

## SOMATIC EMBRYOGENESIS IN OZARK CHINQUAPIN

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Chinquapins (or chinkapins) are shrubs or small trees closely related to the American chestnut, commonly found throughout the southeastern U.S. The Ozark chinquapin (*Castanea pumila* var. *ozarkensis*), is a variety of chinquapin that in the Ozark Highlands of eastern Oklahoma, southwestern Missouri and Arkansas. Like American chestnut, Ozark chinquapin populations have been devastated by chestnut blight, and most individuals now exist as stump sprouts of variable size and age. Because Ozark chinquapin is closely related to American chestnut, some of the same biotechnological approaches currently under development to help conserve and restore American chestnut may be useful for conservation and restoration of Ozark chinquapin. Immature (green) burs were collected in August 2015 from five Ozark chinquapin source trees growing in the Nature Conservancy's Nickel Preserve near Tahlequah, OK, and shipped to UGA. Burs were opened and nuts were surface-disinfested and dissected to remove the immature seeds, which were cultured on semisolid chestnut induction-maintenance medium (IMM) supplemented with 4 mg/l 2,4-D. After one month, explants were transferred to IMM with 2 mg/l 2,4-D, and proliferating cultures were transferred to medium of the same composition every three weeks thereafter. Overall, 19 embryogenic cultures, representing all five source trees, were obtained from 1368 immature seed explants, for an overall initiation rate of 1.4 percent. Suspension cultures were initiated from the cultures, and these were subsequently size-fractionated and plated to produce populations of somatic embryos. Over 1000 somatic embryos were produced from 15 lines and given 12 weeks of pre-germination cold treatment at 8° C before being transferred to a lighted incubator for germination. To date, over 70 of the embryos have germinated *in vitro*. The first somatic seedlings were transferred to potting mix and are growing in the greenhouse.

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