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*Research note*

# Barley anther culture using membrane rafts

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**Abstract**

When compared to agarose solidified media in small petri dishes, membrane rafts used in conjunction with liquid induction media significantly improved anther culture response in the Australian, malting-quality, spring barley cultivar Clipper. In contrast, the German cultivar Gimpel did not show an increased response on rafts.

*Abbreviations:* BA – 6-benzylaminopurine, IAA – indoleacetic acid, DH – doubled haploid

The advent of membrane raft technology (Herman 1991; Luckett *et al.* 1991) allows media manipulation in tissue cultures to be performed with minimal effort and, by avoiding direct handling, without causing damage to the tissues (Luckett & Darvey 1992).

In this note we report an experiment designed to establish whether membrane rafts are a potentially superior support method to agarose-solidified media for barley anther culture.

Two spring cultivars (Clipper and Gimpel) of barley (*Hordeum vulgare* L.) were grown in the glasshouse at Wagga Wagga (35° S) in large pots (Luckett & Smithard 1992). Clipper was chosen for inclusion since it had been used as a standard in previous experiments and had shown an intermediate level of response (Luckett & Smithard, unpubl.). Gimpel was used since its response was reported as high (Jähne *et al.* 1991) and it was easier to use than its fellow-European cultivar, the highly-responsive Igri, which is a winter type and requires vernalisation. The glasshouse donor plants produced immature spikes suitable as explants during the period November 1991 to January 1992.

The so-called 'FHG' induction media of Hunter (1987; 1988) was used in this experiment, as modified by Luckett & Smithard (1992). In addition, BA was omitted and IAA at 1.0 mg $l^{-1}$, and kinetin at 0.2 mg $l^{-1}$, were included.

Induction medium was either solidified with SeaKem LE agarose (FMC BioProducts) at 3 g $l^{-1}$, with 2.5 ml of medium used per 35 mm Ø Petri dish (Luckett & Smithard 1992), or used as a liquid medium under membrane rafts (Sigma Chemical Company) (Luckett *et al.* 1991).

The care of donor plants and regenerants has been described elsewhere (Luckett & Smithard 1992). All spikes were cold pre-treated at 4 °C in the dark for 14 days prior to anther dissection and plating (Luckett & Darvey 1992; Luckett & Smithard 1992). Approximately 30 anthers were plated in each dish and on each membrane raft.

A number of variates were scored:

1) the proportion of anthers responding to produce at least one embryo (pr);
2) the number of embryos per 100 anthers plated (epa);
3) the number of green regenerants per 100 anthers plated (gpa);
4) the number of albino regenerants per 100 anthers plated (apa);
5) proportion of total regenerants that were green (pg);
6) number of fertile DH lines per 100 anthers plated (fpa); and
7) number of sterile lines per 100 anthers plated (spa).

Non-orthogonal analysis using generalised linear regression methods was conducted using the Genstat 5 computer package. Variables pr and pg were analysed as binomial-distribution variates; and epa, gpa, apa, fpa and spa were analysed as Poisson-distribution variates. The character pg was used because the more common 'green/albino regenerant ratio' causes major loss of data during computation since if one or both of the contributing variates is zero, then the ratio must be set to missing. The analysis of pg as a binomial variate preserves all data so that it contributes to the analysis.

The significance of the main effects (genotype, support) and the interaction was assessed by analysis of deviance and F-tests. The models were then used to predict mean values and associated standard errors for the effects and interactions.

A total of 47 spikes (1375 anthers) were plated across the 4 treatment combinations (2 cultivars × 2 support systems). 1021 embryos were produced, of which 297 (29%) regenerated to produce green plantlets and 128 (13%) albino plantlets. Only 20 of the green regenerants grew on to produce fertile DH lines, while a further 14 survived to adulthood but were sterile.

Contamination of jars containing membrane rafts was a problem, possibly due to the high occurrence of static electric charge building up on the vessels during handling. A total of 11 rafts were contaminated (11/28 = 39%) whereas no dishes were lost. Handling methods to overcome this problem are being developed. As soon as they were identified as contaminated, jars and rafts were discarded and did not contribute further to the analysis.

A summary of the main effects and interactions from the analysis of deviance (Table 1) showed no clear pattern across the seven variates measured. Support main effects were particularly significant for the character gpa. Genotype effects were highly significant for gpa and pg.

Gimpel anthers responded most on solid media in dishes whereas Clipper anthers responded best on rafts over liquid media (Table 2). Clipper produced markedly more embryos per 100 anthers plated on rafts (epa = 265.1 ± 71.3) than on dishes (epa = 67.6 ± 33.7). Conversely, Gimpel's embryo production on rafts was lower than in dishes, and much lower than Clipper's (Table 1).

