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WHEAT CALLUS CULTURE: INDUCTION AND GROWTH OF CALLUS DERIVED FROM CARYOPSES*

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Abstract

A method for rapid callus induction and subculturing is described. Using this direct method, a number of calli from one single caryopsis of wheat can be obtained for rapid multiplication and building up of stocks for various studies.

Introduction

Callus and organ formation in wheat through isolated embryo and explants has been reported (Shimada *et al.*, 1969; Dudits *et al.*, 1975; Chin & Scott, 1977; O'Hara & Street, 1978; Sears & Deckard, 1982; Ozias-Akins & Vasil, 1983) but there still is a need for a simple and rapid method for callus induction and its multiplication. In our programme for developing salt tolerant plant, through selection of tolerant lines of callus and or cells, studies were carried out to explore the possibility of generating callus directly from germinating wheat caryopses.

Materials and Methods

Caryopses of *Triticum aestivum* L. (cv. H–68 and Punjab–81) were transferred to flasks containing 70% ethanol and placed for 2 minutes on a shaker (100 oscillation/min) The alcohol was then decanted, replaced with 0.1% mercuric chloride and after 5 min. shaking the caryopses were washed three times with sterilized distilled water in a laminar flow cabinet.

Two basal media Gamborg's B5(Gamborg *et al.*, 1968) and MS(Murashige & Skoog, 1962) (50 ml in 250 ml Erlenmeyer flasks) were supplemented with 1 mg/l 2,4-

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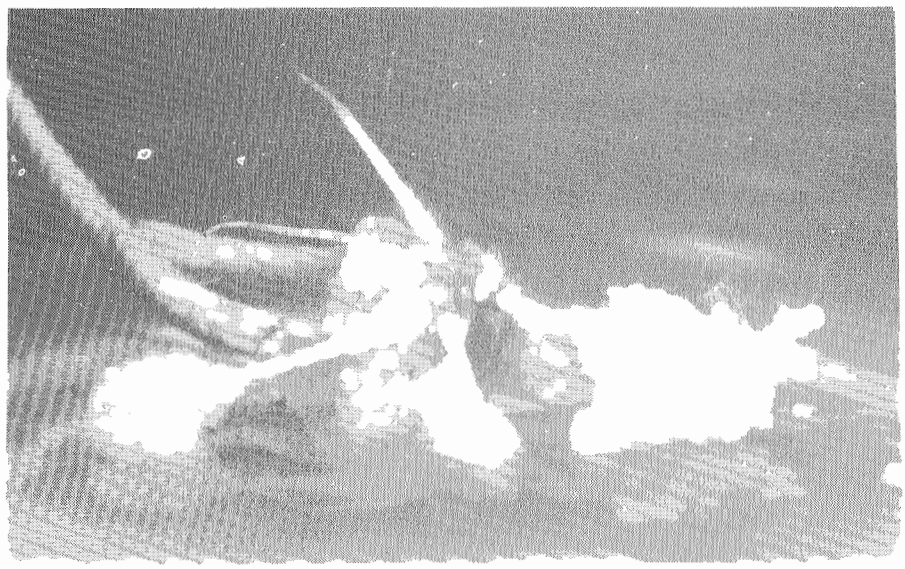

Fig. 1. Induction of callus in roots by direct caryopsis planting (X 2.5)

dichlorophenoxyacetic acid, adjusted to pH 5.5 and 5.7, respectively, and sterilized at 15 lb in^{-2} for 30 min. Sterilized caryopses were aseptically transferred over the solidified surface of 1/2 B5 or MS media and incubated at 25 ± 2°C in either 8 hr light (500 lux, fluorescent tube light) or in complete darkness.

To develop callus from embryo (scutellum), caryopses from current harvest were used. After sterilization the caryopses were aseptically transferred onto 1/2 B5 or MS media and the flasks were placed in a refrigerator (4–6°C) for 48 hr. before they were transferred into the incubator. Other conditions of incubation remained identical.

