CELL AND TISSUE CULTURE, EMBRYOGENESIS, TRANSFORMATION

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INTRODUCTION

Tremendous progress has been made throughout the last decade in the field of cell and tissue culture techniques (Fig. 1). As a consequence, techniques like embryo and anther culture are often considered as routine methods for barley breeding. Nevertheless, some problems still need further consideration. For example, pronounced genotypically different responses need to be solved in order to allow a broader application of these techniques in practical barley breeding programmes. This is also the case for somatic and androgenetic embryogenesis, where still some basic investigations of the phenomenon are required. Even more, techniques for genetic transformation of barley need substantial improvements based on fundamental research, so that efficient techniques will be available, once the respective genes for a reasonable transformation of barley will be available in the future.

In this contribution, recent progress made in the above fields as presented in a poster session at the Barley Genetics Symposium will be summarized and discussed.

ENDOSPERM DEVELOPMENT AND HYBRIDIZATION

In an interesting study of barley endosperm development, Bosnes *et al.* (1991, p. 227) demonstrated that endosperm development can be differentiated into 3 stages, i.e. the syncytial, cellularization and maturation stage. A hierarchical system of three sets of genes is supposed to be responsible for endosperm development, specifying (1) cell wall formation, (2) starchy endosperm and (3) aleurone cell differentiation, respectively.

Embryo culture *in vitro* is a useful technique for production of interspecific but also of intergeneric barley hybrids. By application of embryo rescue, Pershina *et al.* (1991, p. 214) recovered numerous hybrids of barley to *Triticum* and *Secale* species. In addition, some trigeneric hybrids could be successfully raised.

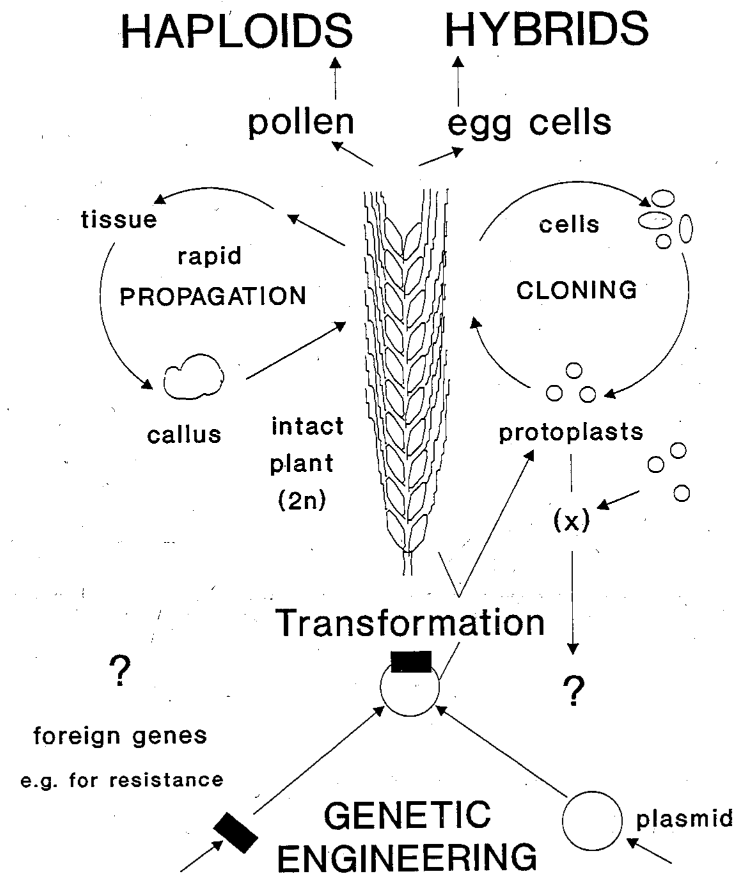

Fig. 1. Overview of cell and tissue culture and transformation methods for barley breeding

DEVELOPMENT AND USE OF HAPLOIDS

Haploid barley plants can be obtained either from microspores or from unfertilized ovaries. Wu and Zhang (1991, p. 223) reported on the production of haploid and diploid progeny via culture of unfertilized ovaries of different Chinese barley materials on maltose containing N6 and MS media.