Many more green regenerants originated from the liquid/raft treatment (gpa = 52.7 ± 12.0) than the solid/dish treatment (gpa = 9.92 ± 4.91). Genotype × support effects were significant (Table 1) with Clipper markedly favouring the liquid/raft treatment (Table 2). The performance of Clipper controls on liquid/rafts was outstanding with over 100 greens per 100 anthers plated and more than 10 times the number obtained on dishes (Table 2). Similarly, the number of albino regenerants was higher on rafts than dishes but not to the same extent as for gpa.

For pg, only genotype was significant (Table 1). Gimpel produced a higher proportion of green regenerants from the dishes (pg = 0.56 ± 0.11) whereas Clipper produced the higher proportion on rafts (pg = 0.78 ± 0.04).

Results for fpa were not significant (overall mean = 1.92) whereas for spa only genotype effects were significant (Table 1) and Clipper produced 2.56 ± 0.83 compared to 0.48 ± 0.30 for Gimpel.

The use of membrane rafts for barley anther culture shows some promise given the significant response of cv. Clipper using liquid media. The figure of over 100 green regenerants per 100 anthers plated (Table 2) compares favourably with other published values (Pickering & Devaux 1992). The Clipper genotype would generally be regarded as "poorly responsive" using established techniques as seen in its poor gpa value in dishes with solid media (Table 2). In the membrane raft system it becomes "highly responsive", however, the efficacy of the membrane raft system was genotype dependent, with cv. Gimpel performing much more poorly.

Generally the results for both genotypes on membrane rafts were more variable than for those anthers on solid media in dishes. When combined with the problems of contamination this illustrates that there are technical improvements to be made, although the potential of this system in barley is evident. The capital cost of the rafts remains an obstacle to their widespread adoption, unless cheaper, more readily-available materials can be found.

In this experiment a large number of embryos failed to germinate (58%). In addition, of the total green regenerants that were produced in the laboratory, only about 7% (20/297) survived to produce spontaneously-doubled seed in the glasshouse. Many regenerants died on transfer to soil due to the difficulty in maintaining adequate glasshouse conditions. Clearly, the embryo germination and the transplantation stages of the DH production system require careful attention.

The proportion of soil-established regenerants that were spontaneously doubled was, however, encourag-

*Table 1.* Variance ratio F tests from analysis of deviance for seven variates measured in this experiment

| Source | Variates | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | df | pr | epa | gpa | apa | pg | fpa | spa |
| Genotype (G) | 1 | 0.00 ns | 6.08 * | 15.98 *** | 3.13ns | 9.56 ** | 0.58 ns | 7.14 * |
| Support (S) | 1 | 1.92 ns | 2.13 ns | 12.62 ** | 7.27 * | 0.00 ns | 0.13ns | 3.22 ns |
| G × S | 1 | 6.63 * | 6.16 * | 3.15 ns | 1.99 ns | 0.95 ns | 2.76 ns | 2.28ns |
| Residual | 32[a] | | | | | | | |
| Total | 35 | | | | | | | |

[a] for pg, df = 22; ns – $p > 0.05$
*: $0.05 > p > 0.01$
**: $0.01 > p > 0.001$
***: $0.001 > p$

*Table 2.* Predicted (or fitted) means for six variates showing significant effects calculated from the generalised linear regression model. Standard errors are given in parentheses.

| Variate | Genotype | Support | |
|---|---|---|---|
| | | Liquid/raft | Solid/dish |
| pr | Clipper | 0.44 (0.11) | 0.29 (0.09) |
| | Gimpel | 0.19 (0.07) | 0.50 (0.08) |
| epa | Clipper | 265.2 (71.3) | 67.6 (33.7) |
| | Gimpel | 33.8 (22.8) | 81.8 (31.6) |
| gpa | Clipper | 112.9 (27.3) | 11.5 (8.2) |
| | Gimpel | 9.7 (6.7) | 8.8 (6.1) |
| apa[a] | | 19.9(4.6) | 6.4 (2.5) |
| pg[b] | Clipper | 0.76 (0.04) | |
| | Gimpel | 0.48 (0.09) | |
| spa[b] | Clipper | 2.56 (0.83) | |
| | Gimpel | 0.48 (0.30) | |

[a] only support effects were significant.
[b] only genotype effects were significant.

ing (20/(20 + 14) = 59%) and similar to the levels in previous experiments (Luckett & Smithard 1992).

The experimental comparison of anther culture protocols which utilise different culture vessels (as in this study) presents several difficulties:

1) Anther plating densities were not equal. One would need approximately 144 anthers/raft to equal the density per ml of media in petri dishes, or 60 anthers per raft to equal the density per $mm^2$ of plating area. This difference was a consequence of the desire to preserve the use of one spike per vessel.

2) Air (head space) volumes were not equal in the two vessels and culture response could have been influenced by the accumulation or depletion of various gases.

Despite these reservations it appears that for certain genotypes the membrane raft support system is a distinct improvement over agarose-solidified induction media. The raft system is particularly useful where short-term exposure to particular media is required without the associated risk of directly handling the cultured tissue. The tissue is moved *en masse* saving time and labour costs.

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