Theor Appl Genet (1986) 71:631–636

Tissue culture and plant regeneration from immature embryo explants of Barley, *Hordeum vulgare**

C. S. Goldstein[1] and W. E. Kronstad[2]

[1] Del Monte Corporation, Agricultural Research Center, 850 Thorton St., San Leandro, CA 94577, USA
[2] Department of Crop Science, Oregon State University, Corvallis, OR 97331, USA

Accepted July 20, 1985
Communicated by G. Melchers

Summary. Immature embryo explants taken 8 days after anthesis were used to establish callus cultures of spring barley. Two types of calli were observed. A soft, watery callus produced a limited number of shoots and a harder, more compact, yellowish callus gave rise to numerous green primordia and shoots. Gamborg's B5 basal medium supplemented with either 2,4-D (2,4-dichlorophenoxyacetic acid) or Cl_3 POP (2,4,5-trichlorophenoxypropionic acid) was found to give good callus growth and shoot initiation. Media containing 2,4-D at 1.0 mg L^{-1} or Cl_3 POP at 5.0 mg L^{-1} produced numerous cultures resulting in regeneration of plants. Plantlets developed roots on basal medium with Cl_3 POP at 1.0 mg L^{-1} or on auxin-free medium. Twenty genetically diverse genotypes were screened to determine if these techniques were suitable for a wide range of spring barley cultivars. Regeneration of plantlets was obtained for 19 of the 20 genotypes approximately 4 months after culture initiation. Lines differed in the ability to develop vigorously growing calli and in the ability of calli to develop large numbers of shoots and regenerated plantlets.

Key words: Immature embryos – Spring barley – Plantlet regeneration – Auxin – Genotypes

Introduction

Reproducible regeneration of plants has recently been reported for a number of cereal species including wheat (Sears and Deckard 1982; Ahloowalia 1982; Eapen and Rao 1982a), rye and triticale (Eapen and Rao 1982b), proso millet (Heyser and Nabors 1982a), oats (Heyser and Nabors 1982b), and barley (Deambrogio and Dale 1979; Bayliss and Dunn 1979). A high rate of regeneration from callus cultures is a prerequisite for the use of tissue culture as a tool in crop improvement.

Specific differences exist in the in vitro response of genetically diverse genotypes. Distinctions can be made among cultivars for their callus growth response, ability to initiate shoots and roots, and regeneration potential. Varieties and breeding lines have been identified for their ability to yield numerous regenerated plantlets. The identification of specific genotypes that are capable of rapid callus production and high rates of plantlet regeneration is an important step toward the application of tissue culture techniques to agriculture.

Results will be presented to substantiate the successful regeneration of a genetically diverse range of spring barley cultivars. Experiments were conducted to determine optimal explant stage of maturity, growth regulator requirements, and distinct genotype responses. Detailed explanations of culture responses at various stages of callus growth and plantlet development are provided.

Materials and methods

Plants used in these experiments were grown under greenhouse conditions. Measures were taken to avoid environmental stress. Pots containing a greenhouse soil mixture of peat, leaf mulch, and soil were watered daily. A 15-15-15 fertilizer was incorporated into the soil mixture at the time of planting and a one-tenth strength Hoagland's fertilizer solution was applied one week prior to anthesis. Plants for hormone studies were kept at constant temperatures of 35 to

* Contribution from Department of Crop Science, Oregon State University, Corvallis, OR 97331. Oregon Agric. Exp. Stn. No. 7582

38 °C with a 14 h day-length. Plants for genotype studies were held at temperatures ranging from 26 to 38 °C and approximately 14 h daylight. The seed sources for all plants were acquired from breeders seed maintained as pure lines.

Spikes were bagged a few days before anthesis. Immature spikes were collected 8 days after anthesis. The caryopses were surface-sterilized with 20-fold diluted Clorox (5.25% sodium hypochlorite) for 5 min and rinsed 5 times with sterile distilled water. Embryos were excised using a dissecting microscope under aseptic airflow conditions of a laminar flow hood. Three immature embryo explants were taken from individual spikes and placed in the same petri dish.

