In Vitro Cell. Dev. Biol.—Plant 33:195–199, July–August–September 1997

1071-2690/97 $05.00 + 0.00

GELRITE AS AN ALTERNATIVE TO AGAR FOR MICROPROPAGATION AND MICROTUBERIZATION OF *SOLANUM TUBEROSUM* L. CV. BARAKA

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(Received 11 September 1996; accepted 26 February 1997; editor M. A. L. Smith)

SUMMARY

Phytagel™ allowed the production of longer internodes, faster *in vitro* tuberization, and larger tubers in *Solanum tuberosum* L. cv. Baraka as compared to Difco Bacto-agar during both an 8-h photoperiod or in darkness. It also allowed a higher tuberization percentage in the dark. Only a 0.2% (wt/vol) Phytagel allowed optimal micropropagation and microtuberization under the photoperiod regime used. Water availability does not account for the observed differences in growth and tuberization between media containing the above gelling agents. In consequence, Phytagel appears as an advantageous alternative to agar for micropropagation and microtuberization.

Key words: Gelrite; *in vitro*; micropropagation; potato; tuberization.

INTRODUCTION

There is a common tendency in plant biological research in general, and in tissue culture in particular, to routinely repeat protocols and recipes published for other plant materials and experimental systems, assuming that such protocols might still yield optimum results. In this sense, agar has routinely been used as a gelling agent.

However, agar from different sources contains various amounts of contaminants (Debergh, 1983; Pierik, 1987; Scherer et al., 1988) that may contribute to increase experimental errors and make comparisons between different results more difficult.

The gelling agent "Gelrite" (the trade name) is a gellan gum PS-60 isolated from the bacterium *Pseudomonas elodea*. It is a linear polysaccharide, composed of glucuronic acid, glucose, and rhamnose that requires the presence of either monovalent or divalent cations for gelation to occur. Gelrite is free from phenolic compounds but has a higher ash content than agar (Scherer et al., 1988).

It has been reported to yield better results than agar in: regeneration and shoot multiplication (Henderson, 1987; Goldfarb et al., 1991; Van Ark et al., 1991; Welander and Maheswaran, 1992), production of somatic embryos (De Wald et al., 1989; Yeh and Chyuan, 1992), and callus growth (Ichi et al., 1986; Huang and Chi, 1988), but it may favor vitrification (Ladyman and Girard, 1992). Gelrite has been considered an alternative to agar (Fisher and Bessler, 1988).

The purpose of our study was to determine whether the select grade of Gelrite, Phytagel™ (Sigma Chemical Co., St. Louis, MO), which produces colorless and high strength gels, is a favorable alternative to agar for *in vitro* micropropagation and tuberization of potato currently used in germplasm processing and potato breeding programs.

MATERIALS AND METHODS

Plant material. Single-node stem sections of potato (*Solanum tuberosum* L. cv Baraka) cultured *in vitro* were used throughout the experiments. Protocol for tuber sprouting and culture initiation was as previously described (Mingo-Castel et al., 1991) with the following changes. Potato tubers were surface-sterilized with a 1% (vol/vol) NaClO, 0.1% (vol/vol) Tween-20 solution for 5 min, and rinsed with water.

Tissue plugs of 2 cm in diameter and 2.5 cm thick, each containing a single bud, were individually excised from the tuber with the aid of a cork borer. Plugs were incubated in plastic trays containing moist vermiculite in the dark and sprayed with $Ca(NO_3)_2 \cdot 4H_2O$ (27.7 μM). Single-node sprout sections were aseptically cultured in Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) containing 2% (wt/vol) sucrose and 0.8% (wt/vol) Difco Bacto-agar. Cultures were kept at 20° C under 16 h daily photoperiod and a 70 $\mu mol\ m^{-2}s^{-1}$ photosynthetic photon flux (PPF).

Micropropagation. Single-node sections were aseptically cultured in MS medium containing 2% (wt/vol) sucrose and one of the following gelling agents: 0.8% (wt/vol) Difco Bacto-agar, 0.2% (wt/vol) or 0.3% (wt/vol) Phytagel. Considerably less Phytagel is needed to achieve equivalent physicochemical gel properties (Huang et al., 1995). Twenty ml of medium were dispensed in 125 × 25 mm culture tubes and closed with Bellco Kap-uts. Culture conditions were similar to those previously described. After 2, 3, and 4 wk of culture, the following parameters were measured: sprout length, number of usable nodes (nodal sections 2 mm or more in length), and number of roots. Twenty-four explants were cultured for each treatment. Two explants were planted per tube. The experiment was repeated twice, and the mean value of the data is reported.

