

Work Tool
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Hybrids of Sugar Pine by Embryo Culture

A modified embryo culture technique was used to facilitate germination of seed obtained after pollinating sugar pine with pollen from blister-rust-resistant Armand and Korean pines. Resulting seedlings appear to be hybrids.

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SUGAR PINE (*Pinus lambertiana* Dougl.), a prized species in California and Oregon forests, is highly susceptible to blister rust attack. For this reason repeated efforts have been made to cross sugar pine with several of the blister-rust-resistant Eurasian white pines. In 1946, the Institute of Forest Genetics attempted a cross using pollen of Armand pine (*P. Armandi* Franch), a native of China. Two mature cones from this attempt were harvested in 1947; they yielded 115 normal-sized seeds, of which 4 were apparently filled. These were stratified for 13 weeks at 5° C. in a mixture of moist sand and peat and were planted in the Institute's nursery on May 13, 1948. None of these seeds germinated. When 8 wind-pollinated seeds from the same sugar pine seed-parent were treated in the same manner, 6 germinated. Why the seeds resulting from the cross pollination failed to germinate was not immediately apparent. Therefore, the following year it was decided to try an em-

bryo culture technique on 7 sound seeds which had resulted from pollinating sugar pine with pollen from Armand pine and the one sound seed which had resulted from pollinating sugar pine with pollen from Korean pine (*P. koraiensis* Sieb. and Zucc.). If this technique failed it was hoped that the dissections involved might at least reveal some morphological reason for the failure.

Reported methods of increasing and hastening germination of sugar pine seed both by the embryo culture technique and by treatments other than stratification are rather confusing. Show (3) reported that soaking the seed in sulfuric acid was the only effective method and that this gave poor results in comparison with fall planting. Jacobs (2) reported better germination after using a particular water-soaking routine, but neither he nor anyone else has since been able to repeat this treatment successfully. Swensen (5) and Haddock (1) were able to obtain a

small increase in germination by removal of the seed coat. Haddock (1) was also successful in excising embryos from fresh seed and growing a few to transplantable size on nutrient agar. On the other hand, Stone (4), working with 2-year-old seed, was unable to do this unless the seed had previously been stratified for 3 months at 5° C.

A recent study has somewhat clarified this picture. Data¹ from this study indicate that up to 3 or 4 months after harvest from mature cones, seed stored either at 5° C. or at room temperature will germinate immediately provided the seed coat and inner integument are removed. (Germination also took place when, in addition, the endosperm was removed. However, a more vigorous, rapidly growing seedling resulted when germination took place with the endosperm present.) As the seed becomes older the presence of the endosperm becomes inhibitory and it is necessary to remove it and use a nutrient agar to

¹Unpublished data from a current study by E. C. Stone.

achieve germination. In still older seed (the age depending upon whether storage has been at 5° C. or room temperature) the embryo by itself is dormant, and stratification of the seed at 5° C. for 3 months is necessary.

Therefore, because the seed for this experiment had been held in storage less than 3 months after harvest, it was decided to first remove only the seed coat and inner integument. If germination failed to take place within 10 days, then the endosperm would also be removed and the embryo placed on a nutrient agar.

Procedure and Results

Seed coats were sterilized by soaking in saturated bromine water for thirty minutes, after which they were thoroughly washed in sterile water. Removal of the seed coat and all subsequent transfers were carried out in a sterile culture room; hands of the operator were covered with sterile rubber gloves.

The seed coat was cracked by pressure exerted with the thumb and forefinger along the edge of the seed; previous experience had shown that breaking in any other fashion usually resulted in damage to the embryo. After the seed coat was removed, the inner integument and the remains of the nucellus were removed with a pair of forceps. The embryo with the surrounding endosperm tissue (Fig. 1, c) was then placed on a 1.5 percent plain agar slant contained in a 25 by 150 millimeter rimless test tube. The test tubes were stoppered with cotton plugs and placed on racks in the laboratory in diffused light at a temperature of approximately 26° C.

The slants were examined daily; growth was first noted on the sixth day with the appearance of the radicle. The presence of the endosperm was apparently not inhibitory in this instance.

When the roots were approximately 3 cm. in length (Fig. 1, e) the seedlings were transferred to quart cans filled with previously fumigated soil. These plants were then placed in the greenhouse for six weeks (Fig. 1, f), transferred to gallon cans, and then placed outside in the nursery.

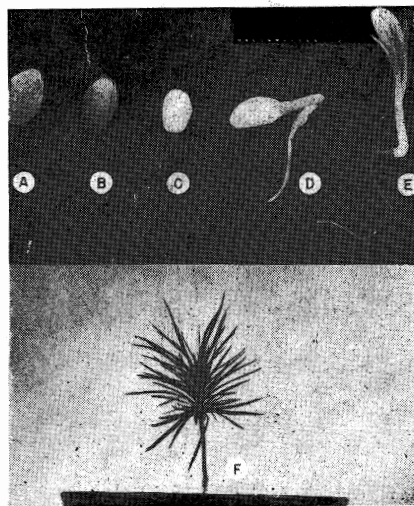


Fig. 1.—Various stages of embryo culture. a. intact seed; b. seed coat removed; c. seed coat, inner integument, and nucellus removed; d. embryo at end of 12 days; e. embryo at end of 16 days, immediately before transfer to soil; f. seedling 40 days after transfer to soil.

Two seedlings resulting from the cross between sugar pine and Armand pine died during the first summer, probably as a result of injury in handling; the remaining five, and the one seedling from the cross between sugar pine and Korean pine are growing well.

Discussion

The failure of the 1947 seeds to germinate cannot be explained; the embryos in the 1948 seeds were normal in every way, and there was no evidence of incompatibility between the embryo and endosperm.

Work on this phase of the problem is being continued.

All the seedlings have produced needle fascicles during the first summer and the dorsal surfaces of the needles are without stomata. As this is not characteristic of sugar pine but is of both Armand and Korean pine, it suggests but does not prove that the seedlings are indeed true hybrids.

There is no reason for believing that in this case germination would not have taken place had the seed coat been removed and the endosperm-encased embryo placed directly in soil. However, this would still require sterile conditions which are more conveniently obtained with agar slants than with cans of soil. In this respect it is interest-

ing to note that once the embryo starts growing it thrives quite well on nonsterile soil provided it is protected from damping off against. Another reason for using agar slants was the chance that the embryo might be dormant when the endosperm was present. Then it would have been necessary to excise the embryo and place it on a sterile nutrient agar—a procedure impossible to follow if the endosperm-encased embryo had been originally started in soil.

Use of an embryo culture technique is of definite value when only one or a few seeds are available and a high germination percent is necessary. It is quite possible that if in addition to this practice, a chilling treatment is given the greenhouse-grown seedling when it enters the dormant period, a 2-year-old sized plant can be obtained within a year. This is an important consideration in any plant-breeding program.

Summary

1. Seeds were obtained after pollinating *P. lambertiana* with pollen from *P. Armandi* and *P. koraiensis*.
2. Germination of these seed soon after harvest was facilitated by a modified embryo culture technique.
3. Resulting seedlings appear to be hybrids because (a) they produced fascicles in the first summer and (b) the dorsal surfaces of the needles are without stomata—both characteristic features of *P. Armandi* and *P. koraiensis* but not of *P. lambertiana*.

Literature Cited

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