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Grain growth in wheat ears cultured in saccharose solution

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Abstract

Ears of uniculm wheat (*Triticum aestivum* L. cv. Gigas) grown in liquid medium for 11 d absorbed more solution when the saccharose concentration was 2 % than when it was10 %. When the ears were grown in 6 % saccharose solution, the rate of uptake from the solution was between that from the 2 and 10 % saccharose medium. Dry mass per grain increased with the saccharose concentration in the medium and the reduced uptake of solution did not decrease the moisture percentage of the grain. The culture of ears decreased pH of the solution with 2 % saccharose more than with 10 %. Addition of 0.5 % chloramphenicol to the culture solution had no adverse effect on grain mass; it prevented contamination of the solution and maintained a higher pH.

Additional key words: absorption, chloramphenicol, pH.

Introduction

There is a close interrelationship between source and sink for photosynthetic assimilates. Investigation of the two as separate entities within the intact plant is difficult. The culture of detached ears of wheat in solutions of saccharose, the main sugar translocated in phloem, has been performed in studies on the accumulation of dry matter and nitrogen in grain (Jenner 1968, Donovan and Lee 1977, Barlow *et al.* 1983, Singh and Jenner 1983) as well as on the regulation of grain numbers (Waters *et al.* 1984). Barlow *et al.* (1983) and Waters *et al.* (1984) observed that increases in the saccharose concentrations of the medium are accompanied by a decreased uptake of solution.

The approach of liquid culture has been used in experiments of a few hours duration with wheat grains (Jenner 1974). However, such short-term studies may well be dominated by transitory artifacts related to wounding and osmotic shock. Long-term culture in liquid media (several days or more), should minimize these influences. Long-term culture is more suitable for whole grains and short term culture for endosperm slices or homogenates and isolated amyloplasts or starch granules.

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Gifford and Bremmer (1981) reported a decline in pH of the medium by about 0.2 units along a 3-d experiment and no significant influence of pH on saccharose uptake or starch synthesis when the initial value ranged between 5.5 and 7.2. The change with time in the pH of media with varying saccharose concentrations has not been examined clearly so far in relation to grain growth.

The aim of the present work on wheat ear culture were (*1*) to find out the cause of the decreased uptake of solution when the saccharose concentration in the medium is increased, and its possible effect on grain growth; (*2*) to examine the inpact of chloramphenicol, used to prevent microbial contamination of the medium, on grain growth and pH of the solution.

Materials and methods

Plant and ear culture: The wheat (*Triticum aestivum* L.) uniculm cultivar Gigas was raised in earthen pots under natural conditions. NPK were applied at the recommended doses. Anthesis took place on 2nd February, 1990. Fifteen days later, shoots with 8 to 12 spikelets per ear were selected for liquid culture.

Detached ears were cultured for 11 d following the procedure of Donovan and Lee (1977) with some modifications. Stems were cut at the base, surface sterilized by wiping with a 10 % sodium hypochlorite solution after removing the flag leaf blade and cut again just below the last node (Singh and Jenner 1983) under sterile distilled water. Flasks (100 cm^3) with the Hoagland nutrient solution and containing three ears were placed in a growth chamber (irradiance 500 $\mu mol\ m^{-2}\ s^{-1}$, photoperiod 14 h, day/night temperature and relative humidity 20/10 °C and 65/80 %, respectively). Three different concentrations of saccharose were used and the amino acid mixture was substituted by NH_4NO_3 (0.1 %) as the nitrogen source as reported by other authors (Donovan and Lee 1978, Waters *et al* 1984). Chloramphenicol (0.5 %) was added to the solution in all experiments except in the control (distilled water). The pH was adjusted to 5.5 with KOH.

