Theor Appl Genet (1991) 82:417–420



# Nuclear genes affecting percentage of green plants in barley (*Hordeum vulgare* L.) anther culture

**E.T. Larsen, I.K.D. Tuvesson and S.B. Andersen**

The Royal Veterinary and Agricultural University, Department of Agricultural Science, Section of Plant Breeding and Biotechnology, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

Received January 7, 1991; Accepted April 5, 1991
Communicated by G. Wenzel

**Summary.** The genetics behind response in barley anther culture was studied with 22 reciprocal and one single cross between three varieties with high and four varieties with low capacity for green plant formation. Effects of genotypes dominated embryo formation and percentages of green plants, accounting for 62 and 76% of total variation, respectively, with almost no genetic effect on the ability to regenerate plants from pollen embryos. Nuclear genes could explain all genotype effects in this plant material, since no reciprocal effects were indicated. The three parents with high and the four parents with low capacity for green plant formation formed two phenotypically homogeneous groups, producing 27–52% and 0–7% green plants, respectively. Genetic variation within hybrids for both embryo and green plant formation could be explained completely by general combining ability (GCA). The results are discussed with respect to a previous similar study in hexaploid wheat and the reported existence of DNA deletions in the plastid genomes in albino plants from anther culture of wheat and barley.

**Key words:** Barley – Anther culture – Androgenetic responsiveness – Albinism – Genetic control

## Introduction

In anther and microspore culture of cereals and grasses, the frequent formation of many albino plants is a peculiar and serious problem. With recent progress in anther culture techniques, reasonable numbers of embryos and plants can be obtained in many genotypes of wheat and barley (Andersen et al. 1987; Knudsen et al. 1989; Kuhlmann and Foroughi-Wehr 1989; Foroughi-Wehr and Zeller 1990). In many cases, however, the majority of plants obtained are albinos.

In hexaploid wheat it has been demonstrated that the ratio of green to albino plants obtained is strongly affected by genes in the parent material (Andersen et al. 1987). From studies of albino frequencies in anther culture of reciprocal hybrids between lines with high and low capacity for green plant formation, it has been shown that the capacity for green plant formation is inherited on the chromosomes in this species (Tuvesson et al. 1989). It has also been demonstrated in the case of barley that the capacity for formation of high percentages of green plants is a heritable character. Seven barley varieties were divided into one group with low percentages of green regenerants (5–8%) and another group with high percentages of green plants (30–40%) (Knudsen et al. 1989), indicating the possibility that major genes control the trait. Among the lines with high capacity for green plant regeneration was the variety 'Igri,' which has been used in many studies as a model variety due to its ease in responding in anther culture (Foroughi-Wehr and Friedt 1984; Olsen 1987; Hunter 1987; Ziauddin et al. 1990). Apparently, 'Igri' does not have a genetic capacity for green plant formation that is superior to several other widely grown barley varieties, but it has combined the low albino frequency trait with high capacity for embryo formation (Knudsen et al. 1989).

Studies of the genetics behind the ability to respond in anther culture are of interest for two reasons. Understanding the main genetic components and knowing the parental material of their crosses, breeders may to some extent predict the level of response from their hybrids and thus optimize the allocation of resources for haploid production. If a characteristic pattern of genetics can be deduced into major genes, valuable information about the anther or microspore culture process may be obtained by studying the mechanism of action of such genes. Since albinism affects the development of plastids in plants, the possibility of a cytoplasmic mode of inheritance of the trait must be considered. This paper presents a study of albino frequencies in barley anther culture of reciprocal hybrids between three lines with high capacity for green plant formation ("green-type parents") and four lines with low capacity for green plant formation ("albino-type parents"), to determine whether the genes regulating the percentage of green plants in barley anther culture are inherited on the chromosomes or in the cytoplasm.

## Materials and methods

The parental plant material consisted of seven widely cultivated varieties of barley (*Hordeum vulgare* L.). It has been previously demonstrated that the three winter varieties, 'Igri,' 'Plaisant,' and 'Marinka,' produce high percentages of green plants in anther culture, while the three spring varieties, 'Triumph,' 'Sewa,' and 'Grit,' will produce only low percentages of green plants (Knudsen et al. 1989). In addition, the variety 'Jupiter,' which is presumed to produce only low percentages of green plants, was included in the experiment among the "albino type parents." All the 3 × 4 crosses between the two groups of parents were produced reciprocally, with the exception of the combination 'Marinka' (female) × 'Jupiter' (male). The 23 different hybrids and their seven parents were evaluated for anther culture response in two replications, with donor plants raised in field and growth chamber, respectively. Winter types were germinated and vernalized at 1°–3 °C for 2 months before planting, and five donor plants were raised for each genotype for each environment and planted at the beginning of June. Growing conditions for donor plants in field and growth chamber were as described previously (Knudsen et al. 1989).