With regard to the culture of microspores, Engell (1991, p. 197) made morphogenetic comparisons between zygotic and androgenetic embryos (embryoids) of barley cv. Bomi. The results indicate that there are clear-cut differences between both types of embryos: a 22 d old embryoid resembles an 14 d old zygotic embryo. Furthermore, Pedersen (1991, p. 213) has shown that androgenetic and zygotic embryos possess common but also different isozymes.

Anther-pollen culture *in vitro* was found to be an ideal system for both genetic research and practical application once its frequency is enhanced fully (Hu *et al.* 1991, p. 203)). With the current progress made in embryogenesis, the system is considered to be much more efficient than before and is thought to have great prospects.

The work of Cho and Kasha (1991, p. 194) demonstrated that antisenesecense compounds, i.e. n-propylgallate, $AgNO_3$ and aminooxyacetic acid, are beneficial in anther culture with regard to the plantlet regeneration frequencies in three cultivars, i.e. Bruce, Klages and Elrose.

Besides other compounds, the carbohydrate source in media has been shown to be an important factor for response of cultured anthers and microspores. Similar to earlier findings, high androgenetic response was achieved by Li *et al.* (1991, p. 209) with malting barley by using induction medium with maltose (6.4%). Out of 16,790 anthers cultured 1,963 green androgenetic plants could be regenerated in different experiments. The average frequency of anther response was almost 17% and the average rate of green plants was about 12%. More than 90% of the progeny were spontaneously doubled haploids.

A study with 270 androgenetic DH-lines regenerated on maltose medium from cvs. Tweed, Tyne and Natasha revealed that DH-families exhibit a high degree of stability. Preferential transmission of specific regions of the barley genome - as indicated by segregation for specific markers - may be associated with genetic factor(s) conditioning anther culture response (Finnie *et al.* 1991, p. 200). However, further studies are necessary in order to demonstrate whether agronomic characters are affected, too.

Furthermore, results of experiments with three spring barley crosses demonstrate again that anther culture progeny may not represent random samples of gametes as demonstrated for SSD-progeny and DH-lines derived via the "bulbosum method" (Björnstad and Skinnes 1991, p. 191). Improvements of anther culture technique are suspected to be due to the replacement of sucrose by maltose.

IN VITRO SELECTION

As long as cell and tissue culture techniques exist, selection *in vitro* i.e. independent of variable field or greenhouse conditions and in a minimum time interval, has attracted the interest of plant breeders. The contribution of Hunold (1991, p. 206) shows, that selection *in vitro* with *Drechslera teres* toxin applied to callus derived from immature embryos resulted in progeny which are more resistant to both, the toxin and the pathogen. Selection was even more efficient after mutagenic treatment.

It has often been speculated that *in vitro* selection should be of maximum success, if new genetic variation (socalled "somaclonal variation") would be induced *in vitro*. Ullrich *et al.* (1991, p. 220) assessed the potential of somaclonal variation in progeny of immature embryo-derived callus from different barley cultivars as compared to mutagenic sodium azide treatment. Somaclonal variation was demonstrated to occur in barley, but at a lower rate than induced by azide. As a consequence, *in vitro* selection methods with callus cultures are considered to be potentially useful for simply inherited traits, such as herbicide and disease resistance.

Furthermore, tolerance to environmental stress might be a feasible target as indicated by the results on *in vitro* selection for Al-tolerant barley by Zhu *et al.* (1991, p. 224) Calluses derived from anthers and immature embryos were selected on MS media containing different concentrations of Aluminum. Tolerant calli showed increase respiratory intensity on Al-media than original ones (control). However, no plant could be regenerated from suspected tolerant calli, so far.