Tuberization. Single-node sections were cultured in MS medium containing 6% (wt/vol) sucrose, 11.6 μM kinetin, and one of the gelling agents previously mentioned. Cultures were maintained at 20° C either under continuous dark or 8 h daily photoperiod providing a PPF of 70 $\mu mol\ m^{-2}s^{-1}$. Cultures were incubated for 8 wk. Tuberization percentage (number of explants with at least one microtuber/total number of explants) was measured weekly. After 8 wk, the following parameters were measured: microtuber fresh and dry weights, presence or absence of roots, and fresh weight of basal callus in the explant. Twenty-four explants were cultured for each treatment. The experiment was performed twice, and the mean value of the data is reported.

Relative matric potential and gel expressibility measurements. The relative matric potential of gelled media was measured as previously described (Owens and Wozniak, 1991), with the following changes: air-dry filter paper discs of 21 mm diameter were used to overlay the gel surface in culture tubes (125 × 25 mm) containing 20 ml of medium; incubation was performed at 20° C for about 24 h. Measurements of the liquid expressed from the gel were done as previously described (Owens and Wozniak, 1991).

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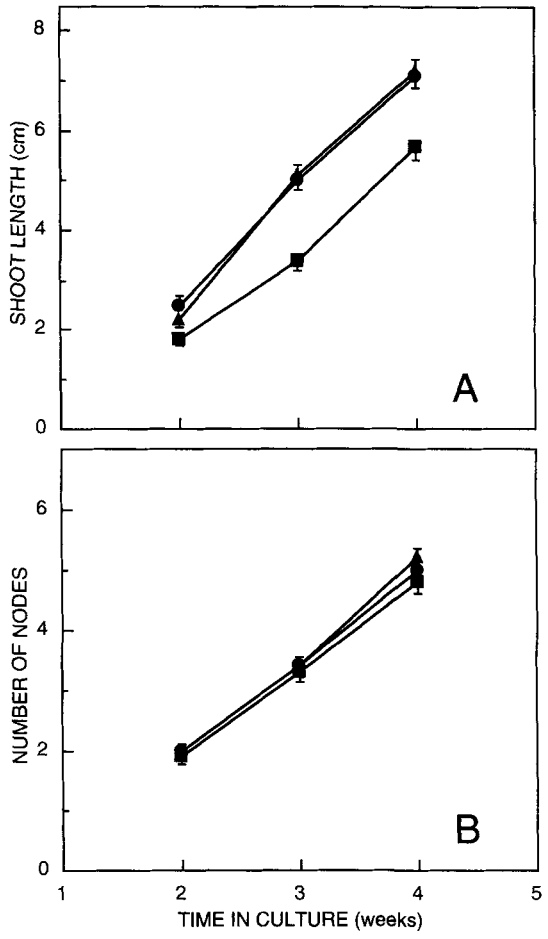

FIG. 1. Effect of gelling agents on shoot length (*A*) and number of usable nodes of potato (*B*): 0.8% (wt/vol) Bacto-agar (■), 0.2% (wt/vol) Phytagel (●), 0.3% (wt/vol) Phytagel (▲). Values are means of two experiments with 24 explants each. *Bars* stand for standard errors.

RESULTS

Micropropagation. Shoot length of explants cultured in Phytagel was significantly higher than that of those cultured in Difco Bacto-agar (Fig. 1 *A*). The difference of growth could be observed after 2 wk in culture. The difference in length, after 4 wk, was 1.5 cm. There were no significant differences between 0.2 and 0.3% Phytagel.

Unlike shoot length, the number of nodes was nearly the same throughout the culture (Fig. 1 *B*). After 4 wk, the average number of nodes in 0.3% Phytagel was 5.2 against 4.8 in Bacto-agar. No significant differences were observed. It follows that Phytagel promotes growth by increasing internode length, without causing any changes in the number of nodes. The number of roots per explant was significantly lower in Phytagel than in Bacto-agar (Table 1).

