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Chromosomal Characteristics of Barley Root Meristems Differentiated from Calli

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Abstract. Callogenesis was induced in mature embryos. The efficiency of induction and the growth of calli were dependent on 2,4-D concentration. No regeneration of buds or shoots was observed in 720 calli studied, but most calli showed intensive root proliferation on the regeneration medium. Most of the root meristem cells maintained diploid chromosome number. Only a low proportion (about 3.5%) of tetraploid cells was found. No other chromosomal changes were observed. Chromosomal variability does not contribute to the inability of calli derived from mature barley embryos to form buds and shoots.

The basic requirement for the practical use of plant tissue cultures is their ability to regenerate plants. This ability in barley, however, is still lower than in other economically important cereals like wheat, rice or maize (YAMADA 1977). CHENG and SMITH (1975) were the first to refer plant regeneration from barley calli derived from apical meristems, and DALE and DEAMBROGIO (1979), ORTON (1980), ORTON and STEIDL (1980) and NOVÁK (1982) ascertained regeneration ability in the calli derived from immature barley embryos. Several authors found only root differentiation in the calli derived from other barley tissues (GAMBORG and EVELEIGH 1968, CHIN and SCOTT 1977), and this is also restricted only to certain barley genotypes (SCHEUNERT *et al.* 1977).

Barley calli possess a considerable chromosomal variability which includes polyploidy as well as aneuploidy (SAALBACH and KOBLITZ 1977, SCHEUNERT *et al.* 1978, NOVÁK 1980) and this chromosomal variability is perhaps one of the factors which block the regeneration ability of buds, shoots and plants. NOVÁK (1982) showed that the calli derived from immature barley embryos cv. Korál possess the ability to regenerate shoots whereas those derived from mature embryos of the same genotype show only root formation. We tried to find out whether chromosomal variability could be responsible for the inability of the calli derived from mature barley embryos cv. Korál to

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induce shoots. Perhaps, root meristems can tolerate a higher degree of chromosomal variability than shoot meristems. The main aim of our study was to estimate the chromosomal variability of roots regenerated from calli that were derived from mature embryos; such analysis was never performed before.

MATERIAL AND METHODS

Spring barley *Hordeum vulgare* cv. Korál was used throughout the experiments. Mature seeds which were not in dormancy stage, were sterilized in 2% solution of Chloramine B for 24 h. After several washings in a sterile distilled water embryos were dissected under stereomicroscope and sterilized repeatedly in 2% Chloramine B for 5 min and washed again. Calli were induced on the medium R 8 of SOZINOV *et al.* (1981) fortified with 0.8% agar. Concerning growth regulators, the medium for callogenesis contained 2,4-D (2—3 mg l^{-1}) and kinetin (0.05 mg l^{-1}) and pH was adjusted to 5.5. Cultures were kept in the dark. Regeneration medium had an identical composition of inorganic additives, vitamins, sucrose and the pH value, but it contained IAA (0.5 mg l^{-1}), kinetin (1—2 mg l^{-1}) or BAP of the same concentration range. Cultures were kept under natrium fluorescent light bulbs with light intensity 5 000 lx and 16 h photoperiod. Experiments were replicated three times.

The cytological study of root meristems was performed on the calli with differentiated roots. They were put into the liquid medium and shaken 24 h on a horizontal gyratory shaker (1 Hz). During the last three hours of cultivation, the cultures were pretreated with 0.15% colchicine in saturated α-bromonaphthalene solution. Root tips and calli were fixed in ethanol-acetic fixative 3 : 1, stained by Feulgen method and squashes were made. Chromosome numbers were scored in the metaphase figures and the presence of chromosomal aberrations as well as other chromosome disturbances were checked.

RESULTS

Callus Induction

Extirped sterile embryos were placed with scutelar side down on the medium for callogenesis. Calli appeared first in the region of contact with the medium, after 1 week of cultivation. After 6 weeks of cultivation, the proportion of embryo-forming calli was evaluated (Table 1). This proportion was higher on the medium with 3 mg l^{-1} 2,4-D than on that with 2 mg l^{-1} 2,4-D. The growth of calli on the medium with a higher content of 2,4-D was more vigorous. The calli were of creamy colour, compact appearance and firm consistency. Their mass, on the average, was on the verge of about 100 to 200 mg. In most cases callogenesis did not interfere with the development of seedlings.

An important presumption for efficient callogenesis was a proper contact of the whole scutelar region with the medium. If the growth caused relocation

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Abbreviations used: IAA = indolyl-3-acetic acid; 2,4-D = 2,4-dichlorophenoxyacetic acid; BAP = 6-benzyl-aminopurine.

TABLE 1

The frequency of callogenesis in barley embryos at different concentrations of 2,4-D

Concentration of 2,4-D [mg l^{-1}]	No. of embryos tested	% of embryos forming calli
2	820	56.5
3	1 200	79.3

The difference is significant on 1% level ($\chi^2_{1]} = 121.6$).

of the scutelum and the embryo was not put back to the original position, callogenesis was severly reduced. Such cases were not included in Table 1.

Differentiation of Roots from Calli

After 6 weeks of the growth on the medium for callogenesis, calli were detached from the original explants and transferred to the regeneration medium.

