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# Anther culture of *Hordeum vulgare* L.: a genetic study of microspore callus production and differentiation

**J. M. Dunwell [1, *], R. J. Francis [1] and W. Powell [2]**

[1] John Innes Institute, Colney Lane, Norwich, England
[2] Scottish Crop Research Institute, Pentlandfield, Roslin EH25 9RF, Scotland

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**Summary.** The inheritance of the ability of barley anthers to produce microspore-derived callus in vitro was investigated. The genotypes selected were the two spring cultivars 'Dissa' (D) and 'Sabarlis' (S), the two $F_1$ hybrids (D×S, S×D), the two backcross generations [D×(D×S), S×(D×S)], and an $F_2$ generation derived from D×S. From a number of individuals of each generation, the first five spikes were harvested sequentially and after pre-treatment the anthers were removed and placed in culture. Cultures were scored for microspore callus production and plantlet differentiation. Although 'Dissa' gave a significantly higher level of callus production than 'Sabarlis', the overall frequencies of green and albino plant production were higher from 'Sabarlis'. There was no significant difference between reciprocal $F_1$ hybrids. Analysis of variance revealed significant differences in response between the spikes sampled from the plants. This was the major source of variation in the experiment. Spike to spike variation also appeared to be a heritable character.

**Key words:** Barley – Anther culture – Pollen – Haploid – Callus

## Introduction

The production of microspore-derived plants from cultured anthers is one of several methods for the isolation of doubled-haploids in barley (Choo et al. 1985).

However, it has not yet achieved widespread acceptance in practical plant breeding programmes because of the overall low yield of plants achieved (Dunwell 1985, 1986a, b). Amongst the many factors that determine this yield, the genotype of the plants used for culture is one of the most important. A number of studies have been conducted in order to quantify this genetic component and these have included that of Foroughi-Wehr et al. (1982) who concluded in a study of 55 hybrids and four cultivars that culture responsiveness is a complex character involving at least two different, and separately inherited mechanisms; first, the ability of microspores to divide and produce callus, and secondly, the ability of calli to differentiate into plantlets.

The present study, which was designed to analyse further these characters, utilised two cultivars, 'Dissa' (Foroughi-Wehr et al. 1982) and 'Sabarlis' (Huang and Sunderland 1982) known to respond well in anther culture, and various generations derived from these cultivars.

## Materials and methods

The genotypes selected for this study were the spring cultivars 'Dissa' (D) and 'Sabarlis' (S), the two $F_1$ hybrids (D×S, S×D), the two backcross generations [D×(D×S, S×(D×S)], and an $F_2$ generation derived from D×S. Plants were grown in a greenhouse under a natural photoperiod with one plant per 100 mm square plastic pot containing John Innes compost. Five plants were selected from each of two cultivars, 10 from each $F_1$ and 12 from each backcross and $F_2$ generation. From each plant, the first five spikes were harvested when the interligule length (75–100 mm) signified pollen at the early to mid uninucleate stage. Spikes were placed in polythene bags and pre-treated in darkness for 28 days at 4 °C (Huang and Sunderland 1982). After pre-treatment, tillers were sterilised and the spikes dissected out. The 30 anthers from the 10 central spikelets of each half of each spike were placed on 10 ml modified MS medium solidified with Sea-Plaque agarose (8 g $l^{-1}$) (Lyne et al. 1986) in plastic Petri dishes (52×18 mm). The two dishes from each spike were incubated at 25 °C in darkness until callus emergence, at which time they were transferred to illuminated conditions (Grolux fluorescent tubes: 30 μ E $m^{-2}$ $s^{-1}$). After a 42 day incubation, differentiating calli/embryos were transferred to 1/2MS (Huang and Sunderland 1982) medium for further growth.

* Present address: ICI International Seeds Business, Tealott's Hill Research Station, Bracknell, Berks.

Cultures were scored for callus production after 42 days and for plantlet differentiation after a further 14 days. Data were analysed in order to compare the relative effects of genotype, plant to plant variation, and spike number.

