

## Corn starch as an alternative gelling agent for plant tissue culture

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**Abstract.** Growth and differentiation of plant cell cultures was increased when media were gelled with corn starch instead of agar. Dry weight of tobacco and wild carrot cell cultures on media gelled with starch was more than three times that of cultures on media gelled with agar. Higher yield of anthocyanin and dry weight of embryos were found in wild carrot cultures grown on media gelled with corn starch. The starch-mediated increase in growth and differentiation of wild carrot cells was accompanied by an increase in density of the cultures shown by higher dry weight/fresh weight ratios.

### Introduction

Agar is the most frequently used gelling agent for plant tissue culture media. It has desirable characteristics of high gel clarity, stability, and resistance to metabolism during use. Agar has been considered biologically inert, although this belief has increasingly been questioned [1, 2, 3]. In anther culture of *Nicotiana tabacum*, inhibitory effects of agar, resulting in embryo abortion, have been reported [4].

Sorvari [5, 6, 7] has investigated starches from barley, corn, potato, rice and wheat as alternative gelling agents to agar. Embryo formation from barley anthers was greater on media gelled with starch than on agar-solidified media. Of the starches examined, barley was the most effective. Frequency of plantlet production from anthers cultured on media with barley starch was five times higher than from anthers on agar-solidified media. Subsequently Sorvari showed that shoot differentiation from potato tuber discs was obtained within three weeks after inoculation on media gelled with barley starch. In contrast, other investigations have found that 5–14 weeks of culture was necessary for potato shoot formation on agar media.

In our studies, cell cultures of tobacco and wild carrot have been grown on media gelled with agar and corn starch to determine if Sorvari's observations could be confirmed and whether secondary product formation was improved on starch-gelled media.

## Materials and methods

### *Cultures*

The origin of cultures of wild carrot (*Daucus carota* L.), cell line WC 63-2, and the culture media used to study anthocyanin accumulation and embryogenesis has been described by Kinnersly & Henderson [8]. The same reference describes the origin and culture conditions for suspension cultures of *Nicotiana tabacum* cv. Burley 21.

### *Starch*

Corn starch, Corn Products Code 3005, was used in the studies with tobacco cell cultures. This starch is essentially food-grade corn starch available at most supermarkets. A 60 fluidity acid-modified corn starch, Code 5061, was derived from the same material, but with a reduced molecular weight. This was prepared by treating 162 g of 3005 starch in an equal volume of water with 4.5 ml 12 N HCl for 6–8 h at 128 °F. The mixture was then neutralized with NaOH to a final pH of 4.4–4.6, filtered through # 1 Buchner funnel, and air-dried. This starch was used in the experiments with wild carrot cell cultures.

### *Corn syrup*

Globe® 1632 corn syrup was obtained from CPC International, Englewood Cliffs, New Jersey. A description of this syrup is provided in ref. [8].

### *Preparation of culture media*

Difco Bacto-agar (Difco Laboratories, Detroit, Michigan) at 0.9% w/v was used as a control in all experiments. Starches were made into slurries and added to media before adjusting volume and pH. Media were dispensed 20 ml per culture tube (25 mm diameter, 150 mm long). An automatic media pump and dispenser such as a manostat-veristatic pump (Manostat Corp., NY) is ideal for handling this material. Tubes were autoclaved at 121 °C for 15 min, slanted and cooled at room temperature. Tubes were inoculated with 2.0 ml aliquots of suspension culture from which media had been removed. Cultures were stored in the dark at room temperature. Embryogenic cultures were exposed to 16 h photoperiods from G.E. cool-white fluorescent lights with an irradiance of  $40 \mu\text{E m}^{-2} \text{s}^{-1}$ .