The calli showed root formation already after 1 week cultivation on the regeneration medium. The roots were of defective appearance; they often contained chlorophyll and never showed hairy roots or branching. The proportion of calli which differentiated roots after 7 weeks of cultivation is evaluated in Table 2. The medium with kinetin induced roots more efficiently than with BAP and the concentration 2 mg l^{-1} of both growth regulators was more efficient than 1 mg l^{-1}. The number of roots per callus was about ten. The length of roots varied on the verge of 1—20 mm.

No regeneration of buds or shoots was observed in 720 calli.

Chromosome Studies of Root Meristem Cells

The stained squash preparations of calli and root meristems, differentiated from calli, cultivated for 7 weeks on the regeneration medium were observed. No mitoses were found in the undifferentiated calli of this stage.

The root tip meristems were much smaller than those in the root tips of barley seedlings. Meristems were found in short roots only (up to 3 mm).

TABLE 2

The frequencies of root regeneration from barley embryo primary calli

	% of root forming calli on the regeneration media with
1. BAP 1 mg l^{-1}	63.7
2. BAP 2 mg l^{-1}	72.8
3. kinetin 1 mg l^{-1}	79.9
4. kinetin 2 mg l^{-1}	82.8

Number of calli in each variant of experiment 180.
Significance of differences:
1 × 2 and 3 × 4 insignificant
1 × 3 significant on 1% level ($\chi^2_{[1]} = 10.6$)
2 × 4 significant on 5% level χ ($\chi^2_{[1]} = 4.6$)

TABLE 3

Chromosome numbers in metaphases of the meristems of roots differentiated from calli cultivated on media with different growth regulators

Growth regulator in the cultivation medium	No. of diploid metaphases	No. of tetraploid metaphases
Kinetin 1 mg l^{-1}	94	6
BAP 1 mg l^{-1}	99	1
Total	193	7

Mitotic divisions were relatively rare, mostly 1—2 metaphases per root meristem. In a small part of the root tips, the number of metaphases was considerably higher (up to 17). Two experimental variants were evaluated karyologically: calli cultivated in 1 mg l^{-1} kinetin and those cultivated in 1 mg l^{-1} BAP. In the first one, the frequency of mitoses was somewhat higher than in the second. One hundred of metaphases were tested in each experimental variant. Results are seen in Table 3. Except tetraploid cells no other chromosomal irregularities, including chromosomal aberrations, were found. Tetraploid metaphases occurred coincidently in some root tips; out of altogether 56 root tips with active meristem there were 4 roots with tetraploid metaphases. Three tetraploid metaphases were ascertained in a single meristem where, however, no diploid metaphase was found. In another meristem, two tetraploid and one diploid metaphases were present. In the third root tip one tetraploid and 15 diploid metaphases were found. In the last one, a single tetraploid metaphase and no diploid metaphases were observed. Altogether, about 7% of root meristems were tetraploid and/or mixoploid.

DISCUSSION

Cereals require only 2,4-D as an indispensable substance for callus induction and maintenance (YAMADA 1977), even though the media used in this study also contained a low concentration of kinetin for improvement of the growth regulators balance. The concentration of 2,4-D in the media used by different authors varies in the range of 2—5 mg l^{-1} (SCHEUNERT *et al.* 1977, 1978, DALE and DEAMBROGIO 1979, ORTON 1979, NOVÁK 1980, ORTON and STEIDL 1980). The results in Table 1 show the differences in the effects of two concentrations of 2,4-D which differed within narrow limits.

No differentiation of plants, shoots or buds was found in any out of 720 calli of independent origin, cultivated on the regeneration medium. NOVÁK (1982) induced plant regeneration in 60% of calli of the same genotype originated from immature embryos and root formation exclusively in calli derived from the mature embryos. Our results, together with those of DALE and DEAMBROGIO (1979) and NOVÁK (1982), confirm that calli derived from mature embryos never differentiate shoots.

The formation of roots in barley calli is a well known phenomenon. Root formation was observed either on calli on the media for callus induction and maintenance (SCHEUNERT *et al.* 1977), or after transfer on the medium without

growth regulators (CHIN and SCOTT 1977) or, in the studies of NOVÁK (1982) and ours, when the ratio of growth regulators with cytokinin activity to auxins in the medium was increased.

The root tip meristems diminished during the root growth and eventually disappeared; this phenomenon is obviously the consequence of hormonal imbalance. This situation resembles that in isolated barley roots (LANDOVÁ and ONDŘEJ 1979).

Chromosomal variability of the regenerants is always much lower than that in original calli (*cf.* ORTON 1980). In conditions favourable to the differentiation, the selection pressure gives some specific advantage to diploid cells, which are physiologically capable of differentiation. If such cells are present in a too low proportion or the differentiation efficiency is too low, no differentiation occurs. However, this is not the case of calli derived from mature barley embryos. Out of 200 metaphases scored, 7 (*i.e.* about 3.5%) were tetraploid, the other were normal diploid metaphases without apparent irregularities. Altogether about 7% of roots were tetraploid or mixoploid.

We have shown that organized meristems with mostly diploid chromosome numbers differentiate in calli originated from mature barley embryos. These meristems, however, do not give rise to shoots but exclusively to roots. The cause of the absence of differentiation of plants is not karyological, but most probably physiological.

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