In vitro Induction of Vegetative Buds on Inflorescence Segments of *Haworthia*

Although many investigators have been able to grow monocotyledonous tissues in vitro[1–6], it is seldom that the regeneration of complete plants from such tissue cultures has been achieved. Recently[7], the production of embryoids and their subsequent development into complete plants has been reported in cultures of *Asparagus* hypocotyl. We report in vitro induction of vegetative buds on callus and segments of the inflorescence axis of another monocotyledon, *Haworthia* (Liliaceae).

In *Haworthia* the inflorescence is a scape; flowers are borne in the axil of bracts (fertile bracts) present on the distal part of the inflorescence axis. Bracts on the proximal part of the axis do not bear any flowers in their axil and are referred to as sterile bracts in this paper.

Inflorescence segments of *Haworthia variegata* L. Bol., *H. chloracantha* Haw., *H. truncata* schoenl. × *H. setata* Haw., *H. atrofusca* G. G. Smith, *H. angustifolia* var. *albensis* (Schoenl.) V. Polln., *H. maughanii* V. Polln., *H.* sp. aff. *baccata*, *H.* sp. nov. (Retusae Sect.), *H. turgida* var. *pallidifolia* G. G. Smith, and *H. retusa* (L.) Haw. were surface sterilized with 4% sodium hypochlorite solution for 8–10 min, washed with sterile water and aseptically planted on various agar media (Table) which had been sterilized by autoclaving at 15 lb pressure and 120 °C temperature for 15 min.

On media I–III, the segments of inflorescence did not show any callusing response. The axis became pale yellow and the flowers shrivelled.

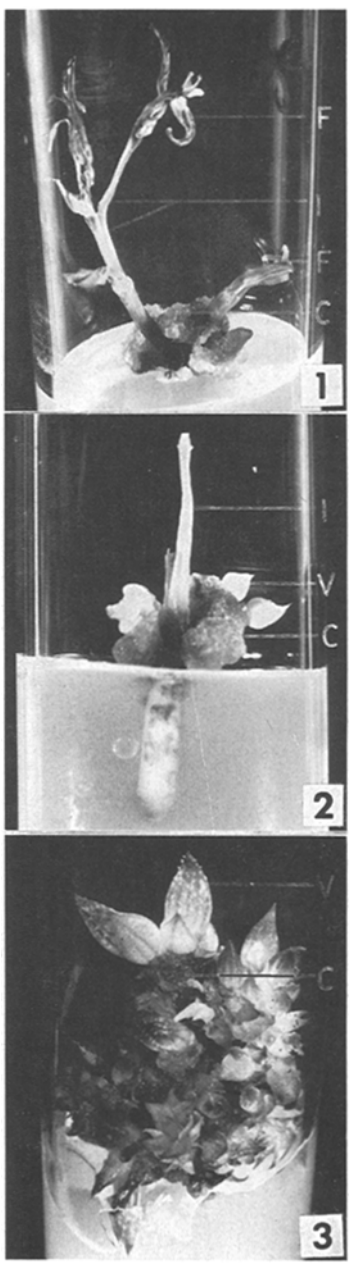
Occasionally 1–4 vegetative buds were induced in the axil of sterile bracts 12–15 weeks after inoculation on media IV and V. No callusing was observed on these media.

The cultures on media VI, VII, and VIII showed 3 nonspecific types of growth responses. (a) In some cultures vegetative buds were produced in the axil of sterile bracts without the formation of callus. (b) In many cultures callus was produced in the axil of fertile (Figure 1) or sterile bracts (Figure 2) 6–10 weeks after inoculation. This callus either continued to grow or showed organogenesis within the next 2–3 weeks. Upon subculture the callus differentiated into vegetative buds (Figure 3), some of which produced roots. On being transferred to soil the rooted buds grew into mature plants. When subcultured on the same media, those buds without roots gave rise to callus which again regenerated to form additional buds with or without roots. Thus, the cycle of differentiation, dedifferentiation and redifferentiation continued. (c) Callus and vegetative buds were also produced from the cut end of the inflorescence segments in some cultures on media VI–VIII all of which contained coconut milk.

The responses of inflorescence segments of different sizes from the distal flower-bearing region and the proximal sterile region were similar in terms of callusing and formation of vegetative buds.

Histological examination of the inflorescence axis of *H. variegata* and *H. turgida* var. *pallidifolia* plants grown in green-houses, indicated that vegetative buds or meristematic zones were absent in the axil of the bracts. These observations, along with the fact that callus and vegetative buds can be produced from the cut end of the inflorescence segments, show that callus and bud formation in vitro are a result of growth induced by the composition of these media.

Induction of callus and vegetative buds occurred exclusively on media containing coconut milk. Growth on medium VIII (BM + CM) was similar to, or even better than, that on medium VI (BM + CM + IAA + K). Addition of IAA and kinetin to a medium containing coconut milk



(1) Inflorescence segment of *Haworthia maughanii* showing callusing in the axil of a fertile bract. × 1.75.

(2) Proximal segment of inflorescence axis of a hybrid (*H. truncata* × *H. setata*) showing differentiation of vegetative buds from the callus produced in the axil of a sterile bract. × 2.