Immature embryo explants were placed in 15 by 60 mm pre-sterilized petri dishes. Cultures were established and maintained on Gamborg's B5 basal medium with the addition of the following components (concentrations are mg L^{-1}): $NaH_2PO_4 \cdot H_2O$, 150; KNO_3, 2,500; $(NH_4)_2SO_4$, 134; $MgSO_4 \cdot 7H_2O$, 250; Ferric EDTA, 40; Gamborg and Wetter (1975) supplemented with 2% sucrose, and 0.7% to 0.8% Sigma agar-agar. The pH of the medium was adjusted to 5.5 with either NaOH or HCl before the addition of agar.

Any embryo explants that showed signs of precocious germination were discarded. Cultures were transferred every 3 to 6 weeks. Once shoots began to develop cultures were placed in autoclaved glass test tubes or pre-sterilized baby bottle liners. All cultures were maintained in the same growth chamber. The controlled environment chamber was kept at 24 to 26 °C, with a 12 h day-length.

Hormone experiments

A randomized complete block design consisting of six replicates, four treatment levels and three subsamples was used. Six plants of the cultivar 'Klages' were established in the greenhouse. Four randomly selected spikes were collected from each plant and labelled according to source for testing the four hormone treatment levels. The spikes were from both primary and secondary tillers.

Three immature embryo explants were taken from a single spike and placed in a petri dish supplemented with a specific hormone concentration. These three embryos were subsamples of a replicate. The hormone treatments were 2,4-D (2,4-dichlorophenoxyacetic acid) at 1.0 mg L^{-1} and Cl_3 POP (2,4,5-trichlorophenoxypropionic acid) at concentrations of 1.0, 2.5, and 5.0 mg L^{-1}. Measurements were taken for callus gain in fresh weight, appearance of green primordia, shoot and root initiation, and plantlet regeneration.

Genotype experiments

A randomized complete block design consisting of five blocks and 20 genotypes was used. One spike was randomly selected from each of the 100 plants in the investigation. Three immature embryo explants were excised from the spike and placed in a culture dish. These three embryos were subsamples of the blocks.

Cultures of 20 genetically diverse genotypes were initiated on Gamborg's B5 medium supplemented with 1.0 mg L^{-1} 2,4-D. Cultures were maintained on this same medium from initiation of callus through plantlet regeneration stages. Regenerated plantlets were transferred to hormone-free media to stimulate root development. Evaluations of successful growth and regeneration were based on measurements of callus fresh weight gain, shoot and root initiation, and plantlet regeneration.

Results

No differences were detected in callus growth, whether the scutellum was placed downward in contact with the medium or facing up. There were obvious differences in the response of embryos taken at various developmental stages. Embryos taken 7 to 8 days after anthesis developed into vigorously growing calli. This is the equivalent of stage II in wheat caryopsis development as described by Rogers and Quatrano (1983), when the caryopsis is at the milky stage. Not only were the number of days after anthesis important, but the size of the explant had a distinct effect on the success of establishing cultures. Embryo lengths of 0.5 to 1.0 mm were best for eliciting rapid callus development while avoiding the precocious germination of the embryo.

Hormonal responses

All auxin concentrations tested were suitable for the initiation of vigorously growing callus cultures. Significant differences existed in the effect of the growth regulators on callus fresh weight gains at both 4 weeks (Table 1) and 9 weeks (Table 2) after explants were established. Supplements of either 2,4-D at 1.0 mg L^{-1} or Cl_3 POP at 1.0 mg L^{-1} caused the greatest callus production by explanted embryos.

Table 1. Hormone treatment means for fresh weight of callus cultures 4 weeks after initiation for the cultivar 'Klages'

Treatment (mg L^{-1})	Fresh wt (g)[a]
2,4-D 1.0	0.289 a[b]
Cl_3 POP 1.0	0.228 a
Cl_3 POP 2.5	0.149 b
Cl_3 POP 5.0	0.118 b

[a] Mean of six replications
[b] Means with a letter in common are not significantly different. $LSD_{0.05}$ for comparison among these means = 0.078

Table 2. Hormone treatment means for fresh weight of callus cultures 9 weeks after initiation for the cultivar 'Klages'

Treatment (mg L^{-1})	Fresh wt (g)[a]
Cl_3 POP 1.0	0.956 a[b]
Cl_3 POP 2.5	0.724 b
2,4-D 1.0	0.633 bc
Cl_3 POP 5.0	0.483 c

[a] Mean of six replications
[b] Means with a letter in common are not significantly different. $LSD_{0.05}$ for comparison among these means = 0.182