Tuberization under 8 h photoperiod. Earlier tuberization was obtained in Phytagel (Fig. 2 *A*). After 3, 4, and 5 wk of culture, the tuberization percentage was significantly higher in Phytagel. After 4 wk of culture, tuberization was not observed with Bacto-agar while 35% tuberization was measured with Phytagel. A tuberization percentage of 50% was reached 1 wk in advance in Phytagel as compared to Bacto-agar. However, after 8 wk, tuberization was similar for all three treatments (differences were not significant).

Microtubers obtained in Phytagel were larger than those obtained in Bacto-agar (Fig. 3). Also, fresh and dry weights (FW, DW) of microtubers cultured in Phytagel after 8 wk were significantly higher than those cultured in Bacto-agar (Table 2). Tuber fresh weight was fivefold in 0.2% (wt/vol) Phytagel as compared to Bacto-agar. No differences were observed between the results obtained with 0.2 and 0.3% (wt/vol) Phytagel. No hyperhydration symptoms whatsoever were observed in Phytagel grown material.

TABLE 1

EFFECT OF THE GELLING AGENT ON ROOTING DURING MICROPROPAGATION

Gelling Agent	Number of Roots/Plant;[1]
0.8% Bacto-agar	3.4 ± 0.1c
0.2% Phytagel	2.4 ± 0.1a
0.3% Phytagel	2.9 ± 0.1b

[1]Values are the mean ± standard error after 4 wk of growth in micropropagation medium, computed from two experiments with 24 explants each. Treatments without a letter in common are significantly different at $P < 0.05$ according to least significant difference (LSD) test.

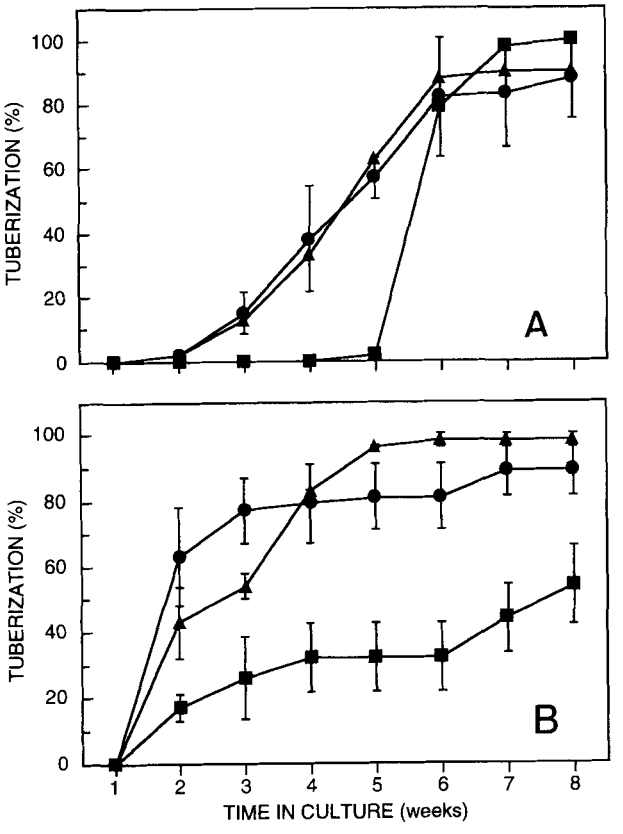

FIG. 2. Effect of gelling agents on microtuberization of potato in light (*A*) and in the dark (*B*): 0.8% (wt/vol) Bacto-agar (■), 0.2% (wt/vol) Phytagel (●), 0.3% (wt/vol) Phytagel (▲). Values are means of two experiments with 24 explants each. *Bars* stand for standard errors.

