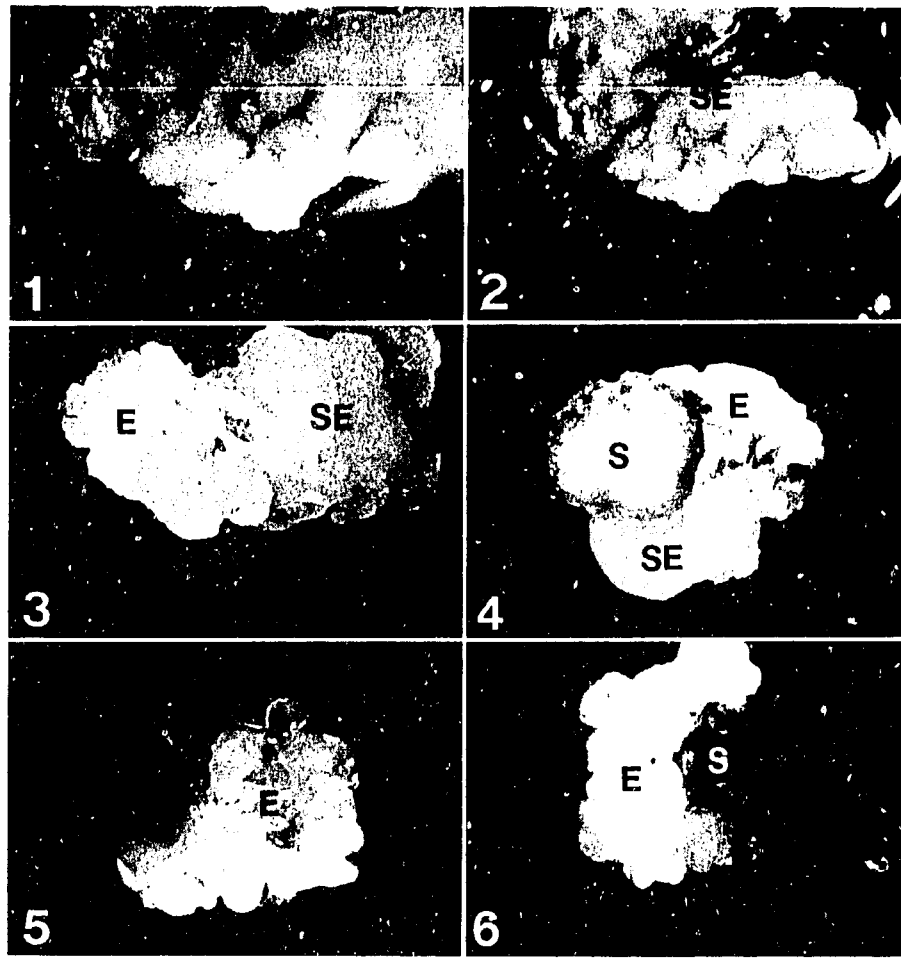

Fig. 9 (1, 2) SE callus occasionally develops a bumpy appearance. (3—6) The shoot axial region of immature embryos sometimes develops SE and nodular E callus when the scutellum is placed toward the medium. S is scutellum with no callus formation. (5, 6) Different views of same cultured embryo

does. Part of the regeneration from smooth E callus appears to be attributable to the growth of the suppressed shoot apex. Since the regeneration potential of smooth E callus is low compared to nodular E and since mature wheat embryos only occasionally produce nodular E, immature embryos placed scutellum up currently are the best source material for obtaining regenerable callus in wheat. Further experimentation may produce a medium which results in high-frequency development of nodular E callus from smooth E (*Fig. 9*) and a consequent increase in plant regeneration from mature-embryo-derived callus.

Smooth E callus can develop a bumpy appearance (*Fig. 9*), and in 0.9% of calli, will form nodular E callus. Smooth and nodular E callus have in common a small cell size and a potential for plant regeneration. They differ in

the potential to form embryoids directly. It is tempting to hypothesize that smooth E callus represents a relatively undifferentiated tissue beginning to differentiate into embryoids. It is also possible that smooth E represents a third distinct type of callus along with nodular E and NE. This issue may well be resolved by cytological studies in progress and by a search for a medium which promotes the development of smooth into nodular E callus at high frequencies.