Green primordia began to appear 4 to 6 weeks after embryo explants were established. The addition of Cl_3 POP at 1.0 mg L^{-1} often resulted in the formation of green roots from these primordia rather than the more desirable initiation of shoots. Hormone treatments did not differ in their effect on green primordia initiation. The ranking of hormone treatment effects changed from evaluations at 9 weeks compared with those measured at 12 weeks. At 9 weeks, Cl_3 POP at 1.0 mg L^{-1} gave the greatest number of green primordia. The measurements recorded at 12 weeks show 2,4-D to be the better treatment for green primordia initiation. Cultures exposed to Cl_3 POP at 5.0 mg L^{-1} consistently ranked lowest for formation of green spots.

The best auxin treatments for induction of shoot-producing calli were Cl_3 POP at 2.5 and 5.0 mg L^{-1}. These growth regulator treatments resulted in the largest numbers of shoots. Although these results were not statistically different at the 0.05 probability level, the greatest mean value for shoot emergence at 9 weeks was for cultures with Cl_3 POP levels of 2.5 mg L^{-1}, and at 12 weeks for Cl_3 POP levels of 5.0 mg L^{-1}.

Plantlets were regenerated approximately 12 to 16 weeks after initial embryo excision. Plantlets developed for all hormone levels tested. Analysis of data collected 6 months after culture establishment proved that significant differences existed for regenerative capacity (Table 3). The greatest number of plants were regenerated for those cultures with Cl_3 POP concentrations of 5.0 mg L^{-1}.

Genotype responses

Callus formed within the first 3 weeks. Genotypes ranged in their relative callus-forming ability when scored 6 weeks after culture initiation (Table 4). The cultivar 'Gus' had twice the weight gain compared to the mean of all twenty genotypes. The cultivars 'Klages', 'Himalaya', 'Triumph', and 'Morex' ranked in the top 25% for the barley populations evaluated.

Table 3. Hormone treatment means for regeneration of plants 6 months after initiation of cultures for the cultivar 'Klages'

Treatment (mg L^{-1})	No. of plants[a]
Cl_3 POP 5.0	1.6 a[b]
Cl_3 POP 2.5	1.1 a
2,4-D 1.0	0.6 b
Cl_3 POP 1.0	0.1 c

[a] Mean of six replications
[b] Means with a letter in common are not significantly different at 0.05 level of probability. $LSD_{0.05}$ for comparison among these means = 0.42

Formation of green spots was first noted four weeks after explanting. Shoots and occasionally roots arose from these green primordia. Significant differences in shoot initiation from the green primordia were observed (Table 5). Cultivars and advanced breeding lines have been selected for their prolific shoot development. 'Apam Dwarf' and 'Advance' were two cultivars that produced an average of more than five shoots from a single culture. 'Multum' and the breeding line 'OSB

Table 4. Mean value for gain in fresh weight of callus cultures 6 weeks after initiation for 20 cultivars of spring barley

Genotype	Fresh wt (g)[a]
'Gus'	0.645 a[b]
'Klages'	0.452 b
'Himalaya'	0.450 b
'Triumph'	0.410 bc
'Morex'	0.403 bcd
'Apam Dwarf'	0.359 b–e
'Or 7334-3'	0.341 b–e
'Minn 66-102'	0.299 b–f
'Diamant'	0.288 c–f
'OSB 763390'	0.281 c–f
'Multum'	0.235 d–g
'Akka'	0.227 e–h
'ORSS-2'	0.224 e–h
'Karl'	0.205 e–h
'Advance'	0.134 fgh
'Short Wocus'	0.134 fgh
'Benton'	0.116 gh
'C2-79-198'	0.103 gh
'Steptoe'	0.088 gh
'Mex 79132-Hk'	0.071 h

[a] Mean of five replications
[b] Means with a letter in common are not significantly different at the 0.05 probability level. $LSD_{0.05}$ for comparison among these treatment means = 0.159

Table 5. Treatment means for shoot development 12 weeks after culture initiation for 20 spring barley cultivars