Experimental design: To examine the effect of saccharose concentration on solution uptake, the first set of experiments compared ears cultured in the presence of 2, 6 and 10 % of saccharose. The flasks contained 20 cm^3 of solution which was renewed daily, after recording the change in volume. In the second set of experiments, the flasks contained 100 cm^3 of medium with 10 % of saccharose and with or without chloramphenicol. The pH of the solution was recorded on days 7 and 10. In the third set of experiments, the three saccharose levels in the medium (2, 6 and 10 %) were compared with the numbers of grains per ear. The culture media, 20 cm^3 per flask were renewed daily, and the volume and pH recorded. All the sets of experiments were performed in duplicate. The fresh and dry mass of glumes and rachis and remaining grains and grain numbers per ear were also recorded.

Results

Uptake of solution: Volume of solution taken up was highest from 2 % saccharose solution and decreased with increasing saccharose concentration. After one day of culture in 2 % saccharose solution, the uptake rate of solution was 0.094 mm^3 s^{-1} $flask^{-1}$. It declined gradually, thereafter. In 10 % saccharose solution the uptake was approximately 32 % less than from 2 % saccharose solution. In 6 % saccharose solution, the volume of solution taken up by the ears was intermediate of the two. The volume of solution taken up by the ears also fell progressively with time (Table 1).

Table 1. Uptake rate of solution by ears cultured in media with different concentrations of saccharose (2, 6 and 10 %).

Saccharose [%]	Uptake rate [mm^3 s^{-1} $flask^{-1}$] 1 d	2 d	3 d	4 d	5 d	9 d	10 d
2	0.098	0.094	0.094	0.081	0.060	0.031	0.024
6	0.086	0.061	0.056	0.046	0.042	0.019	0.015
10	0.070	0.057	0.043	0.038	0.033	0.038	0.012
SE ($P < 0.05$)	0.012	0.021	0.013	0.012	0.007	0.007	0.004

Growth of detached wheat ears: Increase in dry mass of the grains was greater in solution with 10 % saccharose than with 2 % saccharose and intermediate in 6 % saccharose solution. There was gradual increase in the grain mass upto day 10. The dry mass of glumes and rachis in 2 % saccharose solution was maximum at day 7. Thereafter, it declined slowly upto day 10. In 10 and 6 % solution the dry mass of glumes and rachis increased upto day 7 and 10 of culture, respectively (Table 2).

Table 2. Effect of different concentrations of saccharose (2, 6 and 10 %) in the medium on growth of detached wheat ears.

Culture age [d]	Saccharose [%]	Grain [mg(d.m.) ear^{-1}]	Glumes and rachis [mg(d.m.) ear^{-1}]	Grain [mg(d.m.)]
0		647.5	506.0	25.7
3	2	869.0	506.5	29.3
	6	775.0	574.0	31.3
	10	749.0	462.0	30.2
7	2	884.5	524.0	35.5
	6	847.5	545.0	34.6
	10	767.5	533.0	35.9
10	2	881.0	515.0	37.9
	6	843.5	562.5	39.3
	10	718.1	613.5	42.5

Differences were not significant at $P < 0.05$.

The dry mass of grain increased in all the treatments progressively with time. In 2 % saccharose solution it was 25.7 mg at day 0 and 37.9 mg at day 10. Similar pattern of grain mass increase was observed at 10 and 6 % saccharose (Table 2).

Nitrogen content: The nitrogen percentage and total nitrogen content increased in all the treatments with time of culture. In 2 % saccharose concentration, nitrogen percentage varied from 1.52 to 2.34 from day 0 to day 10. Similarly, at 10 % saccharose it varied from 1.54 to 2.14 % from day 3 to day 10 (Table 3).

Table 3. Nitrogen and protein percentage in grains of detached wheat ears cultured in 2, 6 and 10 % saccharose solution.