Results and Discussion

The caryopses readily germinated within 2 days and seedlings were established in 4 to 5 days. After about 10 days, callus formation was visible from the meristematic region near the root tips. A week later, callus formation was also observed at the junction between root and shoot and all of them proliferated with the passage of time (Fig.1). These calli were usually compact and reached the maximum of their growth in 4–5 weeks. The shoot system remained alive for 5–6 weeks and then eventually died. Both the media were found to induce callus but 1/2 B5 was comparatively better than MS.

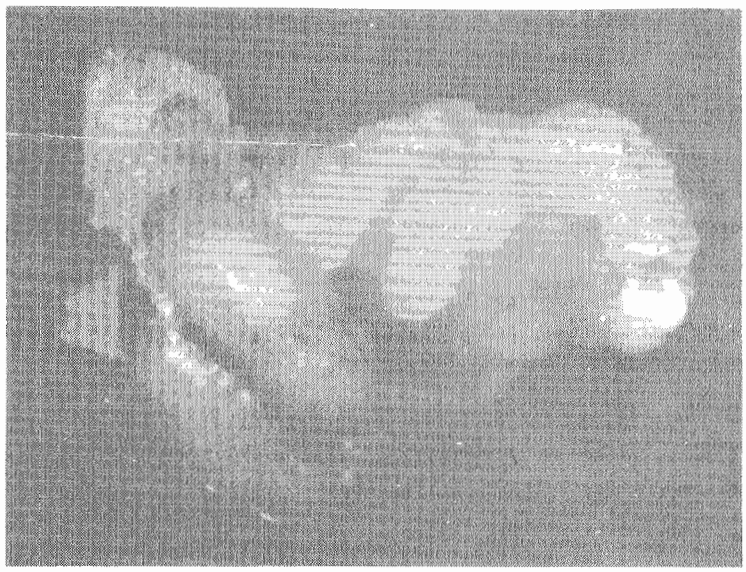

Fig. 2. Induction of embryo callus in wheat by direct caryopsis planting (X 10).

In our other experiment only 30–40% of the embryos developed into callus which proliferated with the passage of time (Fig. 2). The remaining caryopses germinated and produced seedlings. Here again 1–B5 was better than MS and embryo callus mass was ready within 7–8 weeks for subculturing. Compared with the preceeding method, this was found to be less efficient and produced only one callus. The total callus mass produced per caryopsis was about 1/8 of the callus mass obtained from the seedling method.

Callus mass obtained from both the systems has been successfully sub-cultured on to the similar media for more than 180 days but in this case MS was found to be comparatively better than 1–B5. Light or dark condition did not materially affect callus induction or its growth under our experimental conditions.

Successful callus induction has been reported for wheat from explants such as isolated embryo (mature or immature), shoot segments (nodal or internodal), rachis and root segments (Dudits *et al.*, 1975; Chin & Scott, 1977; O'Hara & Street, 1978; Shimada *et al.*, 1969; Sears & Deckard, 1982; Ozias-Akins & Vasil, 1983). In these studies as well as in our own embryo callus there is a limitation that besides efficiency only a limited callus mass can be obtained from each explant. However, our first method saves time and yields a number of calli from a single caryopsis for sub-culturing and or regene-

rating whole plant. Similar method of callus induction has been reported by Carter *et al.*, (1967) for *Avena* but again only one callus mass was obtained which replaced the root system. Nabors (1982) found that callus mass obtained from root, by direct seed planting, was superior for plant regeneration than calli obtained from explants or embryo culture. Under our experimental system a number of roots developed normally and each of them formed callus whenever they came in contact with the media. Thus a number of calli were available for sub-culturing and regenerating plants and building up of stock from a single caryopsis.

Our method of callus induction provides a simple and time saving technique for rapid multiplication of calli from a single caryopsis.

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