The anthers from ten spikes of each hybrid and 15 spikes from each parent were plated as one experimental unit for each environment, the anthers from two spikes together in one petri dish, 10 cm in diameter. The methods for selection and sterilization of spikes, and the conditions for anther culture were as described previously (Knudsen et al. 1989), except that the substrate for culturing the anthers contained 6% maltose instead of sucrose (Hunter 1987). Since the maltose-containing substrate caused a huge number of embryos in each petri dish, only embryos from a 1-cm wide, randomly selected band, containing approximately 40 anthers in each dish, were recorded and transferred to regeneration substrate.

For the statistical analysis, the square-root transformed numbers of embryos/100 cultured anthers, numbers of plants/100 embryos, and percentages of green plants (green plants × 100/total plants) from each experimental unit were subjected to an analysis of variance with main effects from genotypes, environments, and reciprocals, and interactions between genotypes and environments. Residuals were checked graphically. For further analysis of genotype effects, the combined error *b*, consisting of residuals and genotype × environment interactions with 29 *df*, was used for testing.

## Results

From the 17,108 anthers cultured, 7,141 anthers (41.7%) responded with a total of 110,014 embryos, from which 2,543 green and 13,414 albino plants were regenerated.

**Table 1.** Analysis of variance

| Source | *df* | Embryos/ 100 anthers MS | Plants/ 100 embryos MS | Percent green plants MS |
|---|---|---|---|---|
| Genotypes (G) | 18 | 120.64*** | 1.13 | 6.83** |
| Environments (E) | 1 | 52.80 | 0.89 | 7.10* |
| Reciprocals | 11 | 55.69 | 0.29 | 0.33 |
| G*E | 18 | 27.52 | 0.52 | 0.96 |
| Error *a* | 11 | 16.25 | 0.58 | 1.06 |
| Subdivision of genotypes SS | | | | |
| Within parents | 6 | 138.57*** | 1.39* | 12.67*** |
| Within hybrids | 11 | 120.60*** | 0.92 | 4.26*** |
| Between groups | 1 | 13.50 | 1.88 | 0.06 |
| Subdivision of hybrid SS | | | | |
| GCA green parents | 2 | 254.97*** | 1.49 | 11.20*** |
| GCA albino parents | 3 | 98.54* | 0.95 | 6.61** |
| SCA | 6 | 86.84** | 0.73 | 0.77 |
| Error *b* | 29 | 23.25 | 0.54 | 1.00 |

* Significant at the 5% level
** Significant at the 1% level
*** Significant at the 0.1% level

Thus, from the whole material, plants were regenerated from 14.5% of the embryos, 15.9% of the plants obtained were green, and 14.9 green plants were obtained on average per 100 cultured anthers.

The analysis of variance (Table 1) showed significant effects of genotypes for embryo formation and green plant percentage at the 0.1 and 1.0% level, respectively, while showing nonsignificance for regeneration. Effects of the two environments were significant only for percentage of green plants at the 5% level. This environmental effect was an improved percentage of green plants from the anthers of the field-grown material (15.1%) relative to the material from the growth chamber, where 9.9% of the plants obtained were green. Reciprocal effects and interactions between genotypes and environments were nonsignificant for all three response characters.

Subdivision of the genotype effects showed highly significant differences both within parents and within hybrids for embryo formation and green plant percentages. In addition, differences within parents for regeneration ability significant at the 5% level were revealed (Table 1). There were no indications of average difference between parents and hybrids for any of the three response characters (between group effects, Table 1).

From Table 2 it can be seen that the parents were well selected for the study, because all three "green-type parents" responded with high percentages of green plants between 27 and 52% and the four "albino-type parents" all produced low percentages of green plants between 0 and 7%. The sum of squares of parents for percentage of green plants when subdivided was shown to be caused