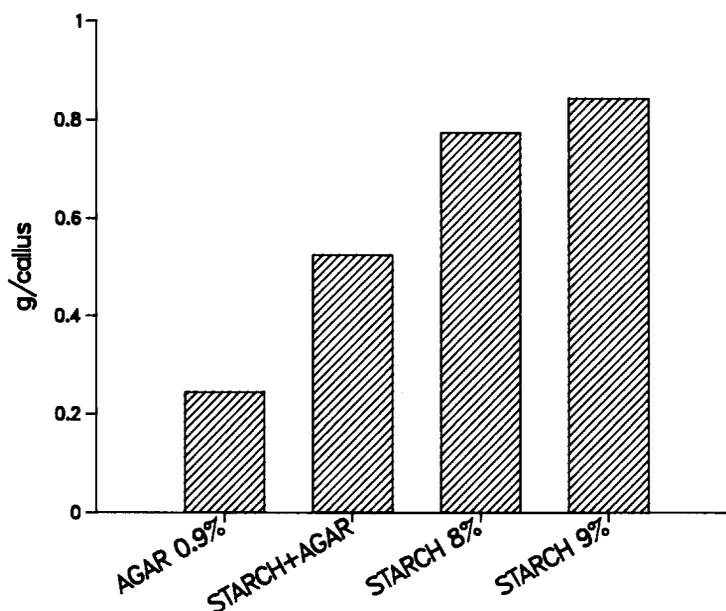
### *Analytical procedures*

Anthocyanin accumulation in wild carrot cultures was determined as described by Kinnersley & Dougall [9] with the following modification. Following determination of calli fresh weight, the tissue was suspended in a volume of extractant equal to twice the callus weight. Embryogenesis was studied by determining the dry weight of embryogenic cultures. Measurements of dry weight in embryogenic cultures of wild carrot have been shown to be directly correlated with embryo number [10].

### **Results**

The effects of 3005 corn starch on dry weight of tobacco cells after six months culture are shown in Fig. 1. The starch-agar mixture consisted of 0.45% w/v agar + 3.5% w/v starch. Results show an increase in culture dry weight with increasing levels of starch. On media gelled with 8% and 9% starch, dry weight was more than 3 times greater than on agar-gelled medium.

A similar starch-mediated promotion of cell dry weight was found in wild



*Fig. 1.* Dry weight of tobacco callus cultures after 6 months growth on media solidified with corn starch, agar, and a starch (3.5% w/v)-agar (0.45% w/v) mixture. Each bar is the average weight of 10 replicates.

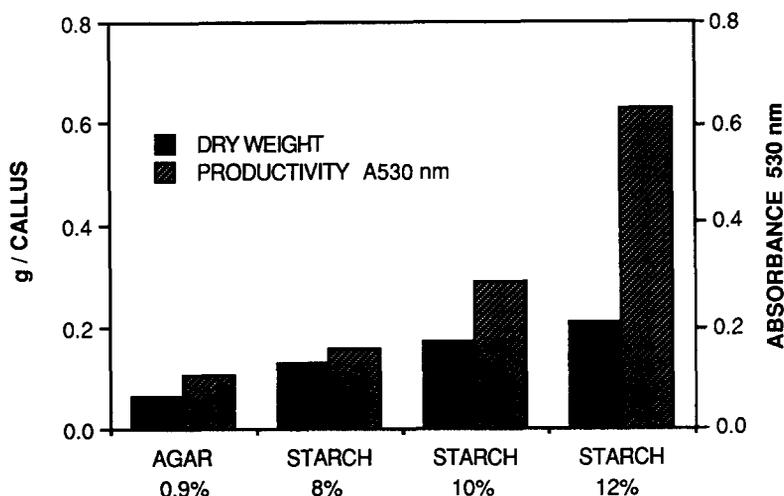


Fig. 2. Effect of acid-modified corn starch and agar on dry weight and pigment production in callus cultures of wild carrot. Each bar is the average of 5 replicates.

carrot cultures inoculated with suspension tissue and grown for 78 days (Fig. 2). Gels were easier to prepare and handle with acid-modified starch than with the 3005 starch used above. An increase in anthocyanin accumulation that was accompanied by an increase in cell dry weight was found with increasing levels of starch. Cultures grown on 12% starch had 3 times the growth and almost 6 times the anthocyanin content of cells grown on agar. The increases in growth and pigment formation was accompanied by an increase in density of the cultures. Fresh weight of agar-grown cells was 25 times the dry weight. Fresh weight was only 10 times the dry weight of cells grown on media gelled with 12% starch (Table 1).