Regenerated plants were selfed to obtain S_1 progeny. These plants were grown to increase seed stock at CIMMYT, and root-tip squashes were analyzed to establish chromosome number. S_1 progeny obtained from cultures tolerant to 3000 mg/l NaCl had 18.6% with an abnormal chromosome number (43 or 44). S_1 progeny from cultures not exposed to salt also had 18.2% with an abnormal chromosome number (41, 43, 44). The sample sizes were 11 and 43 plants, respectively. Salt-tolerance testing of regenerated plants and their progeny is in progress.

Obtaining salt-tolerant plants

Currently, the origin of plants obtained from cereal calli is a subject of debate. Some workers (STREET 1979) feel that tissue-culture regenerated plants always arise from single cells. Recently, however, WERNICKE et al. (1982) have presented evidence that in sorghum at least, regenerated plants (derived from leaf-tissue callus) arise from multicellular, pre-existing meristems which proliferate in callus culture. In terms of obtaining non-chimerical mutant plants from tissue cultures, no problem arises if embryos are of single-cell origin. If embryos are of multicellular origin, chimerical plants could result unless selection was continued for a length of time sufficient to insure that all cells in the culture were mutant.

NABORS et al. (1975) showed that in suspension cultures of tobacco, salt tolerance occurs in only a few cultures. In these cultures the growth rate under salt stress gradually increases over a 17-week period. This is exactly the type of behavior which would be expected if a salt-tolerant cell line with increased division and growth rates gradually increased its frequency in the cell population. Similar selective behavior has been noted for a euploid mutant cell line in calli of *Haworthia* (OGIHARA 1982).

In some cases cereal tissue cultures appear to be composed of partially dedifferentiated proliferating meristems (CURE and MOTT 1978, WERNICKE et al. 1982). Whether this is the case for the highly regenerative E callus we discuss has not yet been determined. Selection of non-chimerical mutant plants from a callus composed of proliferating meristems would easily be possible if long-term selection were utilized. Under conditions of long-term selection, a meristem containing one mutant cell would gradually come to be composed, by its proportionally higher rate of division and growth, entirely of mutant cells. As selection continued and the meristem proliferated, the entire culture would come to consist of mutant cells. The importance of the selection of somatic mutations in the evolution of whole plants has been recently discussed by WHITHAM and SLOBODCHIKOFF (1981).

Zusammenfassung

Lang andauernde Pflanzenregeneration mit hoher Reproduktionskapazität und die Induktion somatischer Embryogenese in Kallus-Kulturen von Weizen (*Triticum aestivum* L.)

Eine lang andauernde Pflanzenregeneration mit hoher Reproduktionsrate ist notwendig, wenn Gewebekulturmethoden nutzbringend in der Pflanzenzüchtung angewendet werden sollen. Kallus-Kulturen aus keimenden Samen des Weizens und unreifen Embryonen produzierten zwei Zelltypen. Embryogener Kallus (E) besteht aus kleinen isodiametrischen Zellen und zeigte eine kompakte Struktur. Nichtembryogener Kallus (NE) enthielt lange tubuläre Zellen von lockerer Struktur. Die Pflanzenregeneration aus embryogenen Regionen war hoch, während aus nichtembryogenem Kallus nur gelegentlich Pflanzen regenerierten. Die Regeneration hielt so lange an, wie die Kalli Bereiche mit embryonalen Zellen ausbildeten und diese auf ein definiertes Regenerationsmedium übertragen wurden.

Unreife Embryonen bildeten einen strukturierten E-Kallus und NE-Kallus aus dem Scutellum. Wurden unreife Embryonen in Scutellum, Sproßspitzenregion und Wurzelspitzenregion zerteilt, kam es zur entsprechenden Bildung von strukturiertem E-Kallus und weichem E- und NE-Kallus. Reife Embryonen bildeten einen weichen E-Kallus aus der Sproßspitzenregion, hingegen keinen Kallus aus dem Scutellum.

Der relative Anteil E- und NE-Kallus aus reifen und unreifen Embryonen konnte durch unterschiedliche 2,4-D-Konzentrationen signifikant beeinflußt werden.

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Authors' addresses: Dr. J. W. HEYSER, Los Alamos National Laboratory, Los Alamos, New Mexico 87545 (USA); Dr. M. W. NABORS, C. MACKINNON, T. A. DYKES, K. J. DEMOTT and D. C. KAUFMAN, Department of Botany and Plant Pathology, Colorado State University, Fort Collins, Colorado 80523 (USA); Dr. A. MUJEEB-KAZI, Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), Apartado Postal 6-641, Mexico 6, D.F. (Mexico).