SOMATIC CELL CULTURE AND REGENERATION

Plant regeneration from somatic cells still can be considered as a very ambitious goal in cereal species. Now, success is reported with *Hordeum marinum* suspension cultures (Omelianchuk *et al.* 1991, p. 211). On a CC medium (described by Lührs and Lörz 1988), supplemented by 1 mg/l IAA and 0.05 mg/l zeatin, green plants could be regenerated out of immature embryo-callus derived suspension (shaking) culture of *H. marinum*. Double salt concentrations in CC medium supplemented by 2 mg/l IAA and 0.1 mg/l zeatin only led to embryoids with root-like structures.

However, plant regeneration was even reported from protoplast-derived callus of barley (*H. vulgare* L.) by Satoh *et al.* (1991, p. 217). Stable embryogenic suspension cultures were obtained from barley cv. Dissa and Tsuyushirazu. Protoplasts isolated from the suspension cells divided efficiently and formed colonies at comparatively high frequencies (0.5-1%). After all, 5 green plants were regenerated from protoplast-derived colonies of Dissa. As a consequence, the following factors are considered to be critical for protoplast regeneration: (1) genotype, (2) rapid establishment of suspension culture, (3) selection of embryogenic callus, and (4) appropriate culture and adaptation of calluses to light conditions.

It was also demonstrated by Lazzeri and Lörz (1991, p. 235) that regeneration from barley protoplasts is possible now, although in low frequency. Nevertheless, the feasibility of regeneration from protoplasts opens new possibilities for genetic transformation of barley. By combining various modifications to the L1 medium plating efficiencies up to 25% could be obtained (Lazzeri and Lörz 1991, p. 235). With the use of a modified transformation procedure, stable transformation frequencies up to 5.9×10^{-4} (absolute value 9×10^{-5}) were achieved. Successful transformations were demonstrated by NPT and GUS assays.

GENETIC TRANSFORMATION

After all, an optimum transformation system for barley remains to be established. Kaneko *et al.* (1991, p. 231) studied and compared electroporation and laser perforation. Transformation of callus cells of *Hordeum bulbosum* line B53 was achieved after electroporation and laser perforation, where the latter is considered to be more advantageous, since maintenance of competent cell lines is not required. The presence of transferred reporter genes could be confirmed by PCR technique.

Alternatively, gene transfer in barley may be facilitated by microprojectile bombardment. For example, exponentially growing cell culture of barley cv. Borwina were bombarded by tungsten microprojectiles with plasmid DNA. Expression of reporter genes could be demonstrated by GUS and NPT assay (Kolesnikov 1991, p. 234).

Correspondingly, Mannonen *et al.* (1991, p. 237) cultured cell suspensions of barley cv. Pokko in Gamborgs B5 medium and bombarded by using Du Ponts biolistic device PDS-1000 with tungsten particles carrying the foreign DNA. The β-glucuronidase gene *uidA* from *E. coli* was used as a reporter, which was fused to the CaMV 35S promoter and the first intron of the maize *adh1* gene. Transient expression of the reporter gene was achieved in suspension cells.

Since the optimum procedure is still not available, Mendel *et al.* (1991, p. 240) compared different methods for gene transfer to barley. Transformation of barley was approached by (1) DNA transfer to protoplasts, (2) by transfer into suspension cells by particle gun, (3) by macroinjection into floral tillers, and (4) by transfer into growing pollen tubes. Progeny expressing the marker gene NPT could be derived by the latter two approaches. Positive specific signals were found in selfed progeny by PCR and Southern hybridization methods.

CONCLUSIONS

The poster presentations, comments and discussions during the poster session may be summarized with the following conclusions:

- Various cell and tissue culture techniques are now used more or less routinely in plant breeding programmes, e.g. anther and microspore or embryo culture.
- Embryogenesis, either somatic or androgenetic is principally inducible in barley with sufficient frequencies, however, it still strongly depends on the genotype.
- Due to improvements of protoplast culture and regeneration, the genetic transformation of barley is principally possible now. After further improvements, it is expected that any barley genotype can be transformed in the (near) future.

However, for transformations leading to improvements of agronomic characters, respective genes will be required, (Fig. 1). Hopefully, such genes will be made available in the nearer future by the extensive molecular work carried out at the present time.

