SIGNIFICANCE OF SUGARS ON CALLUS INDUCTION OF PARSLEY ENDOSPERM

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Despite a considerable amount of studies on establishment of endosperm callus originated from mature seeds[1,4,9], relatively little attention has been directed to a role of carbon source in the initial medium on which the seeds were placed for germination. In a previous paper[6], it has revealed that endosperm tissues of parsley seed sloughed off from embryo axis during germination were readily capable of inducing callus formation if the seeds were germinated on nutrient medium. The fact prompts us to further evaluate physiological significance of sugar availability in the initial germination medium as related to the callus induction of parsley endosperm tissues.

Material and Methods

Seedlings of parsley (*Petroselinum hortense* Hoffm. cv. Green Ace) were aseptically grown and handled as previously[6]. For the germination of parsley seeds 100-ml Erlenmeyer flasks containing 30 ml of solid Murashige-Skoog medium (MS medium) with 3% sucrose were employed at an initial pH of 5.8[7]. The seeds were allowed to germinate on the medium until the end of 4th week when the germination rate reached its maximum. The endospem tissues being enveloped by seed coat were still survived at this stage if the tissues were situated in contact with the surface of the medium. These viable endosperms with seed coat were removed from embryo axis and transferred to the solid hormone-free MS medium for callus proliferation. The proliferation of callus from the transferred endosperm tissues was visible within 4 weeks of subculture. All cultures were conducted in a growth chamber at 26°C with a continuous irradiation of fluorescent light at ca 1000 lux (Toshiba, Type FLR 40 S/M) and each experimental treatment consisted of at least 15 cultures.

Results and Discussion

Effect of sugars: With the intention of studying on what components in the MS medium are required to induce callus proliferation of parsley endosperm tissues, the following experiment was performed by modifying ingredients of the germination medium as follows: A) complete MS medium, B) MS medium omitted nitrogen and potassium source, C) MS medium omitted all mineral elements except nitrogen and potassium, D) 0.09 M sucrose alone. As showing in Table 1, the endosperm tissues appeared to require the presence of sucrose in the germination medium by all means to induce callus proliferation in the succeeding culture, while neither mineral elements nor vitamins were necessary for it. Although a possible availability of sucrose as carbon source of callus induction is suggested, it cannot be excluded an alternative possibility that sucrose may produce a beneficial effect on the callus induction through osmotical adjustment of medium. The seeds were allowed to germinate on a medium osmotically secured by the addition of polyethylene glycol 6000 (Wako Chemicals Ltd. Japan) in place of sucrose. The result, given in Figure 1, reveals that if osmotic value of medium was regulated by polyethylene glycol there was no significant improvement of callus induction as compared to that by sucrose. In considering a documented fact that the reserve food in endosperm was digested and utilized during germination, it can be concluded that there was either too little or insufficiently available carbon source to support induction of callus growth. Accordingly, the result described above supports the assumption that an external supply of sugar as a carbon source is prerequisite for the callus incuction of parsley endosperm during its germination period. Subsequently, if we used several kinds of sugar as carbon source for the callus induction, it

TABLE 1. Effect of germination medium on callus induction of parsley endosperm

Medium	Callus induction (%)*
Control: Distilled water	2.7
A: MS basal medium	36.4
B: MS basal medium free from N and K	33.3
C: 0.09 M sucrose with N and K	45.3
D: 0.09 M sucrose	41.0

* Callus induction was measured at the end of 4th week subculture after transferred to hormone-free MS medium.

should be expected some differences in efficiency of individual sugars. The results presented in Table 2 show that sucrose was a most pronounced efficiency for callus induction, followed by glucose and fructose. Maltose exerted similar but less effect as compared to the other sugars stated above, while galactose produced no appreciable effect.

Effect of intact embryo: Although a role of embryo in the callus induction of mature endosperm is at present a matter of speculation, some factor(s) may be contributed by the germinating embryo which is essential

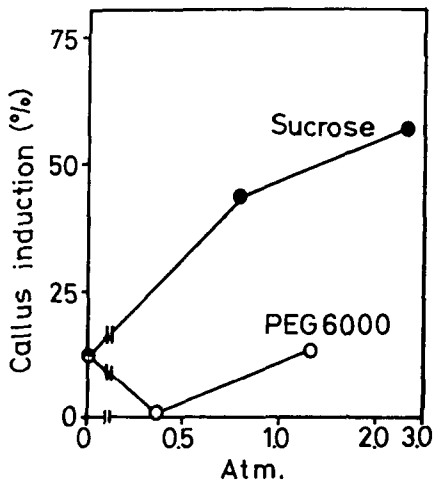

Fig. 1. Comparison of callus induction of parsley endosperm at different osmotic values of the germination medium. Each osmolarity of medium was adjusted by using sucrose and polyethylene glycol 6000 (PEG 6000). Percentage of callus induction was counted at the same time as showing in Table 1.

TABLE 2. Comparison of response of callus induction to sugars supplemented to germination medium

Sugar*	Callus induction (%)**
sucrose	42.9
maltose	17.9
glucose	24.2
fructose	21.2
galatose	2.9

* All sugars were added at 0.03 M into water agar medium.

** Percentage of callus induction was counted at the same time as shown in Table 1.

for the activation of mature caster bean[2]. In view of the reports that the embryo releases gibberellin-like substances during germination[3,8], these substances seem to be invoked as explanation of the embryo factor(s). In fact, BHOJWANI[5] reported that gibberellic acid was able to replace the embryo factor for inducing proliferation of mature endosperm of *Croton bonplandianum*. It would be interesting to see, therefore, whether or not the intact germinating embryo of parsley seeds will be required for induction of proliferation from mature endosperm. When the seeds were carefully cut into transverse pieces with or without intact embryo and each group of the pieces was explanted separately, the cultures of the half-split endosperm with the embryo resulted in callus induction. In contrast, the other halves of endosperm without embryo failed to be incited the induction even if being supplied exogenously with gibberellic acid at different concentrations (10^{-4}, 10^{-5} and 10^{-6}M). The results have an important bearing on the fundamental problem which governs the success of endosperm callus culture, suggesting that some unknown factors other than gibberellin may be anticipated since the intact embryo is required to induce the endosperm callus. Although conclusion as to the presence of embryo factors must be drawn with caution, of interest is the idea that a physiological role of intact embryo of parsley seed seems to be different from the other plant stated above, in spite of the similarity in the pattern of the callus induction.

Summary

The presence of sugar in the medium where parsley seeds were placed to germinate was prerequisite for inducing endosperm to proliferate callus during subculture in hormone-free Murashige-Skoog medium, and sucrose was most efficient sugar for the callus induction. The induction of callus appeared to be imposed by the intact embryo during its germination and a role of the embryo could not be replaced by application of gibberellin. Thus, not only exogeneous application of sugar but some endogenous factor(s) owing to the intact embryo were found to be indispensable for this callus induction.

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