Induction of embryogenesis without exogenous hormone-supplement in barley microspore culture

(Short communication)

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SUMMARY

Induction of embryogenesis without exogenous hormone-supplement has been reported in isolated microspore cultures of wheat and triticale so far. Here we report about haploid embryogenesis and plant regeneration without exogenous hormone-supply in isolated microspore cultures of barley. In both induction media and both genotypes ('Igri' and 'Kymppi') applied, however, induction media containing 1 mg/l BAP lead to haploid embryoids of higher regeneration capacity than media without hormone-supplement. Highest rate of green plant regeneration (353 green plants/100,000 microspores) was achieved in cultivar 'Igri' using a medium of optimized N-composition, supplemented with BAP. These results suggest that the induction of haploid embryogenesis is possible without exogenous hormone-supplement, while high level of plant regeneration requires the stimuli provided by hormones/growth regulators added to the induction medium.

Key words: *Hordeum vulgare* L., barley, androgenesis, microspore culture

INTRODUCTION

In dicotyledonous species, exogenous hormone-supply is not essential for the induction of microspore embryogenesis (Nitsch, 1977, Touraev and Heberle-Bors, 1999). Among cereals, induction of androgenesis without exogenous hormone-supplement has been reported in anther cultures of barley (Cai *et al.*, 1992) and oat (Kiviharju *et al.*, 1997) as well as in isolated microspore cultures of wheat (Touraev *et al.*, 1996a) and triticale (Pauk *et al.*, 2000) so far. These results raise the question, whether exogenous hormones are essential for the induction of androgenesis and/or for plant regeneration in microspore culture. Reports on tobacco and wheat microspore cultures suggest that the signal for the induction of embryogenesis is provided by various stress factors during pretreatment. Further stimuli such as those given by exogenous hormones are not necessary (Touraev *et al.*, 1996a,b). Induction of androgenesis in hormone-free media may indirectly confirm the proposed decisive role of stress signals in switching microspores from gametophytic to sporophytic development. On the other hand, the evaluation of plant regeneration rates in cultures induced with or without exogenous growth regulators can elucidate the promoting role of hormones in haploid embryogenesis.

Here we report about induction of embryogenesis in isolated microspore cultures of barley without exogenous hormone-supplement. The effects of hormone-supplemented and hormone-free induction media on haploid embryogenesis and plant regeneration was studied in two cultivars. Two basic media, among them that successfully used earlier for triticale microspore culture have also been tested in barley.

MATERIALS AND METHODS

Microspores of the barley cultivars 'Igri' and 'Kymppi' were isolated by microblending of cold-pretreated spikes as described elsewhere (Pauk *et al.*, 2000). Isolated microspores of 'Igri' were cultivated in N24A2.7G3 (further referred to as N24-BA) medium as well as in modified 190-2 medium, both with and without hormone supplement (Fig. 1). N24-BA is a medium of optimized nitrogen composition established exclusively for microspore culture of barley, containing 1 mg/l BAP (Mordhorst and Lörz, 1993). Hormone-free N24-BA is designated as N24-0. 190-BA and 190-0 media - with and without BAP-supplement, respectively - are based on 190-2 medium (Zhuang and Jia, 1983). 190-0 medium gave superior plant regeneration results in microspore culture of certain triticale genotypes. In other genotypes its derivatives supplemented with growth regulators gave the best result (Pauk *et al.*, 2000). In another series of experiments isolated microspores of the cultivars 'Igri' and 'Kymppi' were induced in N24-BA medium as well as in 190-0 medium (Fig. 2). Plants were regenerated on hormone-free LA_3 medium (Mordhorst and Lörz, 1993).

RESULTS

High level of embryogenesis has been observed in each culture of both 'Igri' and 'Kymppi'. The number of embryoids was 76 (190-0), 35 (N24-BA), 22 (N24-0) and 11 (190-BA) at the first transfer to solid induction media ('Igri'; means of two experiments). Haploid embryoids were further treated as bulk, thus their total number was not determined.

Figure 1. shows green plant production from microspore cultures of 'Igri' induced in four different media. Due to the high values of CV% (>100%), statistical analysis of data was not possible (data not shown). Mean values are given for information only (Fig. 1.). Induction on N24-BA medium resulted in the highest number of green plants (75 plants/100,000 microspores). The 190-0 medium resulted in the lowest number of green plants (6.5 plants/100,000 ms). Application of N24-0 and 190-BA media led to green plants at similar level (10 and 9.86 plants/100,000 microspores, respectively).

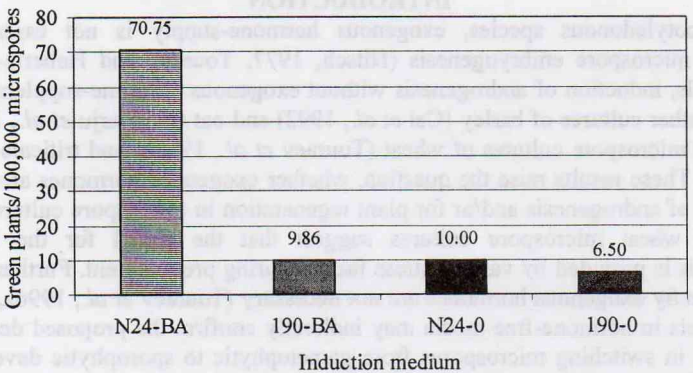

Figure 1. Effect of different induction media on the green plant regeneration in isolated microspore culture of cultivar 'Igri'.
Means of eight, seven, six and four experiments from left to right, respectively. Values of standard deviation (s) are 78.45, 8.61, 18.84 and 10.69 from left to right, respectively.