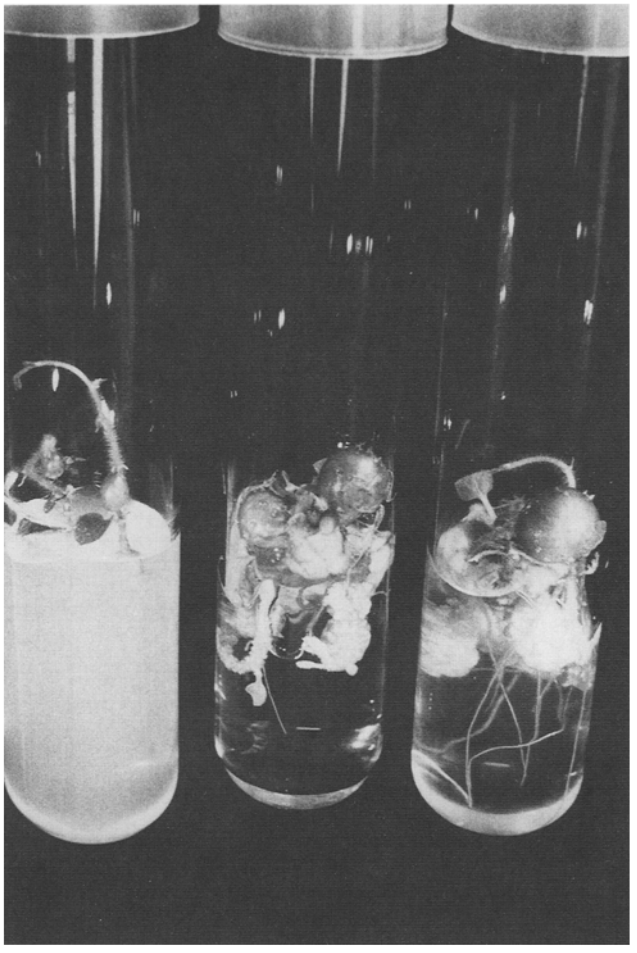

FIG. 3. Tuberized explants of potato under 8 h photoperiod after 8 wk of culture. From left to right: 0.8% (wt/vol) Bacto-agar, 0.2% (wt/vol) Phytagel, 0.3% (wt/vol) Phytagel.

TABLE 2

EFFECTS OF GELLING AGENT AND PHOTOPERIOD ON FRESH WEIGHT (FW) AND DRY WEIGHT (DW) OF MICROTUBERS AFTER 8 WK IN CULTURE

Photoperiod	8 h[1]		Dark[1]	
Weight (mg/tuber)	FW	DW	FW	DW
0.8% Bacto-agar	36 ± 2.5a	8 ± 1.4a	7 ± 0.7a	1.0 ± 0.6a
0.2% Phytagel	180 ± 25.0b	36 ± 5.3b	18 ± 1.2b	3.1 ± 1.3b
0.3% Phytagel	130 ± 1.4b	25 ± 2.5b	29 ± 1.4c	5.2 ± 1.7c

[1]Values are the mean ± standard error from two experiments with 24 explants each. Within each column, treatments without a letter in common are significantly different at $P < 0.05$ according to least significant difference (LSD) test.

White friable callus developed over the base of the explants. The mass of callus developed in Phytagel was larger than that in Bacto-agar (Fig. 3). Fresh weight of callus in Phytagel was approximately three times higher than in Bacto-agar (60 mg and 20 mg, respectively). Despite callus production, sprouts grew vigorously and tuberization percentage was not affected.

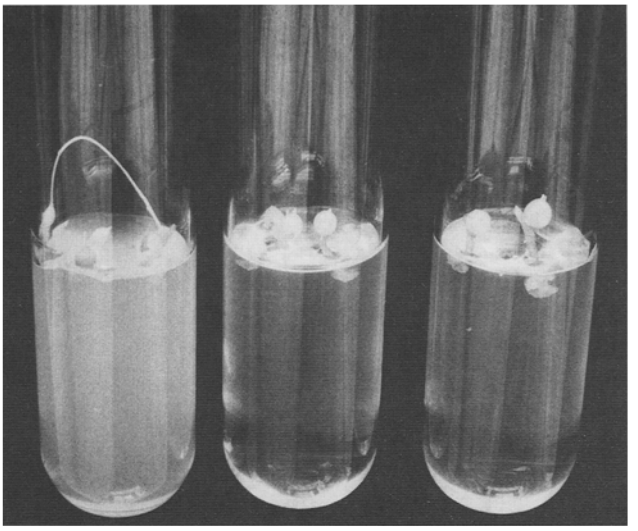

FIG. 4. Tuberized explants of potato in the dark after 8 wk of culture. From left to right: 0.8% (wt/vol) Bacto-agar, 0.2% (wt/vol) Phytagel, 0.3% (wt/vol) Phytagel.