REFERENCES

Björnstad, A. and Skinnes, H. (1991) Genetic studies of anther culture derived lines in barley. In Barley Genetics VI, Vol. I: 191-193.

Bosnes, M., Weideman, F. and Olsen, O.-A. (1991) Barley endosperm development: sub-division into syncytial, cellularization, differentiation and maturation stages. In Barley Genetics VI, Vol. I: 227-230.

Cho, U.H. and Kasha, K.J. (1991) Effect of antisenescence compounds on regeneration from andogenesis of barley. Barley Genetics VI, Vol. I: 194-196.

Engell, K. (1991). Zygotic and somatic embryos in barley. In Barley Genetics VI, Vol. I: 197-199.

Finnie, S.J., Forster, B.P., Thompson, D.M., Dyer, A.F., Chalmers, K.J. and Powell, W. (1991) Genetic stability of microspore derived families of barley: a cytological, biochemical and molecular study. In Barley Genetics VI, Vol. I: 200-202.

Hu, H., Li, W.Z. and Jing, J.K. (1991) Anther/pollen *in vitro* culture of barley. In Barley Genetics VI, Vol. I: 203-205.

Hunold, H. (1991) *In vitro* selection on disease resistance in barley callus cultures. In Barley Genetics VI, Vol. I: 206-208.

Kaneko, T., Kuroda, H., Hirota, N., Kishinami, I., Kimura, N. and Ito, K. (1991) Two transformation systems of barley - electroporation and laser perforation. In Barley Genetics VI, Vol. I: 231-233.

Kolesnikov, V. (1991) Introduction of foreign genes into barley cells by high velocity microprojectiles. In Barley Genetics VI, Vol. I: 234.

Lazzeri, P.A. and Lörz, H. (1991) Protoplast culture and transformation in barley. In Barley Genetics VI, Vol. I: 235-236.

Li, A., Shao, Q., De, W., Yu, J., Cao, L., Fu, Z., Ying Lin, X. and Xu, W. (1991) Studies on malting barley anther culture. In Barley Genetics VI, Vol. I: 209-210.

Lührs, R. and Lörz, H. (1988) Initiation of morphogenic cell suspension and protoplast cultures of barley (*Hordeum vulgare* L.). Theor. Appl. Genet. 175: 71-81.

Mannonen, L., Ritala, A., Salmenkallio, M., Aspegren, K., Teeri, T.H. and

Kauppinen, V. (1991) Optimization of gene transfer in barley by microprojectile bombardment. In Barley Genetics VI, Vol. I: 237-239.

Mendel, R.R., Schulze, J., Clauss, E., Nerlich, A., Cerff, R. and Steinbiss, H.H. (1991) Gene transfer to barley. In Barley Genetics VI, Vol. I: 240-242.

Omelianchuk, N.A., Philippova, G.I. and Vershinin, A.V. (1991) Plant regeneration from *Hordeum marinum* suspension cultures. In Barley Genetics VI, Vol. I: 211-212.

Pedersen, S. (1991) Expression of isozymes in barley anther culture. In Barley Genetics VI, Vol. I: 213.

Pershina, L.A., Belova, L.I., Numerova, O.M. and Shumny, V.K. (1991) *In vitro* techniques for production of intergeneric barley hybrids. In Barley Genetics VI, Vol. I: 214-216.

Satoh, N., Kuroda, H., Kihara, M. and Kishinami, I. (1991) Plant regeneration from protoplast-derived callus of barley (*H. vulgare* L.). In Barley Genetics VI, Vol. I: 217-219.

Ullrich, S.E., Kleinhofs, A., Jones, B.L. and Johnson, J.J. (1991) Somaclonal variation in barley: theoretical and practical implications. In Barley Genetics VI, Vol: I: 220-222.

Wu, H. and Zhang, F. (1991) Induction of haploid barley plants from unpollinated ovaries. In Barley Genetics VI, Vol. I: 223.

Zhu, M., Huang, C., Xu, A., Yuan, M. and Yu, Z. (1991) *In vitro* selection and characterization of Al-tolerant variants of barley callus. In Barley Genetics VI, Vol. I: 224-226.