Embryogenic cultures of wild carrot showed a similar increase in dry weight and density when grown for 106 days on media gelled with acid-modified starch (Fig. 3). Dry weight of cultures was further increased when corn syrup was substituted for sucrose in the culture media. Previous studies

Table 1. Effect of acid-modified corn starch on wild carrot dry and fresh weight.

| Starch<br>(% w/v) | Agar<br>(% w/v) | FW <sup>1</sup><br>(g/callus) | DW <sup>2</sup><br>(g/callus) | FW/DW <sup>2</sup> |
|-------------------|-----------------|-------------------------------|-------------------------------|--------------------|
| 0.0               | 0.9             | 1.66 + 0.25                   | 0.067 + 0.12                  | 24.9 + 0.7         |
| 8.0               | 0.0             | 2.12 + 0.24                   | 0.130 + 0.019                 | 16.3 + 0.6         |
| 10.0              | 0.0             | 2.06 + 0.11                   | 0.172 + 0.018                 | 12.0 + 0.6         |
| 12.0              | 0.0             | 2.11 + 0.13                   | 0.210 + 0.022                 | 10.1 + 0.5         |

<sup>1</sup> Mean, s.d. of 10 replicates

<sup>2</sup> 5 replicates

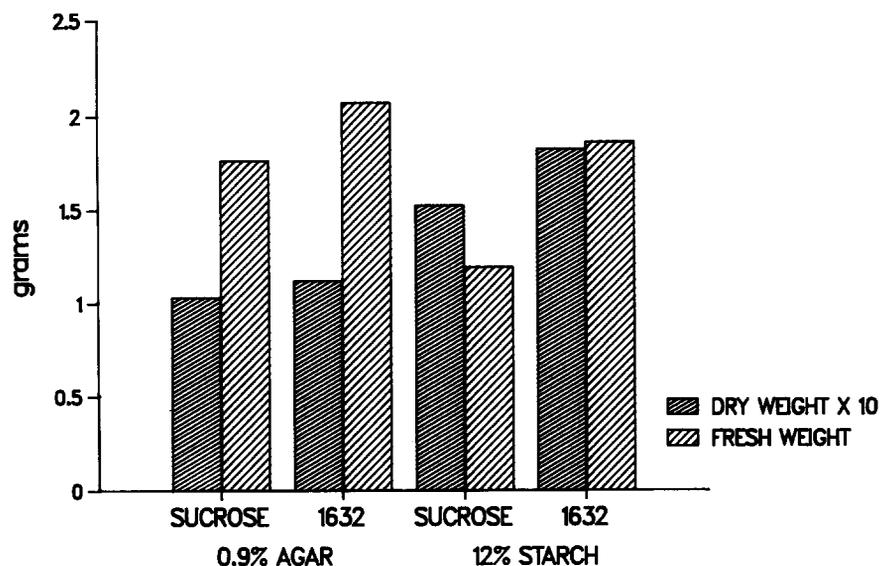


Fig. 3. Effect of carbon source and gelling agent on fresh and dry weight of embryogenic wild carrot cultures. Sucrose and 1632 corn syrup were added to medium at  $20 \text{ g l}^{-1}$ . Each bar is the average of 10 replicates.

with alternative carbohydrates [8] showed that growth of embryogenic suspension cultures was increased when sucrose in the culture media was replaced with corn syrup. Culture fresh weight was not promoted by starch. With 12% starch, fresh weight of embryogenic cultures was less with both carbohydrates as compared to cultures on agar-gelled media.

## Discussion

Experiments reported herein confirm Sorvari's observations on the benefits of starch-gelled media. Unorganized cell growth in tobacco, dry weight of embryogenic cultures and anthocyanin accumulation in cultures of wild carrot were greatly improved on starch-gelled media.

It is unusual to find large increases in both growth and productivity of secondary metabolites from plant cultures. Growth and productivity are more often inversely related [11, 12].

A potential difficulty with using starch as a gelling agent is the softness of the media. Using 5% corn starch, Sorvari [5] found it necessary to use polyester nets to prevent tissue from sinking in the weakly solidified media. In the present study, best growth and pigment productivity was found with 12% starch. At this level there was no problem with solidification of the

media. Stable gels capable of fully supporting tissue were not consistently formed at starch levels of 7% or less. Acid-modified starch, with reduced molecular weight, dispensed more readily than regular corn starch, and produced good gels at 10% w/v (not shown). Handling problems associated with use of starch as a gelling agent can be solved by choice of properly modified starch and by use of appropriate equipment.

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