Genotype	No. of shoots[a]	Genotype	No. of shoots[a]
'Apam Dwarf'	6.1 a[b]	'Minn 66-102'	2.1 c–f
'Advance'	5.3 ab	'Mex 79132-Hk'	1.6 c–f
'OSB 763390'	4.5 abc	'Diamant'	1.6 c–f
'Multum'	4.2 a–d	'Akka'	1.3 def
'Karl'	3.7 a–d	'Benton'	1.1 def
'Gus'	3.2 a–e	'Triumph'	1.1 def
'ORSS-2'	2.9 b–f	'Morex'	0.3 ef
'OR 7334-3'	2.9 b–f	'Short Wocus'	0.3 ef
'C2-79-198'	2.9 b–f	'Steptoe'	0.1 ef
'Klages'	2.6 b–f	'Himalaya'	0.0 f

[a] Mean of five replications
[b] Means with a letter in common are not significantly different. $LSD_{0.05}$ for comparison among these means = 3.1

Table 6. Treatment means for regeneration of plants for 20 spring barley cultivars

Genotype	No. of plants[a]	Genotype	No. of plants[a]
'Multum'	2.1 a[b]	'Akka'	0.9 b–g
'Apam Dwarf'	1.8 ab	'Klages'	0.9 b–g
'C2-79-198'	1.7 abc	'Benton'	0.8 b–g
'Karl'	1.7 abc	'Diamant'	0.8 b–g
'Mex 79132-Hk'	1.7 abc	'Steptoe'	0.7 c–g
'OR 7334-3'	1.3 a–d	'Minn 66-102'	0.5 d–g
'Advance'	1.2 a–e	'Triumph'	0.3 d–g
'OSB 763390'	1.1 a–f	'Short Wocus'	0.3 d–g
'ORSS-2'	1.1 a–f	'Morex'	0.1 fg
'Gus'	0.9 b–g	'Himalaya'	0.0 g

[a] Mean value of five replications
[b] Means with a letter in common are not significantly different at the 0.05 probability level. $LSD_{0.05}$ for comparison among these means = 1.00

Table 7. Simple correlation coefficients between four tissue culture traits for 20 spring barley genotypes

	Shoots (12 weeks)	Roots (12 weeks)	Regeneration (6 month)
Weight gain (6 weeks)	0.06	0.38	−0.37
Shoot initiation (12 weeks)		0.56[a]	0.73[a]
Root initiation (12 weeks)			0.35

[a] Significant at the 0.01 level of probability
[b] Each correlation coefficient is based on 20 means

763390' from Oregon State University both yielded an average of four shoots per initial explant.

Regeneration of plants was achieved for 19 of the 20 genotypes of spring barley investigated with Himalaya being the only genotype that produced no plantlets. Genotypes differed in their capability to regenerate plants with both shoots and roots (Table 6). Fifty percent of the genotypes averaged one regenerated plant from each initial embryo explant. The cultivar 'Multum' produced two shoots per explant, the greatest mean regeneration value found in this study.

Simple correlation coefficients were calculated for four tissue culture traits (Table 7). Correlations between weight gain of 6-week-old callus cultures and shoot initiation were low and non-significant. Root initiation was positively correlated at the 0.10 probability level to weight gain at 6 weeks. A significant positive relationship was found between root and shoot initiation at the 0.01 level of probability. The relationship for fresh weight gain at 6 weeks and plantlet regeneration 6 months after initial establishment was negative at the 0.10 probability level.

Discussion

The physiological maturity of immature embryo explants was found to be of utmost importance in attaining vigorous callus growth and avoiding precocious germination of the explant. A specific size and number of days post anthesis were identified as being optimal for barley explant materials. Dale and Deambrogio (1979) described the relationship between time after pollination and mean embryo length. They concluded that immature embryos ranging in size from 0.7 to 1.4 mm, corresponding to 10 to 13 days after pollination, were best suited for obtaining vigorous callus induction and higher callus yields. Embryos excised 10 to 13 days after anthesis from plants grown under greenhouse conditions in Corvallis, Oregon were found to be much larger than predicted by Dale and Deambrogio. The result was precocious germination of the explant. Under local conditions a narrower window of 7 to 8 days after anthesis was the optimal stage for excising embryos. The excised immature embryo explants in these studies were all 0.5 to 1.0 mm in length. Callus growth from these embryos was vigorous, and rapid gain in fresh weight was measured.

Two distinct callus types were observed. A soft, watery, translucent callus, and a second, yellowish, more friable callus. These two types of calli often appeared side-by-side in cultures derived from a single explant. More than two discrete classes of callus types probably exist in these cultures. Orton (1979) characterized five morphologically distinct categories of callus in barley exhibiting a range of regeneration potentials. The callus can be described according to Orton's system as being of type A or type E.