## Results

Data on anther response and plant regeneration frequencies for the seven generations tested are given in Table 1. Although 'Dissa' gave a significantly higher level of anther response than 'Sabarlis', the overall frequencies of green and albino plant production are higher from 'Sabarlis'. There was no significant difference between reciprocal $F_1$ hybrids for: percentage responding anthers, green plant production and total plant production. This suggests that maternal and cytoplasmic factors are not involved in anther culture response in this particular cross and for subsequent analyses, data from reciprocal $F_1$ hybrids were therefore combined.

The material sampled in this experiment may be classified in an hierarchical manner and the analysis of variance appropriate to this experimental design is given in Table 2. The highest level in the analysis is differences between plants and for this item there are significant differences between individual plants for the 'Sabarlis', $F_1$ D×S and the $F_2$ generations. Differences between spikes within plants is the second item in the analysis of variance and this is significant for all the generations scored. The analysis may be extended to include the components of variance. These may be calculated from the expected mean squares appropriate to the experiment and are also given for each generation in Table 2. The components of variance have been scaled to sum to 100 and provide an estimate of the amount of variation attributable to each item in the analysis. Reference to Table 2 indicates that the between spikes item accounts for between 46 and 95% of the total variation in the experiment.

A genotype's phenotypic variance, (the square root of the variance component over the five spikes sampled) may be used as a measure of a genotype's sensitivity to age of spike. The seven generations examined differ in their sensitivity and reference to Table 3 indicates that 'Dissa' is far more sensitive to age of spike than 'Sabarlis'. These two parameters, mean response (Table 1) and sensitivity (Table 3) provide a two dimensional assessment of a genotype's performance in vitro and have been used previously to examine the response of immature embryos in culture (Powell and Dunwell 1986).

The genetical factors influencing microspore callus production and differentiation, were analysed by using the family means. Weighted least squares model fitting procedures were used on the available generations to estimate the m, [d], [h], [i], [j] and [l] parameters which provide estimates of the main effects and first order interactions of the genes (Mather and Jinks 1982). Considering the character percentage responding anthers (Table 4), the model fitting analysis indicate that the genetic control of this character is complex and non-allelic interactions ([i] and [j]) are involved. Furthermore, the [h] effect (i.e. sum of the dominance effects) is negative. For green plant production the [h] parameter is positive and the [l] parameter negative. Since the magnitude and sign of [h] and [l] are not influenced by the way the alleles are distributed between the parents, these components may be used to classify the predominant type of epistasis (Jinks and Jones 1958). The sign of [h] and [l] are opposite and suggest an excess of duplicate type epistasis. This form of epistasis is generally associated with characters which have been exposed to directional selection for extreme expression. It is also of interest to note that the absence of a significant [d] component would imply little or no additive genetic differences between the parents which are both known to be responsive to anther culture. The absence of a significant [d] com-

**Table 1.** Overall values for anther response and plant production

| Generation | Character | | | | | | |
|---|---|---|---|---|---|---|---|
| | No. anthers responding | (%) | No. plants | | Green plants/100 responding anthers | Albino plants/100 responding anthers | Total plants/100 responding anthers |
| | | | Green | Albino | | | |
| D | 633 | 33.56 | 6 | 6 | 0.95 | 0.95 | 1.90 |
| S | 222 | 21.89 | 6 | 10 | 2.70 | 4.50 | 7.20 |
| $F_1$ (D×S) | 834 | 24.48 | 19 | 12 | 2.28 | 1.44 | 3.72 |
| $F_1$ (S×D) | 909 | 28.25 | 26 | 21 | 2.86 | 2.31 | 5.17 |
| $F_2$ (D×S) | 1,551 | 39.73 | 29 | 20 | 1.87 | 2.29 | 3.16 |
| D×(D×S) | 2,088 | 37.30 | 50 | 24 | 2.39 | 1.15 | 3.54 |
| S×(D×S) | 483 | 16.65 | 28 | 19 | 5.80 | 3.93 | 9.73 |
| Total | 6,720 | 30.27 | 164 | 112 | 2.44 | 1.67 | 4.11 |