Another difference observed with Phytagel was rooting ability. A 17% rooting occurred in Phytagel (there were not significant differences for both Phytagel concentrations), whereas no rooting was observed in Bacto-agar.

Tuberization in the dark. Tuberization in Phytagel was always higher and earlier than in Bacto-agar (Fig. 2 *B*). During Weeks 4, 5, 6, 7, and 8, the tuberization rate was significantly higher in 0.3% (wt/vol) Phytagel than in Bacto-agar. A tuberization percentage of 50% was reached 6 wk in advance in Phytagel as compared to Bacto-agar. No significant differences were observed between 0.2 and 0.3% (wt/vol) Phytagel throughout the culture.

Microtubers obtained in Phytagel were larger than those obtained in Bacto-agar (Fig. 4). Fresh and dry weights of microtubers cultured in Phytagel after 8 wk were significantly higher than those cultured in Bacto-agar (Table 2), the higher concentration of Phytagel being the most favorable. Tuber FW was 2.5-fold in 0.2% (wt/vol) Phytagel as compared to Difco Bacto-agar. No hyperhydration was observed in Phytagel-cultured material.

A very low amount of callus appeared over the base of the explants. No differences were observed between treatments. Neither in Bacto-agar nor in Phytagel was root development observed during tuberization.

DISCUSSION

Phytagel allowed the production of longer internodes, which is a clear advantage over agar with slow-growing cultivars that are difficult to multiply (i.e., cv. Baraka). Phytagel also allowed faster *in vitro* tuberization and larger tubers as compared to agar for both the 8 h photoperiod or dark treatments, and also higher tuberization rates in the dark.

No differences in shoot growth or microtuberization were observed for the two concentrations of Phytagel except for a slight increase in FW and DW of tubers developed in the dark, and a small increase in the number of roots during micropropagation in 0.3% (wt/vol) Phy-

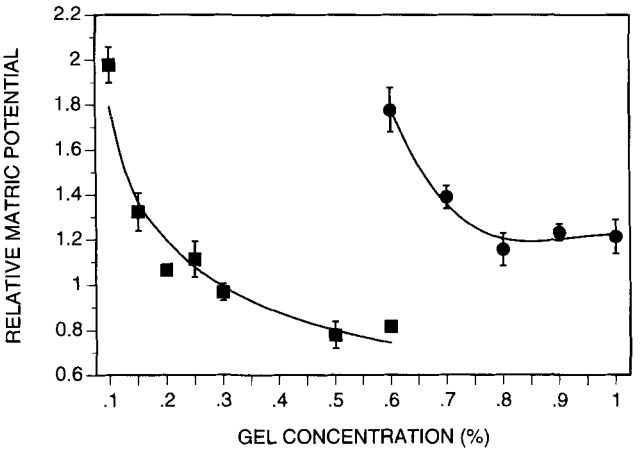

FIG. 5. Relation of relative matric potential to gel type (Phytagel -■-, Bacto-agar -●-) and concentration. Values plotted are means of three measurements. *Bars* (shown when larger than symbol) stand for standard errors.

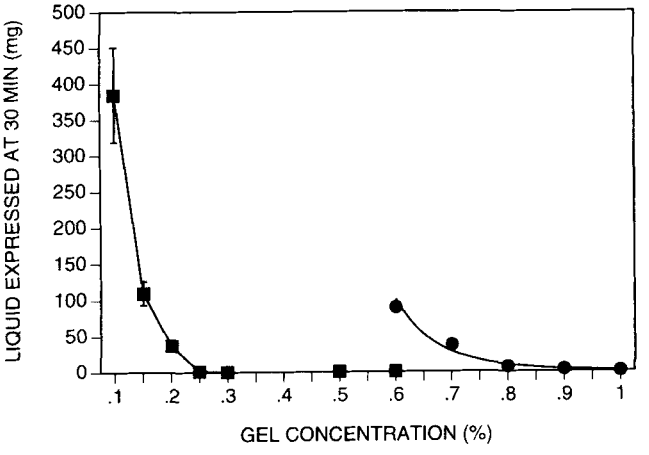

FIG. 6. Relation of gel expressibility to gel type (Phytagel -■-, Bacto-agar -●-) and concentration. Values plotted are means of three measurements. *Bars* (shown when larger than symbol) stand for standard errors.

tagel. Overall, 0.2% (wt/vol) Phytagel seems sufficient when using a photoperiod.