Hormone experiments

Barley cultures initiated on media supplemented with 2,4-D consistently formed calli with shoot development capability. Shoot initiation was stimulated by both auxin sources tested. Rooting was best accomplished by lowering the hormone concentration to 1.0 mg L^{-1} Cl_3 POP or by removing all growth-promoting supplements.

Plant regeneration was obtained for all growth hormone treatments. The greatest capacity for regeneration was observed from cultures with Cl_3 POP at concentrations of 2.5 or 5.0 mg L^{-1} for the single cultivar evaluated. A similar response has been reported for the effect of Cl_3 POP on other cereal crop tissue cultures. Shoot bud regeneration was observed in primary cultures of rye and triticale after 3 to 4 weeks when Cl_3 POP at 5.0 mg L^{-1} was used as a growth promoter Eapen and Rao (1982a). Eapen and Rao (1982b) also tested the effects of Cl_3 POP on callus cultures of 'Durum' and 'Emmer' wheat. Embryos initiated on Murashige and Skoog's medium supple-

mented with 5.0mg L^{-1} CL_3 POP were reported to demonstrate a superior callusing response. Shoot buds with leaves emerged from cultures with Cl_3 POP added as a growth promoting substance. Further studies of barley shoot regeneration utilizing this auxin source are in order.

Genotype experiments

Cultivars can be selected for superior growth and development in tissue culture. Genotypes differed in their callus formation abilities. Genotypes that are capable of rapid and vigorous callus initiation have been identified. These cultivars and advanced breeding lines will be valuable sources of materials for selection studies involving callus screening techniques.

Only one genotype surveyed formed callus cultures that did not produce shoots. The cultures derived from the cultivar 'Himalaya' produced a single callus type that was watery and translucent. Some green spots or sectors appeared in 2 of the 15 Himalaya cultures scored. However, no shoots or roots formed from any of these calli. Regeneration of Himalaya barley has been reported previously by Cheng and Smith (1975). The differing results of this investigation are due to the source of initial explant materials. Apical meristems were the source of materials for culture initiation in the research of Cheng and Smith. This would suggest the importance of selecting an explant source that produces a callus capable of regeneration.

The other 19 genotypes were able to undergo shoot and root development. Fifty percent of the genotypes evaluated were capable of producing at least one plant per initial explant. The regeneration of plants for all but one of the genotypes is a noteworthy finding. The methods tested proved to be successful for the tissue culture regeneration of cultivars from genetically diverse backgrounds. A wide variety of barley cultivars not yet tested are likely to respond favorably to in vitro propagation methods.

A number of chlorophyll deficient mutants were recovered. The cultivars 'Akka', 'Klages', and 'Triumph' each yielded between 5 and 10 chlorophyll mutants. This is not uncommon in barley tissue culture populations (Dale and Deambrogio 1979; Saalbach and Koblitz 1977).

There was a large degree of variability among observations. A range of developmental responses was often observed among the three subsamples of a single replicate. Within any single replication, often only one or two calli generated numerous shoots. A predictable proportion of initial embryo explants would not be expected to demonstrate the capacity for regeneration. This variability must be anticipated when designing tissue culture experiments.

Many investigators have previously reported that regenerative capacity of barley cultures decreases during successive subculturing (Scheunert et al. 1977; Deambrogio and Dale 1980; Jelaska et al. 1984). Their results are confirmed by this study. A decline in totiopotency was observed 6 months after culture initiation. Fewer green primordia appeared and shoots did not arise frequently. Callus growth did continue for most cultures with a few shoots still emerging 9 months after culture establishment.

Cultures of four genotypes established 18 months earlier still demonstrated callus-forming ability. The calli were friable and often had numerous green sectors which no longer produce shoots or roots. This loss of totiopotency after several months in culture has been frequently reported for other cereal crops (Nabors 1983; Ahloowalia 1982; Heyser and Nabors 1982).

The conflicting conclusions drawn from the determination of correlation coefficients is disappointing. Predictions of regeneration potential based on callus growth and fresh weight gain does not seem to be possible. Negative correlations found between fresh weight gain and plant regeneration for the genotype survey are not encouraging. These studies do not support the conclusions of Nabors et al. (1983) suggesting a positive correlation between the presence of green spots and regeneration potential.