Age [d]	Saccharose [%]	Nitrogen [%]	Protein [%]
0		1.52	9.47
3	2	2.01	12.57
	6	1.78	11.09
	10	1.54	9.59
7	2	2.17	13.53
	6	2.10	13.10
	10	1.97	12.28
10	2	2.34	14.72
	6	2.23	13.94
	10	2.14	13.35
CD ($P < 0.05$)		0.32	2.00

Effect of chloramphenicol: Contamination of the medium deprived of chloramphenicol was evident after 6 d of culture, while it was not observed when the antibiotic was present. Neither dry mass of grain or glumes and rachis nor the grain mass per ear were affected by the presence of the antibiotic. In the absence of chloramphenicol, there was a marked decline in pH attributable to presence of the microorganisms (Table 4).

Table 4. Effect of the addition of chloramphenicol to the liquid medium with 2 % of saccharose on the pH of the solution and growth of detached wheat ears.

Age [d]	Chloramphenicol	Grain [mg(d.m.)]	Grain [mg(d.m.) ear^{-1}]	Glumes + rachis [mg(d.m.) ear^{-1}]	Solution pH
7	+	35.25	689.0	507.5	4.52
	-	35.80	726.0	525.0	3.88
10	+	37.80	804.5	534.0	4.87
	-	36.75	778.0	513.0	2.69

Differences in growth were not statistically significant, for solution pH CD ($P < 0.05$) for culture age was 1.09, for effect of chloramphenicol 1.09 and for interaction 1.53.

Discussion

Growth rates of grains in liquid medium with saccharose measured in the present study during the course of 11 d were comparable to those found by Donovan and Lee (1977) and Singh and Jenner (1983) and to those observed for samples originating from intact plants (Martinez-Carrasco and Thorne 1979).

The decreased uptake of saccharose from the medium with its increasing concentration observed in the present study and data reported previously (Barlow *et al.* 1983, Waters *et al.* 1984) suggested that the capacity of grains to absorb saccharose limits uptake. Large increase in mass of glumes and rachis was probably due to an accumulation of carbohydrates, which could restrict photosynthesis, stomatal aperture and thus transpiration and water uptake (Barlow *et al.* 1983). Both the duration and the rate of grain growth were affected by the supply of saccharose per grain, originating in raising the saccharose concentration in the solution or in decreasing the number of grains per ear. The shortening of the growth period of grains at 2 % saccharose in the medium could be due to an insufficient supply of saccharides to allow continued growth, though the influence of some other factors cannot be excluded. The saturation response of the growth rate and thus the grain mass to the supply of saccharose points that grain has a growth dependent on carbohydrate availability and a limited capacity to store dry matter, as found in intact plants (Brocklehurst 1977, Martinez-Carrasco and Thorne 1979, Radley and Thorne 1981) and in detached ears or grains in saccharose solution (Gifford and Bremmer 1981, Barlow *et al.* 1983).

The extent to which nitrogen translocated to the ear was converted to grain protein under different saccharose concentrations has been estimated using the wheat ear culture technique (Donovan and Lee 1978). It has been shown from our studies that, with cultured ears, nitrogen nutrition does not play a major role in grain dry mass accumulation. This is in accord with the commonly held view that application of nitrogenous fertilizer to crop after heading results in little or no increase in yield but does give grain with higher protein content. The cultured ears described in this experiment were not grown to maturity although, in the absence of a stress situation, the pattern of grain nitrogen accumulation once established, tends to remain unchanged throughout the remainder of development. It seems, then reasonable to assume that the pattern of accumulation of grain nitrogen established in the ear culture experiments would have been maintained through to maturity, and that almost all of the nitrogen would ultimately exist in the grain as proteins.

The decrease in pH of the solutions during ear culture may be partially attributed, to the maintenance of internal pH in plant tissues and to the uptake of mineral nutrients (Raven and Smith 1973). The presence of chloramphenicol did not affect grain growth, though the antibiotic maintained a higher pH value in the solution. Addition of chloramphenicol to medium appears to be as efficient as sterilization to prevent microbial contamination and certainly simplifies the culture technique.

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