**Table 2.** Analysis of variance for the percentage of responding anthers (after angular transformation)

| Item | Generation | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | D | | S | | | $F_1$ (D×S) | | | $F_1$ (S×D) | | | $F_2$ (D×S) | | | D×(D×S) | | | S×(D×S) | |
| | df | MS | df | MS | | df | MS | | df | MS | | df | MS | | df | MS | | df | MS |
| Between plants | 4 | 1,536 (0) | 4 | 416 (38)*** | | 9 | 4,705 (37)*** | | 9 | 837 (0) | | 11 | 4,586 (31)** | | 11 | 3,061 (0) | | 11 | 1,079 (0) |
| Between spikes within plants | 20 | 2,554 (95)*** | 13 (7)[a] | 975 (56)*** | | 38 (2)[a] | 1,075 (47)*** | | 38 (2)[a] | 1,256 (81)*** | | 45 (3)[a] | 1,342 (61)*** | | 46 (2)[a] | 2,423 (89)*** | | 38 (10)[a] | 796 (70)*** |
| Between Petri dishes | 25 | 71 (5) | 16 (9)[a] | 49 (6) | | 41 (9)[a] | 160 (16) | | 48 (2)[a] | 133 (19) | | 53 (7)[a] | 80 (8) | | 52 (8)[a] | 147 (11) | | 45 (15)[a] | 142 (30) |

** $P<0.01$; *** $P<0.001$
( )[a] indicates number of missing values
( ) percentage variation accounted for in the Analysis of Variance

**Table 3.** Mean sensitivity ($\sigma$) for the character percentage responding anthers

| Generation | Sensitivity ($\sigma$) |
|---|---|
| 'Dissa' (D) | 21.86 |
| 'Sabarlis' (S) | 5.30 |
| $F_1$ (D×S) | 5.08 |
| $F_1$ (S×D) | 11.10 |
| $F_2$ (D×S) | 8.60 |
| D×$F_1$ (D×S) | 20.11 |
| S×$F_1$ (S×D) | 4.90 |

**Table 4.** Weighted least squares model fitting analysis for the characters; percentage responding anthers and green plant production

| Value | Character | |
|---|---|---|
| | % responding anthers | Green plant production |
| m | 51.53±5.79 | 1.20±0.38 |
| [d] | 6.12±2.63 | – |
| [h] | −25.36±7.02 | 6.13±2.39 |
| [i] | −24.14±6.66 | – |
| [j] | 50.41±9.96 | – |
| [l] | – | −5.03±2.51 |
| $\chi^2$ (n−p) | 0.26 | 1.06 |

ponent could also be due to dispersion of increasing and decreasing alleles between the parents (Jayasekara and Jinks 1976).

The second degree statistics obtainable from the available basic generations have been used to obtain estimates of the heritabilities for the characters scored in the experiment. The narrow sense heritabilities estimated from this study range from 15.5% for green plant production to 28% for percentage anthers responding.

## Discussion

This study represents the first report of a genetical analysis of anther culture response, using the basic $F_2$ and backcross generations derived from an $F_1$ cross between a pair of pure breeding lines. From the data it was possible to calculate the heritabilities of a number of aspects of the in vitro response. Only in one previous study (Charmet and Bernard 1984) have broad sense heritabilities been calculated. Although the concept of heritability provides a convenient way of summarising genetical information, it should be borne in mind that these terms are dependent on both the heritable and non-heritable components.

Thus, the heritability of a character may be increased by reducing the non-heritable component and/or by making the

environment more stable. Caution should be exercised in interpreting the genetical analyses outlined in the present study since differences between spikes accounted for most of the variation in the experiment. Genotypes also differed in their sensitivity to spike number. It is therefore highly probable that analysis based on family means when averaged over spikes is a compromise and a genotype's sensitivity should also be considered when analysing genotypic response to in vitro culture (Powell and Dunwell 1986). The present results emphasise the need for experimental designs which allow the separation of heritable and non-heritable variation.

