Pollen Tube Growth and Embryological Development in *Avena strigosa* × *A. sativa*

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**SEVERAL** interspecific crosses among *Avena* species having different chromosome numbers have been reported. Many of the earlier species crosses in the genus were reported by Kihara and Nishiyama (4) and Nishiyama (6). In more recent years successful crosses have been reported by Brown and Shands (1) and by Zillinsky et al. (9, 10). With few exceptions, successful crosses reported have been between hexaploid *φ* (2n = 42) × tetraploid *δ* (2n = 28) or tetraploid *φ* × diploid *δ* species. Kihara and Nishiyama (4) obtained some crossed seeds of hexaploid *φ* × diploid *δ* species that germinated and produced completely sterile plants. However, the cross was very difficult to make. They crossed diploid *φ* × hexaploid *δ* species with little difficulty but none of the seeds germinated.

This writer encountered a similar experience while attempting to cross diploid *φ* × hexaploid *δ* species in the greenhouse at Urbana in 1958. What appeared to be successful crosses were easily obtained but as seeds matured they became shrivelled and later failed to germinate. These results led to an investigation of the embryological development in the diploid *φ* × hexaploid *δ* cross.

Kihara and Nishiyama (4) observed embryological development in the cross diploid *Avena strigosa* Schreb. (φ) × hexaploid *Avena fatua* L. (δ) at 24, 48, and 72 hours after pollination. According to their report, the embryo and endosperm appeared normal after 24 hours, except that the endosperm had increased more rapidly than in selfed *Avena strigosa*. After 48 hours the embryo had developed more rapidly than in *Avena strigosa* and the endosperm had begun to show abnormal features, especially around the embryo. After 72 hours most of the embryos still had a healthy appearance and many cells were present, but a general degeneration of the endosperm had occurred.

**EXPERIMENTAL PROCEDURE**

*Avena strigosa* Schreb. 'Saia' was the diploid female parent and *A. sativa* L. 'Clintland 60' the hexaploid male parent. Crosses were made in the greenhouse in the 1958-59 season. The procedures used for collecting, fixing, staining, and observing pollen tube growth and embryological development were similar to that reported by Brown and Shands (2).

Pistils were killed and fixed in a solution of 3 parts 95% ethyl alcohol to 1 part glacial acetic acid at the various intervals after pollination. They were left in the fixative for 48 hours and then stored in 70% alcohol until observed. Iodine potassium iodide (IKI) was used to stain pollen tubes on the stigma and in the style of pistils fixed approximately 30 minutes after pollination. Preparations for observing embryological development at 6, 10, 12, 16, and 20 days after pollination were made by imbedding whole pistils in paraffin, sectioning at 15 microns, and staining with Delafield's Hematoxylin.

**OBSERVATIONS**

Pollen grain germination and pollen tube growth appeared normal in pistils of diploid *Saia* φ × hexaploid Clintland 60 δ crosses fixed 30 minutes after pollination. Pollen grains had germinated and some had penetrated the entire length of the style and the ovary by this time. Fertilization was not observed but was likely normal since proembryo and endosperm cells resulted.

A proembryo 6 days after pollination is shown in Figure 1-A. The cells of the proembryo appeared healthy but the shape of the proembryo was somewhat abnormal. According to Bonnett (3) the young proembryo should be obovate (club- or pear-shaped) due to more cell divisions in the apical than in the basal portion. The proembryo of diploid *Saia* × hexaploid Clintland 60 appeared more spherical than obovate 6 days after pollination. The endosperm had degenerated much the same as reported by Kihara and Nishiyama (4).

A proembryo 10 days after pollination is shown in Figure 1-B. The embryo had increased in size but no evidence of normal differentiation was noted. The endosperm was sparse and almost completely degenerated by this time. An embryo 12 days after pollination is shown in Figure 1-C. This was the only one observed in which somewhat normal differentiation of the embryo had occurred. Also the endosperm appeared more nearly normal. It should be noted that all pollinations were made by hand and the possibility of selfing in this one pistil cannot be completely eliminated. An embryo 20 days after pollination is shown in Figure 1-D. The embryo had increased in size but a definite degeneration of some cells had occurred. No evidence of a normal pattern of differentiation was noted. The endosperm was completely degenerated by this time.

**DISCUSSION**

The early embryological development in the diploid × hexaploid cross appeared similar to that reported by Kihara and Nishiyama (4). An interesting feature of later development was the length of time that cells of the embryo multiplied without differentiation occurring. The cells of all embryos appeared healthy for at least 6 days even though the endosperm had more or less degenerated.

On the basis of these observations one might expect some of the proembryos to have the potential for developing into mature plants. This of course suggests the possibility of using embryo culture for obtaining plants from the cross. The writer made several unsuccessful attempts to culture embryos from diploid × hexaploid crosses, using the technique and medium suggested for forage legumes by Keim (5). These attempts were by no means exhaustive and the techniques and medium employed may not be applicable to *Avena*. No attempt was made to culture selfed embryos using the same technique.

Some successful transfers of genes from diploid to hexaploid species of *Avena* have been reported (9, 10), but none of these were accomplished by directly crossing diploid with hexaploid species. In view of the recent reports of Nishiyama (7, 8) one might expect to make better progress by directly crossing the diploid × hexaploid species. Nishiyama crossed tetraploid × hexaploid species and doubled the chromosomes in the *F*₁ hybrid to obtain...