(3) Numerous vegetative buds which have developed in vitro from an explant of undifferentiated callus. × 2.

Figs. 1–3. Responses of inflorescence segments of *Haworthia* on coconut milk medium (C, Callus; F, flower; I, inflorescence axis; V, vegetative bud).

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Supplements added to White's modified[8] medium (BM)

Name of supplement	Media number							
	I	II	III	IV	V	VI	VII	VIII
Casein hydrolysate (CH)	–	1000 ppm	–	–	–	–	–	–
Coconut milk (CM)	–	–	–	–	–	20% v/v	20% v/v	20% v/v
Indoleacetic acid (IAA)	–	1 ppm	–	1 ppm	2 ppm	1 ppm	1 ppm	–
Kinetin (K)	–	0.5 ppm	0.5 ppm	0.5 ppm	1 ppm	0.5 ppm	–	–
Naphthaleneacetic acid (NAA)	–	–	2 ppm	–	–	–	–	–

does not have any significant effect on the callusing and differentiation of these monocotyledonous tissues under culture condition.

Results from these experiments indicate that monocotyledons, specifically the species of *Haworthia*, respond similar to many dicotyledons[9] in forming callus and regenerating whole plants under cultural conditions. This response is stimulated by one or more heat-stable factors present in coconut milk[10].

Zusammenfassung. Teile des Blüttenstandes von verschiedenen Arten der einkeimblättrigen Pflanze, *Haworthia*, liess man in White's Nährlösung wachsen. Die an der Achse des Blütenstandes entstandenen Verhärtungen entwickelten sich entweder weiter oder gingen in viele Pflanzenkeime auf. Kokusnussmilch ist ein wesentlicher Bestandteil der Nährlösung für die Entwicklung von Verhärtungen und der Entstehung von Organismen.

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[10] We thank University of Kentucky Research Foundation for financial support and Dr. H. P. RILEY for allowing us to use his personal collection of various species of *Haworthia*.

Polarity and Symmetry in Composite Oocytes of *Carausius morosus* Br. (Cheleutoptera, Phasmidae)

Material and method. Compound eggs and oocytes, arising from oocyte fusions, have been observed in several insects[1], particularly in Phasmidae[2]. In this paper 53 follicles with composite oocytes of *Carausius morosus* will be described (Figure). They were observed in the panoistical ovarioles of old adults which had been fixed in Carnoy 3:1, embedded in paraffin, sectioned at 10 μ and stained with iron haematoxylin.

Results. The smallest follicles are about 200 μ long and with flat epithelium (5–10 μ thick, Nos. 1–3), the longest are about 850 μ long and with, at any case around the posterior oocyte, columnar epithelium (70–80 μ thick, Nos. 26, 53). The epithelium is either of uniform thickness (apolar epithelium, Nos. 1–26) like the epithelium of normal growing oocytes[3], or higher around the posterior, i.e. older oocyte(s) (polar epithelium, Nos. 27–53).

The ovoid composite oocytes consist of 2–18 growing oocytes (partners) in various stages of development, viz. from newly formed oocytes with cytoplasm (Nos. 1–3) up to oocytes with advanced yolk accumulation. The membranes between 2 oocytes are double and plasma fusions, as observed by CAPPE DE BAILLON[2], were not observed in this material. During early yolk accumulation the granules appear parallel with the surrounding epithelium in those regions of the partners which join the epithelium. In case of apolar epithelium, the volumes of the partners are about equal (except No. 26) and yolk accumulation has advanced equally far in each of the partners (except Nos. 25–26). These composite oocytes resemble normal growing oocytes. In case of polar epithelium the volume(s) of the posterior oocyte(s) is (are) larger than the volume(s) of the anterior oocyte(s) (except Nos. 27–29, 35–36, 47–48, 51) and yolk accumulation has advanced much more in the posterior oocyte(s).

In the normal growing oocyte the nucleus is situated in the centre, but in the composite oocyte (except when newly formed, Nos. 1–3) the nucleus of a partner lies against the membrane as far as possible from the oocyte periphery which joins the epithelium and, eventually also owing to a dent in the membrane, more or less on the anterio-posterior axis of the composite oocyte as far as possible in the centre of the composite oocyte. The nucleus of a partner with advanced vitellogenesis lies in the cortex, as in normal oocytes (Nos. 41, 44–45).

Discussion. According to CAPPE DE BAILLON[2] the composite oocytes of *C. morosus* arise as a result of the non-development and the degeneration of the follicle epithelium between the growing oocytes under the influence of the pressure on each other. However, since composite oocytes occur more frequently in old individuals, hormonal influences on the development of the epithelium may also be important[4]. Since a normal ovariole produces 11 eggs and contains 0–6 (exceptionally 9) growing oocytes in old adults, the great number of oocytes in composite oocytes (Nos. 13, 39, 53) may be also caused by either over-production or blockade of egg development.

There seem to be two kinds of composite oocytes, viz. one kind which consists of equally developing oocytes (Nos. 1–24 with apolar development), and the other one, of which the posterior oocyte(s) develop(s) in advance of the other(s) (Nos. 25–53 with polar development). This difference may be explained by assuming that the oocytes concerned were in the same phase, respectively in different phases, of development at the time of fusion. The

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