Table 2. Genotype means for embryo formation and green plant formation. Means not followed by the same letter are different according to Duncan's range test at the 5% level

| Embryo formation | | Green plant formation | |
|---|---|---|---|
| Genotype | Embryos/100 anthers | Genotype | Percent green plants |
| Triumph | 1,935 A | Igri | 51.6 A |
| Plaisant × Triumph | 1,139 B | Plaisant | 27.7 B |
| Igri × Grit | 1,104 B | Marinka | 26.8 B |
| Igri × Jupiter | 997 C B | Igri × Grit | 25.0 B |
| Igri × Sewa | 951 C B D | Igri × Sewa | 23.0 C B |
| Igri × Triumph | 914 C B D | Plaisant × Grit | 20.3 C B D |
| Sewa | 876 C E B D | Plaisant × Jupiter | 16.1 C E B D |
| Igri | 865 C E B D | Igri × Jupiter | 13.5 F C E B D |
| Marinka × Sewa | 775 C E B D | Plaisant × Sewa | 12.1 F C E B D |
| Grit | 755 C E B D | Igri × Triumph | 10.0 F C E G D |
| Marinka | 754 C E B D | Marinka × Sewa | 8.7 F C E G D |
| Plaisant × Jupiter | 735 C E B D | Marinka × Grit | 7.2 F E G D |
| Marinka × Triumph | 690 C E B D | Triumph | 6.7 F E G D |
| Jupiter | 576 C E F D | Plaisant × Triumph | 6.5 F E G D |
| Marinka × Jupiter | 516 C E F D | Grit | 5.7 F E G |
| Plaisant × Sewa | 465 E F D | Marinka × Jupiter | 4.5 F E G |
| Plaisant × Grit | 401 E F | Marinka × Triumph | 3.3 F H G |
| Plaisant | 318 F | Sewa | 2.0 H G |
| Marinka × Grit | 262 F | Jupiter | 0.0 H |

entirely by differences between the two types of parents, while differences within the two groups were nonsignificant. The group of parents with high percentage of green plants (average 35.4%) also had the superior average regeneration percentage (19.2%) compared to the group with low percentage of green plants (average 3.5%), from which 9.9% of the embryos on average regenerated plants. Also, it can be seen from Table 2 that the hybrids were generally intermediate to their parents with respect to green plant percentage.

The subdivision of hybrid variation into general combining ability (GCA) from the two types of parents and specific combining ability (SCA) (Table 1) demonstrated significant differences in both GCA and SCA for embryo formation, while only differences in GCA were significant for formation of green plants.

A study of all possible correlations between the three response characters, with analysis of covariance, revealed a negative correlation between embryo formation and green plant percentage, significant at the 1% level. However, subdivision of the covariance demonstrated this negative correlation to be due only to correlation within residuals.

## Discussion

The main conclusion from this study of albinism in barley anther culture from reciprocal crosses is very similar to the one obtained in wheat (Tuvesson et al. 1989). Apparently, all the genes controlling percentages of green plants in anther culture with this barley material are inherited chromosomally. Powell (1988) found a major reciprocal effect on percentage of anthers producing embryos, a character which was not measured here, in a 7 × 7 diallel analysis of response in barley anther culture. Smaller reciprocal effects significant at the 5% level were observed in the same study for green plants and albino plants obtained per 100 anthers. These characters are products of embryos per 100 anthers, regeneration percentage, and percentage of green/albino plants as measured in this investigation. A direct comparison of our results with those of Powell (1988) is therefore not possible; however, a discrepancy regarding the results concerning reciprocal effects in the two experiments is indicated. There is a possibility that the use of different barley material or different anther culture substrates for the two experiments could be the cause of such a difference. Another explanation could be that Powell (1988) did replications in the same environment which could lead to estimates of reciprocal effects biased with interactions between the plant material and the environment. It has been shown previously that such interactions constitute a major part of the variation, particularly for embryo formation in cereal anther culture (Andersen et al. 1987; Knudsen et al. 1989).

The major characteristics of the nuclear genes controlling anther culture response from this material are in accordance with the ones found for wheat (Tuvesson et al. 1989) and reported from other genetic studies of the response in barley anther culture (Dunwell et al. 1987; Powell 1988). The major genetic control is on the ability to produce embryos from the cultured anthers and to produce green regenerants from the embryos. The two genetic systems seem to be unrelated since no genetic correlations were observed.

One discrepancy in this study with barley from the results with wheat (Tuvesson et al. 1989) is the lack of detectable SCA for green plant percentage. Specific combining ability would be expected if a few major genes were responsible for the trait, as has been indicated in a previous report (Knudsen et al. 1989). Such major genes, however, would not produce SCA in the kind of cross study used here, if only one gene locus were involved or if the two types of parents, for other reasons, formed two genetically homogenous groups with respect to green plant formation. Another apparent difference from the wheat study is that the correlation between regeneration and percentage of green plants observed previously for wheat (Tuvesson et al. 1989) was not found in this study with barley. However, with the weak genetic differences for regeneration capacity in the barley material used for this study, such a correlation, if it exists, would pass undetected.

