Direct somatic embryogenesis of safflower – a scanning electron microscopic study

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Direct somatic embryogenesis of safflower (*Carthamus tinctorius* L.) is described with a scanning electron microscope (SEM). The SEM ontogeny revealed the normal development of somatic embryos from globular to heart-shaped, torpedo-shaped and finally cotyledonary-stage embryos. Somatic embryo development appeared to be asynchronous in nature. The study also confirmed the direct origin of somatic embryos from cotyledonary explants.

A large number of plant species have been reported to form somatic embryos in culture[1], but a limited number of reports have described the ontogeny of somatic embryogenesis through scanning electron microscopy[2,3]. Somatic embryogenesis in safflower (*Carthamus tinctorius* L.) has been restricted to the hormonal requirements for embryo induction and development[4]. No detailed ontogenic studies have been made either with light or using a scanning electron microscope (SEM). Recently, we describe the ontogeny of shoot-bud development directly from cotyledonary explants, with light microscopy[5]. In this study we describe the ontogeny of somatic embryos at the SEM level and present evidence for direct origin of somatic embryo from cotyledonary explants without an intervening callus phase. The results presented here also supplement our previous work on somatic embryo induction and development.

Seeds of safflower (*C. tinctorius* L.) cv. Girna were aseptically germinated on Murashige and Skoog's (MS) basal medium[6]. Cotyledons were excised from 8 to 10-day-old seedlings and the whole cotyledons were used as explants. Explants were cultured as described previously[4] on MS medium containing 3% sucrose, 0.8% bacto-agar and supplemented with 2 mg/l α-naphthaleneacetic acid (NAA) and 0.5 mg/l 6-benzylaminopurine (BAP). The cultures were maintained under 36 μmol m^{-2} s^{-1} in 16 h photoperiod (white fluorescent tube) at 25 ± 1°C.

The cotyledonary explants with somatic embryos at different developmental stages were fixed in 3% glutaraldehyde in 0.025 M phosphate buffer at pH 6.8 for 24 h at 0°C. Samples were then washed in the same buffer, post-fixed in 1% osmium tetroxide, dehydrated through graded series of acetone for 2 h in each, with three changes in pure acetone. Dehydrated tissues were then critical-point dried, mounted on metal blocks and sputter-coated with gold. Gold-coated samples were observed and photographed under an SEM (Phillip PSEM 500 model) operating at 50 KV.

Swelling of explants was apparent within two days of culture initiation and somatic embryos were visible within 8–10 days. Somatic embryos were developed on the adaxial surface of the cotyledonary explants within 15 days and different developmental stages of the somatic embryos were detected. After three weeks of culture initiation, 51.7% of total explants responded with 11.2 (SD = ± 2.7) number of somatic embryos per responding explant. Scanning electron micrographs of various developmental stages are shown in Figure 1. Somatic embryos at the incipient globular stage can be seen in the vicinity of the cotyledon cut edge (Figure 1 *a*) within six days of culture. At this time, embryos showed a smooth surface. Somatic embryos developed singly and in clusters as well. Globular embryos were seen developing directly on the cotyledonary surface without callus formation. Such direct development of somatic embryos has also been observed in other oilseed crops such as soybean[7,8], peanut[9–12], sunflower[13,14] and niger[15]. Compared to indirect somatic embryogenesis through callus phase, direct somatic embryogenesis appears to be associated with greater genetic and cytological uniformity[16]. While early globular embryo is seen in Figure 1 *b*, Figure 1 *c* shows a mature globular embryo after 10 days of culture. Mature globular embryos as well as the subsequent stages (Figure 1 *c–f*) revealed a rough surface, compared with younger ones. Figure 1 *d* shows heart-shaped embryo with notch on the tip that developed within 13 days of culture. Two incipient cotyledons are also seen in the Figure 1 *d*. The torpedo-shaped and cotyledonary-stage embryos developed within 15 days of culture (Figure 1 *e* and *f*). As the cotyledons of somatic embryos became more prominent, the surface of embryos also became well granulated. Since there is no synchronization of embryo development, different developmental stages are seen in Figure 1 *g*. Per cent somatic embryos at different developmental stages are presented in Figure 2. Somatic embryos with multiseriate suspensor were observed. Our histological observation also revealed the presence of multiseriate suspensor in somatic embryos of this species (unpublished data). The finding indicates the differentiation of multicellular proembryonal complex and its involvement in the induction and development of somatic embryos. Such developmental pattern is in agreement with the previous findings on soybean somatic embryogenesis[17]. About 17% somatic embryos did germinate on the same medium after 21 days of culture initiation[18]. Conversion of germinated somatic embryos into plantlet is reported to be 70% (ref. 4).