There have been numerous previous studies on the genetic control of production of microspore callus in a number of cereal species, notably those on barley (Foroughi-Wehr and Friedt 1981; Foroughi-Wehr et al. 1982), wheat (Lazar et al. 1984) and Triticale (Charmet and Bernard 1984). However, partly because of the different units used to quantify the culture response, it is difficult to compare accurately the results from these various studies. For example, in a single study (Foroughi-Wehr et al. 1982) callus production from different experiments was described in terms of number of calli per 100 anthers, or per 100 spikes or percent of anthers with callus. In this particular study, the most relevant experiment was that conducted using the cultivars 'Dissa' and 'Aramir' and their reciprocal $F_1$ hybrids. It was concluded that anthers of 'Dissa' were significantly more productive (8 calli per 100 anthers) than those of 'Aramir' and that there were significant differences between the reciprocal $F_1$ hybrids. The presence of reciprocal differences was taken as evidence to indicate the involvement of maternal and/or cytoplasmic factors influencing anther culture response. However, in a later publication (Foroughi-Wehr and Friedt 1984) it was concluded that androgenetic response is not dependent on cytoplasmic factors.

No significant reciprocal difference was observed with wheat (Bullock et al. 1982). In this study, anthers from 10 spikes of three pairs of reciprocal $F_1$ hybrids, and their parents, were tested and it was concluded that in each case the hybrids gave values intermediate between the parents and also there was no evidence of reciprocal differences between hybrids.

In the most detailed study so far conducted, that on Triticale (Charmet and Bernard 1984), anthers from a $7 \times 7$ diallel were tested and scored in terms of embryos/100 anthers, green plants/100 embryos and green plants/1,000 anthers calculated. It was found that embryo production from a number of $F_1$'s significantly exceeded either parent – in contrast to the results from barley and wheat referred to above. Also, with the exception of lines carrying *Triticum timopheevi* cytoplasm, there was no reciprocal difference between $F_1$'s. Although the study on Triticale utilised anthers from four spikes of each plant no information was provided on any variation between spikes.

One of the major conclusions to be drawn from the present study concerns the importance of spike number in determining the frequency of callus production. The first comment on possible differences between tillers was the incidental remark made by Orlikowska (1977) who found in Triticale that no embryoids developed in anthers taken from lateral spikes. In a subsequent study on rice, Lupotto (1982) commented that "the best response was obtained with the earliest panicles generated, with a successive decrease for the panicles obtained later". A similar conclusion was made with wheat by Jing et al. (1982), and is supported by evidence from some of the data from the present study.

The only previous quantitative evidence on this subject came from a detailed study on rice by Chen and Tsay (1984) who compared two methods of sampling tillers. The first method involved the successive collection of panicles at the appropriate stage from tillers of different orders within the same plant. The second method was to harvest panicles from tillers of a definite order from different plants. When the first method was used, it was found that contrary to the present study, there was no difference between tillers in terms of the number of anthers producing callus. However, it was found with the second method, that anthers from the tertiary tillers were significantly less productive than those from the main culm or from primary or secondary tillers.

In the only previous mention of this subject in barley, it was stated (Foroughi-Wehr et al. 1982) that "in earlier studies with 'Dissa' no differences were found between the first and subsequent spikes of a plant". This is in contrast with the findings on 'Dissa' in the present study. However, it must be emphasised that these previous conclusions were reached with spikes which were freshly harvested rather than being pretreated as in the present study.

One of the known genetic differences between the two parental genotypes used in the present study is the ear type. 'Dissa' is a six-row barley whereas 'Sabarlis' is a two-row genotype. The inheritance of the two-row versus six-row character is controlled by a single locus (*V-v*) with two-row being partially dominant over six-row (Nilan (1964). In general, two-rowed genotypes have a higher tillering capacity than six-rowed genotypes and the decline in productivity of anthers cultured from older 'Dissa' spikes may relate to this phenomenon. Further genotypes and crosses would need to be examined to substantiate this finding. It would also be of interest to establish whether the *V-v* locus, through pleiotropic or linkage effects, influences anther culture response in barley.

The results from the present study are also of direct relevance to barley breeders, since allocation of resources is of central importance to the success of a breeding programme. This decision on the optimal number of spikes to be harvested from a given plant and the number of plants to be grown per unit area will ultimately influence the cost effectiveness of anther culture techniques in comparison to conventional methods.

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