The choice of plant material for this experiment was based on selection of the lines that were best characterized with respect to embryo formation and green plant percentage in a previous study (Knudsen et al. 1989). From these results it was concluded that capacity for embryo formation from the anthers of this plant material is not genetically related to winter-spring habit, since several of the spring types investigated showed level of embryo formation comparable to the best winter varieties, such as 'Igri.' This phenomenon is clear also from the present study. For the percentage of green regenerated plants, the three winter varieties used as "green-type parents" in the present study were superior to most of the remaining barley varieties. However, several winter-type varieties (i.e., 'Gloria,' 'Sonja,' 'Tapir') were found by Knudsen et al. (1989) to have significantly lower capacity for green plant formation, similar to the level for the spring types. This led us to believe that capacity for green plant formation in barley anther culture is unrelated to winter-spring habit according to the methods used. The subsequent choice of spring × winter combinations in all of the crosses in this study was found to be justified because of the time-saving element of the program, since the hybrids needed no vernalization and winter-type parents could be vernalized during establishment of the hybrid seeds. The possible relationship between capacity for green plant formation and the winter-spring type character, however, may still need some further investigation.

Based on results with DNA hybridization, it has previously been reported that albino plants from anther culture of wheat and barley (Day and Ellis 1984, 1985) carry deletions in their chloroplast genomes. Such deletions could be the reason why these plants have lost their capacity to develop normal green chloroplasts in their leaves. The finding that nuclear genes control the formation of albinos in these anther cultures is not contradictory to the reports that deleted genomes in chloroplasts are the cause of albinism. The reason that the two types of parents have different capacity to produce green plants in anther culture, however, does not seem to be due to different genetic constitution in the chloroplast genomes. Somehow these nuclear genes affect the formation of changes in the chloroplast genomes either before or during the tissue culture, or they else affect the relative survival rate of changed cells or organelles during development. The understanding of the nature and mechanisms of action of these genes may assist in further improving the anther culture procedure (i.e., plant growing conditions, pretreatments, media) to obtain high percentages of green regenerants from all genotypes.

*Acknowledgements.* The authors wish to thank Dr. S. Knudsen for his advice and Mrs. E. Granberg, Miss H. Faarup, and Miss T. Ballin for their help in manipulating the cultures. Financial support from the Danish Agricultural and Veterinary Research Council is warmly acknowledged.

## References


Andersen SB, Due IK, Olesen A (1987) The response of anther culture in a genetically wide material of winter wheat (*Triticum aestivum* L.). Plant Breed 99:181–186

Day A, Ellis THN (1984) Chloroplast DNA deletions associated with wheat plants regenerated from pollen: Possible basis for maternal inheritance of chloroplasts. Cell 39:359–368

Day A, Ellis THN (1985) Deleted forms of plastid DNA in albino plants from cereal anther culture. Curr Genet 9:671–678

Dunwell JM, Francis RJ, Powell W (1987) Anther culture of *Hordeum vulgare* L.: a genetic study of microspore callus production and differentiation. Theor Appl Genet 74:60–64

Foroughi-Wehr B, Friedt W (1984) Rapid production of recombinant barley yellow mosaic virus resitant *Hordeum vulgare* lines by anther culture. Theor Appl Genet 67:377–382

Foroughi-Wehr B, Zeller FJ (1990) In vitro microspore reaction of different German wheat cultivars. Theor Appl Genet 79:77–80

Hunter CP (1987) Plant generation method. European Patent Application No. 0 245 898 A2, 1–8

Knudsen S, Due IK, Andersen SB (1989) Components of response in barley anther culture. Plant Breed 103:241–246

Kuhlmann U, Foroughi-Wehr B (1989) Production of doubled haploid lines in frequencies sufficient for barley breeding programs. Plant Cel Rep 8:78–81

Olsen FL (1987) Induction of microspore embryogenesis in cultured anthers of *Hordeum vulgare*. The effects of ammonium nitrate, glutamine and asparagine as nitrogen sources. Carlsberg Res Commun 52:393–404

Powell W (1988) Diallel analysis of barley anther culture response. Genome 30:152–157

Tuvesson IKD, Pedersen S, Andersen SB (1989) Nuclear genes affecting albinism in wheat (*Triticum aestivum* L.) anther culture. Theor Appl Genet 78:879–883

Ziauddin A, Simion E, Kasha KJ (1990) Improved plant regeneration from shed microspore culture in barley (*Hordeum vulgare* L.) cv igri. Plant Cell Rep 9:69–72