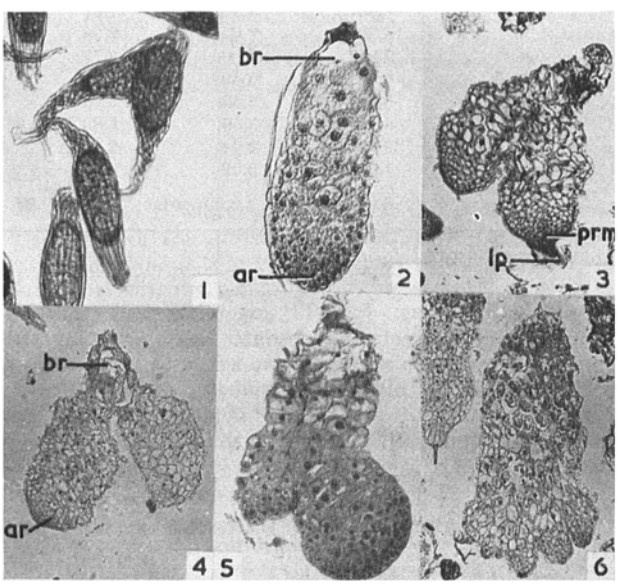
Occurrence of Polyembryony in *Vanda* During *in vivo* and *in vitro* Conditions

Previous reports on the occurrence of polyembryony in orchidaceae have been well summarized[1,2]. There is hardly any published account about polyembryony in the genus *Vanda*. *Vanda* Miss Joaquim is a popular local hybrid (*V. teres* × *V. hookeriana*)[3]. In the mature as well as in some of the germinating seeds in cultural conditions, certain instances of polyembryony were noticed, the description of which forms the subject of this paper.

As in other orchids, the seeds are small and the embryo is a non-differentiated structure; the cells in the basal (micropylar) region being slightly bigger than those of the apical (chalazal) region (Figure 1). About 4000 seeds were examined and in almost 1% of them there were two embryos in each seed, as was also revealed in microtome sections. In some of these, the cleavage was not deep and as such both the embryos had a common central and basal portion; while in others it was deep and the two embryos were almost independent, except for a common basal region (Figures 3, 4). Thus, they were slightly different from one another and were formed as a result of cleavaging of the monozygotic embryo. Further, these clearly resemble the previously described examples of cleavage polyembryony[4].

In order to study the germination and seedling formation, the seeds were inoculated on the agar medium as defined by VACIN and WENT[5], and the cultures were maintained under controlled temperature and light conditions. The seeds germinated readily on the medium and formed young seedlings with 2–3 leaves in 100–120 days. In order to study the organogenesis, tissues were removed from the tubes at periodic intervals and sections were prepared for histological examination. A month-old germinating seed consisted of an enlarged embryo that showed distinct apical and basal regions (Figure 2). At the apical end a promeristem was formed, which by further differentiation gave rise to the shoot apex and leaf primordia. Further details of protocorm development from such embryos and the subsequent organogenesis that lead to the seedling formation will be published elsewhere[6]. The twin embryos present in some of the seeds, formed as a result of a cleavage in the monozygotic embryo, also developed further, resembling the germination of singular embryos of the normal seeds (Figures 3, 4) and gave rise to double seedlings. During the earlier stages of germination, the double embryos of the seeds were bilaterally symmetrical (Figures 3, 4) and the two seedlings formed from them were almost of equal size. In some of the other 1-month-old germinating embryos, a small primordium was organized in the form of a budding, and this grew into an additional embryo (Figure 5). In still other cases, the apical promeristem divided into a number of primordia, all of which further organized into multiple embryos (Figure 6). The plural embryos formed this way varied in number from three to nine; such embryos as developed during the germination could always be distinguished from the single or double embryos developed in the seed by their smaller size, slower growth and asymmetrical structure. Further, the seedlings formed therefrom were also smaller in size. Thus two tendencies were observed with regard to the origin of polyembryony in this orchid hybrid. Twin embryos were present in the seeds organized as a result of cleavaging of monozygotic proembryos. The other tendency was that additional embryos were formed by the process of budding during the seed germination.

The first tendency, namely cleavage polyembryony, is common in orchids and is reported in many genera like *Cymbidium*, *Eulophia* and others[1,2], and all such reported cases refer to the occurrence of polyembryony in the developing or mature seed. However, the second tendency reported here, namely the formation of plural embryos during the seed germination, had not been recorded before in any of the orchids so far studied[1,2,7–10].



Figures 1–6. (ar – apical region, br – basal region, lp – leaf primordium, prm – promeristem). Fig. 1. Whole mount of seeds. In one the twin embryos are present, ×155. Fig. 2. L.s. of a month-old germinating embryo, ×100. Figs. 3–4. One-month-old germinating twin embryos. In Fig. 3 note the formation of promeristem and leaf primordium, ×90, ×93. Fig. 5. Primordium of a second embryo developing from a 40-day-old germinating embryo, ×73. Fig. 6. Development of six independent embryos from a 40-day-old germinating embryo, ×55.

Résumé. Bien que le phénomène de Polyembryonie ait été signalé pour dix-neuf generes de plants de la famille des Orchidaceae, rien n'a été publié à ce sujet sur le genre *Vanda*. Nous avons observé, dans les graines mûres d'une *Vanda* mixte aussi bien que dans celles germant sur de l'agar-agar, certains cas de Polyembryonie. Cet article rend compte du développement des embryons étudiés.

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