Under 8 h of photoperiod, root development occurred in Phytagel but not in agar. Callus growth over the base of the explant under 8 h of photoperiod had no adverse effect on either shoot growth or tuberization.

Hyperhydration has been the main drawback reported in the use of Gelrite media, especially in woody and some ornamental plants (Zimmerman and Cobb, 1989; Zimmerman et al., 1995), and hydric control agents have been developed to prevent it (Nairn et al., 1995). It has been recently reported (Huang et al., 1995) that vitrification, or hyperhydricity, was observed in both Gelrite and agar media and that it may be minimized by increasing the gel concentrations. In potato micropropagation and tuberization, we did not observe any hyperhydricity symptoms under any light treatment.

It is interesting to note that in two physiological processes triggered by cytokinins such as moss protonema bud differentiation (Hahn and Bopp, 1968) and *in vitro* tuberization of potato (Palmer and Smith, 1969), Gelrite as compared to agar seems to change the tissue sensitivity to cytokinins (Hadeler et al., 1995). Hadeler found evidence that the bud protonema differentiation may not be due to differences in salt, glucose, or rhamnose concentrations. The "Gelrite effect" would rather be attributable to physical or chemical properties of the gelling agent itself. Alternatively, the differences observed between agar and Gelrite may be due to growth inhibitory effects of agar-borne impurities.

Comparing the production of embryos and shoots obtained in 0.3% Gelrite versus 0.9% Bacto-agar (Owens and Wozniak, 1991) to results on shoot elongation and tuberization obtained in 0.3% Gelrite versus 0.8% Bacto-agar (Figs. 5 and 6), it turns out that the lower the water availability (produced by a lower relative matric potential plus liquid expressibility), the higher the growth responses. An increase of 0.05 in relative matric potential (same liquid expressibility) between 0.2% versus 0.3% Gelrite (Owens and Wozniak, 1991) produced a larger disc expansion and a stronger morphogenic response (embryo plus shoots) in sugarbeet. However, in our work, an even larger increase in matric potential (0.09) plus a higher liquid expressibility (Figs. 5 and 6) did not result in different shoot elongation or tuberization in potato (Figs. 1 and 2). Water availability for 0.8% Bacto-agar is in between the corresponding values for 0.2% and 0.3% Phytagel (Table 3), and yet 0.8% Bacto-agar results in the lowest growth and tuberization response.

TABLE 3

WATER AVAILABILITY OF THREE GELLED MEDIA[1]

Gelling Agent	Water Not Held (mg)	Water Expressed (mg)	Water Available (mg)
0.8% Bacto-agar	201.3 ± 12.1	7.3 ± 1.3	208.7 ± 10.9
0.2% Phytagel	189.5 ± 5.0	37.8 ± 3.9	226.5 ± 10.2
0.3% Phytagel	167.7 ± 7.6	0.5 ± 0.4	168.1 ± 7.9

[1]Values in the second column represent water available due to matric potential; values in the third column represent water available by expression in response to mechanical deformation of the gel by the explant. Water available is the sum of columns two and three.

Therefore, it follows that water availability (as determined by relative matric potential and liquid expressibility) does not account for the observed differences in growth and tuberization in potato between Gelrite and agar-gelled media. Differences may be attributable to other gel properties.

Our results support recent reports on the effects of Gelrite on tuberization (Nowak and Asiedu, 1992) and extend the application of Phytagel to potato micropropagation.

The use of Phytagel allows remarkable savings. The agar of an MS culture medium accounts for some 67% of its cost (Fischer and Bessler, 1988). Comparing the costs for the concentrations of agar 0.8% (wt/vol) and Phytagel 0.2% (wt/vol) most commonly used, Phytagel is eight to nine times cheaper than Difco Bacto-agar. Phytagel was found to produce exceptionally clear culture media, which was convenient for morphological observations and for the detection of contamination or chemical exudation.

In consequence, it follows that the use of Phytagel in cv. Baraka is advantageous in potato micropropagation, exchange, conservation, and physiological or molecular biology studies of tuberization.

ACKNOWLEDGMENTS

This work was supported by a grant from CICYT (Spain).

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