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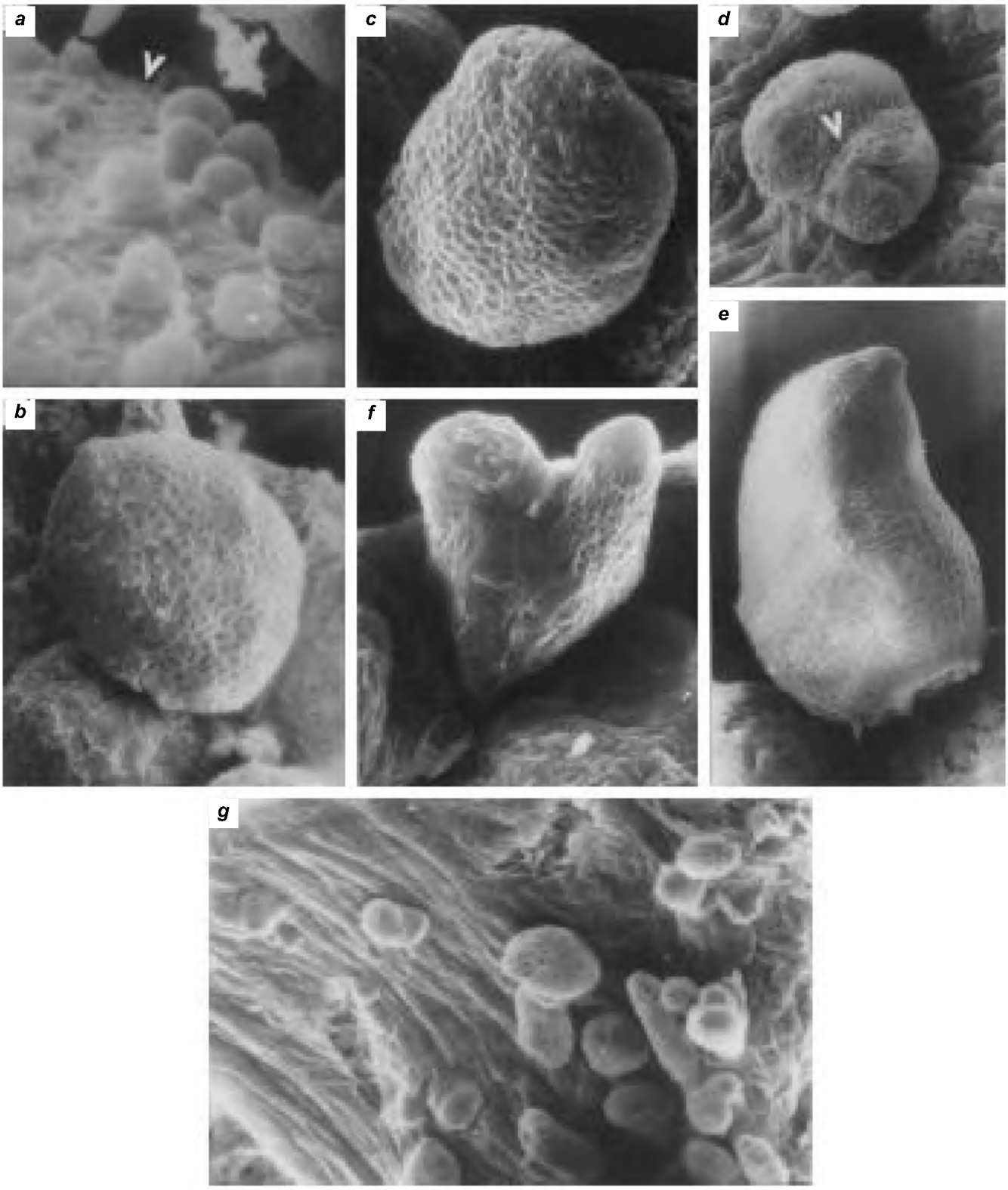