Conclusions

The present data extend the information available on the response of barley genotypes to particular culture media, hormone supplements, and cultural growth conditions. Other studies have focused on the source of explant tissue and the use of a range of concentrations of 2,4-D as a growth hormone. A distinguishing finding of this study is the successful use of Cl_3 POP as an auxin source for the production of calli capable of shoot proliferation. This growth regulator enhanced plant regeneration as well.

The source materials used for this investigation were collected with uniformity of germplasm in mind. Plants were established from breeder seed to ensure genetic purity and minimize sources of variation. Considerable variation was found in these embryo explant cultures. Field populations of spring barley would be expected to exhibit greater genetic uniformity than what was observed in these tissue culture populations. Somaclonal variation in regenerated plant populations has been described repeatedly in cereal crop tissue culture literature (Larkin et al. 1984).

Individual genotypes have been identified for their rapid callus initiation and superior callus yielding ability. Genotypes that have demonstrated predictable shoot proliferation and regeneration potential can be used for further applied tissue culture studies.

It was not the intent of these studies to select and subculture callus types that developed large numbers of green primordia. If callus transfers had been carried out in a selective manner the number of plants regenerated could have been increased. Specific genes for plantlet regeneration are likely to exist. An investigation such as this which selects for genotypes with increased capacity for regeneration will help identify the factors controlling tissue culture regeneration.

References

Ahloowalia BS (1982) Plant regeneration from callus culture in wheat. Crop Sci 22:405–410

Bayliss MW, Dunn S (1979) Factors effecting callus formation from embryos of barley (*Hordeum vulgare*). Plant Sci Lett 14:311–316

Cheng TY, Smith HH (1975) Organogenesis from callus cultures of *Hordeum vulgare*. Planta 123:307–310

Dale PJ, Deambrogio E (1979) A comparison of callus induction and plant regeneration from different explants of *Hordeum vulgare.* Z Pflanzenphysiol 94:65–77

Deambrogio E, Dale PJ (1980) Effect of 2,4-D on the frequency of regenerated plants in barley and on genetic variability between them. Cereal Res Commun 8:417–423

Eapen S, Rao PS (1982 a) Plant regeneration from callus cultures of 'Durum' and 'Emmer' wheat. Plant Cell Rep 1:215–218

Eapen S, Rao PS (1982 b) Callus induction and plant regeneration from immature embryos of rye and triticale. Plant Cell Tissue Organ Culture 1:221–227

Gamborg OL, Wetter LR (1975) Plant tissue culture methods. Published by the National Research Council of Canada, Prarie Regional Laboratory, Saskatoon, Saskatchewan, Canada

Heyser JW, Nabors MW (1982 a) Regeneration of proso millet from embryogenic calli derived from various plant parts. Crop Sci 22:1070–1074

Heyser JW, Nabors MW (1982 b) Long term plant regeneration, somatic embryogenesis and green spot formation in secondary oat (*Avena sativa*) callus. Z Pflanzenphysiol 107:153–160

Jelaska S, Rengel Z, Cesar V (1984) Plant regeneration from mesocotyl callus of *Hordeum vulgare* L. Plant Cell Rep 3:125–129

Larkin PJ, Ryan SA, Bretell RIS, Scowcroft WR (1984) Heritable somaclonal variation in wheat. Theor Appl Genet 67:443–455

Nabors MW, Heyser JW, Dykes TA, DeMott KJ (1983) Long-duration, high-frequency plant regeneration from cereal tissue cultures. Planta 157:385–391

Orton T (1979) A quantitative analysis of growth and regeneration from tissue cultures of *Hordeum vulgare, H. jubatum,* and their interspecific hybrid. Environ Exp Bot 19:319–335

Rogers SO, Quatrano RS (1983) Morphological staging of wheat caryopsis development. Am J Bot 70:308–311

Saalbach G, Koblitz H (1977) Karyological instabilities in callus cultures from haploid barley plants. Biochem Physiol Pflanz 171:469–473

Scheunert EU, Shamina ZB, Koblitz H (1977) Studies on barley calluses cultured in vitro. 1. Establishment, maintenance and growth of two different tissue strains. Plant Sci Lett 10:313–318

Sears RG, Deckard EL (1982) Tissue culture variability in wheat: callus induction and plant regeneration. Crop Sci 22:546–550