The evaluation of N24-BA and the hormone-free 190-0 media was performed in separate experiments. Figure 2. shows that the number of regenerated green plants was nine-fold higher for cultivar 'Igri' (294.33) compared to cultivar 'Kymppi' (33.75), if microspores were induced in the N24-BA medium. In the case of 190-0 medium, however, there was no

significant difference between the two cultivars (11.00 and 11.67 green plants/100,000 ms for 'Igri' and 'Kymppi, respectively). Thus, the regeneration rate of 'Igri' achieved on N24-BA medium is 27-fold higher than on 190-0 medium. For 'Kymppi', however, only a 3-fold difference between the regeneration rates achieved on the two media could be observed.

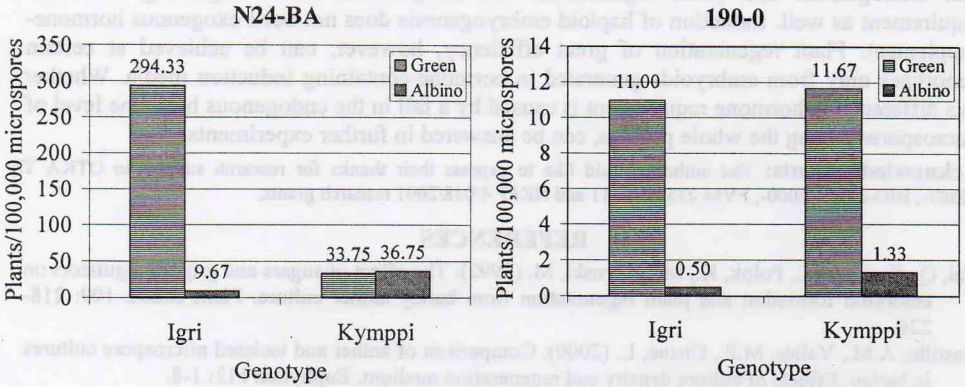

Figure 2. Plant regeneration from microspore cultures of cultivar 'Igri' and 'Kymppi' in N24-BA and 190-0 induction media.
N24-BA: Means of three ('Igri') and four ('Kymppi') experiments. Values of standard deviation (s) are 53.31, 1.70, 12.34 and 10.40 from left to right, respectively.
190-0: Means of two ('Igri') and three ('Kymppi') experiments. Values of standard deviation (s) are 3.0, 0.5, 10.78 and 1.89 from left to right, respectively.

DISCUSSION

The role of hormones, if any, in the induction of androgenesis is less understood, while their role during embryo development has been found to be more evident (for review, see Sangwan and Sangwan-Norreel, 1990). *Gramineae*, such as barley, triticale and wheat, are known to belong to the group of plants, which require hormones in the induction medium of anther cultures. Anthers of this group, however, can also exhibit response if induced in hormone-free medium in the first stage of culture (for review, see Dunwell, 1985).

Haploid embryoids were induced and green plantlets were regenerated from microspore cultures of barley, without exogenous hormone-supplement in the induction and regeneration media. The regeneration rate achieved this way was significantly lower compared to media containing hormones, at both cultivars and media tested. Barley anthers responded similarly to induction media with or without hormone-supplement, but hormone-free medium gave rise to calli of lower regeneration capacity (Cai *et al.*, 1992). Moreover, the auxins indole-3-acetic acid and naphthalene-acetic acid added to the regeneration medium resulted in more vigorous green plants in barley microspore culture (Castillo *et al.*, 2000). In contrast, a positive effect of hormone-free induction medium on embryoid yield has been detected in oat anther culture (Kiviharju *et al.*, 1997) and in triticale microspore culture (Pauk *et al.*, 2000). This is in full concordance with the results reported here, and suggests that induction of androgenesis does not require exogenously added hormones, but hormones can be essential for plant regeneration at great efficiency.

The theory of hormone-free induction of androgenesis can be further explained by the recently suggested role of stress as the single factor required for switching microspores from gametophytic to sporophytic pathway (for review, see Heberle-Bors, 1998). This proposal is

based on the identical conditions required for the pretreatment of tobacco and wheat anthers/spikes prior to isolation of microspores (Touraev *et al.*, 1996a,b).

Androgenesis and plant regeneration are considered to be independent events of different genetic background (Knudsen *et al.*, 1989, Mordhorst and Lörz, 1993). Our results suggest that androgenesis and plant regeneration are independent events regarding hormone-requirement as well. Induction of haploid embryogenesis does not need exogenous hormone-supplement. Plant regeneration of great efficiency, however, can be achieved at certain genotypes only from embryoids generated in hormone-containing induction media. Whether this difference in hormone requirement is caused by a fall in the endogenous hormone level of microspores during the whole process, can be answered in further experiments.

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