Gelrite and Agar Differently Influence Cytokinin-Sensitivity of a Moss

B. HADELER, S. SCHOLZ, and R. RESKI*
Institut für Allgemeine Botanik, Ohnhorststr. 18, 22609 Hamburg, Germany

Received November 11, 1994 · Accepted January 20, 1995

Summary

Physcomitrella patens wild-type as well as its cytokinin-sensitive differentiation mutant PC22 exhibit premature as well as enhanced bud formation when grown on basal medium gelled with Gelrite compared to plants grown on basal medium gelled with agar. Thus, cultivation on Gelrite mimics cytokinin-treatment of mosses. However, plants could only respond to exogenous cytokinin when grown on agar-medium but were inhibited by the growth regulator when grown on Gelrite. These effects could not be mimicked by the addition of inorganic salts, nor by the addition of glucose or rhamnose to agar-gelled basal media.

Key words: Physcomitrella patens (Hedw.)B.S.G., cell differentiation, cytokinin, plant regeneration.

Abbreviations: i°C = i°C (Δ^2-isopentenyl)adenine; p.i. = past inoculation; WT = wild-type; w/v = weight per volume.

Introduction

Mosses are widely used to study the morphogenic potential of different external and internal stimuli (Bopp, 1981; Cove and Knight, 1993). For example, Hahn and Bopp (1968) demonstrated that Funaria hygrometrica specifically reacts to cytokinins with the transition from growth by an apical cell to differentiated growth via a three faced apical cell, the bud. Tempting to unravel the molecular mechanisms of cytokinin action, we concentrate on a moss, Physcomitrella patens (Hedw.)B.S.G. (Reski and Abel, 1985), utilizing cytokinin-sensitive differentiation mutants (Abel et al., 1989). Nevertheless, interactions between cytokinins and several internal and external factors are well documented (Reski et al., 1991; Reski, 1994). We report here that the gel-lant strongly influences cellular differentiation and cytokinin-sensitivity of in-vitro plant cell cultures.

Materials and Methods

Plant material and culture conditions

Physcomitrella patens (Hedw.)B.S.G. wild-type and mutant PC22 have been characterized previously (Abel et al., 1988; Reski et al., 1991; Rother et al., 1994; Reski et al., 1994). Plants were grown under sterile conditions on Knop medium (0.25 g/L KH₂PO₄, 0.25 g/L KCl, 0.25 g/L MgSO₄ × 7H₂O, 1.0 g/L Ca(NO₃)₂ × 4H₂O, 12.5 mg/L FeSO₄ × 7H₂O; pH 5.8; Reski and Abel, 1985) at 25 ± 1°C under a light-dark regime of 16:8 h with 47 μmol photons × m⁻² × s⁻¹. Detailed culture conditions have been described recently (Reski et al., 1994). Eight media have been analysed: «Agar» (Knop medium solidified with 1% w/v agar, Oxoid, Basingstoke, England, Code Nor. L28), «Gelrite» (Knop medium solidified with 0.33% w/v Gelrite, Roth, Karlsruhe, Germany, Code No. 0039), «+ salts» (Knop medium plus 3.6 g/L AlCl₃, 17.9 mg/L Ca(NO₃)₂ × 4H₂O, 74.1 mg/L KCl solidified with 1% w/v agar, s.a.), and «+ sugars» (Knop medium plus 0.5% w/v glucose, Merck, Germany, and 0.5% w/v rhamnose, Merck, Germany, solidified with 1% w/v agar, s.a.). These media were either used directly, or with a supplement of 5 × 10⁻⁴ M N⁴(Δ^2-isopentenyl)adenine (indicated as

* Correspondence.

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«+ i^8 Ade»). The number of buds per protonema were counted using an inverse light microscope. Reliable figures could be obtained up to 35 buds per protonema. The figures presented are mean values from 10 independent plants plus standard deviations where applicable.

Results and Discussion

The wild-type started bud-production at day 11 past inoculation on basal medium gelled with agar. When cultivated on Gelrite, bud-production started two days earlier and more buds per protonema were produced (Fig. 1a). WT on agar plus cytokinin showed massive budding starting 3 days p.i. and reaching uncountable amounts only 3 days later. Cytokinin applied to basal medium gelled with Gelrite likewise induced premature budding. However, the number of buds were even smaller than on media without cytokinin (Fig. 1b). As mutant PC22 is impeded in bud formation, it reacts more slowly as the wild-type upon Gelrite or cytokinin, but essentially in the same manner (Figs. 1 c, d). Gelrite is widely used in tissue culture, because several tissues exhibit promoted growth and differentiation on media solidified with Gelrite when compared to those solidified with agar of different brands (e.g. Ichii et al., 1986; Zimmerman and Robacker, 1988). According to Bornman and Vogelmann (1984) this phenomenon might be due to a better cytokinin uptake, as Gelrite is used in lower concentrations than agar to gel media. However, whereas cytokinins and auxins are routinely used in in-vitro cultures of higher plants, the «Gelrite-effect» was detectable even with basal Knop medium devoid of any phytohormones (Fig. 1a), indicating that physical or chemical properties of the gellant itself have morphogenic effects. Two observations reveal, that this may not be a cyto-
kinin contamination: a) On basal medium gelled with Gel-
rite protonemata produce massively leafy shoots. Applica-
tion of cytokinin led to reduction of the protonemal phase
and thus a decrease in the number of shoots. In contrast, on
agar supplemented with cytokinin, buds did not develop
into shoots, but became callus-like necrotic. b) The decrease
in buds/protonema on Gelrite plus cytokinin may be inter-
preted as a result of an overoptimal cytokinin concentration.
However, these cultures likewise exhibited the cytokinin-effect
of premature budding (compare Figs. 1 a and 1 b).

Scherer et al. (1988) stated that the «Gelrite-effect» on tis-
tue growth is not due to differences in the content of several
inorganic cations, although the amount of these salts are
rather high in this gellant. As a reinvestigation, we calculated
the main differences in inorganic salts of the two gellants,
supplied Knop medium with the additional salts, and gelled
it with agar. As Gelrite is a heteropolymer of glucose and
hammanose, we, likewise, added those sugars to Knop medium
and gelled it with agar. 1% glucose enhanced whereas 1% rhamnose retarded plant growth and differentiation.
However, supplied simultaneously, the retarding effect of rham-
nose predominated in WT as well as in PC22. The addition
of salts slightly enhanced premature budding, but could not
mimic the «Gelrite-effect» (Figs. 2a, c). Moreover, cytokinin
could induce premature budding in both media and in both
genotypes, although the retarding effect of rhamnose was
still discernable (Figs. 2b, d).

The effects observed in this study may not be due to a pro-
moting effect of Gelrite but to an inhibitory effect of agar.
Such contaminates may exist (e.g. Kohlenbach and Wer-
nicke, 1978) and may also inhibit moss differentiation, as the
addition of activated charcoal to agar-gelled basal media leads
to premature budding in Funaria (Bopp and Klein, 1971).
However, our results clearly demonstrate that choice of the
gellant markedly influences cytokinin-sensitivity of mosses.
Above that, they demand caution when analyzing phytohormone
action or performing mutant screens in in-vitro cultures of any plant species. Therefore, since that report from
Reski and Abel (1985) we favour agitated liquid cultures
when analyzing cytokinin effects on moss protonemata.

Acknowledgements

We are indebted to Dres. H. Tantau and J. M. Orsini for valuable
discussions. This work was supported by the Commission of the
European Union as part of EUROMOS.

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