Figure 1. Scanning electron micrographs of somatic embryo development from cotyledonary explants of safflower cv. Girna cultured on MS medium supplemented with 2 mg/l NAA + 0.5 mg/l BAP. ***a***, A group of somatic embryos at incipient globular stage. Note their direct development at the cut edge (arrow head) and absence of callus (× 50); ***b***, Globular embryo at early stage (× 100); ***c***, Typical mature globular embryo (× 50); ***d***, Early heart-shaped embryo with two incipient cotyledons (arrow head, × 100); ***e***, Torpedo-shaped embryo (× 50); ***f***, Embryo at cotyledonary stage (× 100); and ***g***, Somatic embryos at different developmental stages on the surface of the explant indicating asynchronous development (× 37.5).

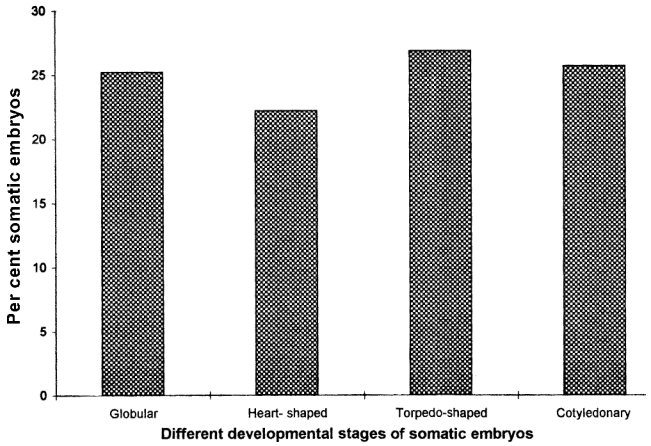

Figure 2. Per cent somatic embroys at different developmental stages after three weeks of culture initiation.

The present work depicts the SEM ontogeny of somatic embryo in safflower. It reveals normal development of somatic embryos from globular to heart-shaped, torpedo-shaped and cotyledonary-stage embryos. Also, precise identification of incipient globular embryo and early heart-shaped embryos, i.e. initiation of the development of two cotyledons was possible. Thus, the present work confirmed the direct development of somatic embryos from the cotyledon surface.

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Role of biological preparations in enhancement of rice seedling growth and grain yield

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The application of five commercial chitosan-based formulations of carefully chosen plant growth-promoting rhizobacteria developed at Auburn University, USA has previously shown demonstrable increase in the growth of nursery-raised plants such as cucumber, pepper and tomato among others. The present study evaluates the beneficial effects of the formulations on the growth of rice seedlings. Seedlings of three indica rice cultivars, IR24, IR50 and Jyothi raised in rice field soil amended with each of the formulations in a 1 : 40 (formulation : soil) ratio have shown significant two-fold increase in root and shoot length, and grain yield. The observations do suggest that application of such commercial bacterial formulations can serve as microbial inoculants for the improvement of rice growth.

As public opinion against the use of chemical pesticides on food crops grows, more and more pesticides have been removed from agricultural use. Hence, establishing new and effective pest control measures is a concern. One such alternative pest control strategy is the use of microorganisms. This strategy has the potential to reduce or eliminate chemical pesticides on agriculturally important crops, and thereby reduce the risk associated with pesticide residues in the environment. Biofertilizers and

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