

Age Dependence for Organogenesis of Seed Explants from Four Cucurbita Accessions

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A genotypic comparison in the in vitro organogenesis of explants from seeds was made on 4 accessions from the genus Cucurbita. Explants of seeds of different ages were tested to determine what developmental stage was most conducive to organogenesis for each of the genotypes.

Cucurbita pepo L. explants from cotyledons of germinated seeds have previously (1,2) been reported to produce callus capable of differentiating into embryoids, shoots, and roots. As of yet, no known information has been reported concerning possible genotypic variation of organogenesis from seed cotyledons of varying developmental stages. Seed was collected from fruit harvested at 10, 17, and 45 days after pollination (DAP) from 3 varieties of Cucurbita pepo ('Early Prolific Straight Neck' summer squash, 'Table Queen' acorn squash, and 'Black Beauty' zucchini squash) and one variety of Cucurbita maxima ('Improved Hubbard' winter squash). Once sterilized in 95% ethanol with the embryonic apex removed, each seed was placed on edge in 10 ml. of MS media (3) supplemented with 4.0 mg./l. BAP and 0.2 mg./l. NAA and maintained at 25°C under 2 klux light exposure. To provide a sufficient opportunity for callus development and differentiation into roots and shoots cultivars were incubated for 3 months before final evaluation.

A correlation between the developmental age of the seed and the extent of callus growth and organogenesis for all 4 genotypes was observed (Table 1). Seed explants with mature cotyledons displayed the greatest ability to form callus and differentiate into shoots and roots. No differentiation of callus was observed from seeds of 10 and 17 DAP. Explants from these seeds did not contain cotyledon tissue.

As shown in Table 2 nearly all explants from mature fully developed seed of the four genotypes formed callus tissue. Explants of the zucchini variety 'Black Beauty' developed the greatest number of vigorous shoots and roots. Organogenesis in explants from the C. maxima genotype 'Improved Hubbard' squash was restricted to root tissue. Formation of minute embryos or embryoids as reported in studies by Jaleska (1) was not observed.

These results reveal the existence of genotypic variation in callus formation and organogenesis among the 4 genotypes of Cucurbita in this study. The use of explants from mature seed dramatically simplifies experimentation in organogenesis since seeds can be procured commercially. Experiments are currently under way to survey for variation in explant organogenesis among an array of genotypes in the family Cucubitaceae. Genotypes expressing enhanced levels of organogenesis will be used in future studies to develop a model system for the generation of somoclonal variants. In addition controlled hybridizations will be made to investigate the genetic basis of the observed variation in organogenesis.

Literature Cited

1. Jaleska, S. 1972. Embryoid formation by fragments of cotyledons and hypocotyls in Cucurbita pepo. Planta 103:278-280.
2. Jaleska, S. 1974. Embryogenesis and organogenesis in pumpkin explants. Physiol. Plant. 31:257-261.
3. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:474-497.

Table 1. Relationship of seed age on explant callus formation and organogenesis

<u>Genotype</u>	<u>DAP</u>	<u>Callus^z</u>	<u>Shoots^z</u>	<u>Roots^z</u>
<u>C. pepo</u> 'Black Beauty'	10	-	-	-
	17	++	-	-
	45	++	++	++
<u>C. pepo</u> 'Table Queen'	10	-	-	-
	17	+	-	-
	45	++	+	+
<u>C. pepo</u> 'Early Profile Straight Neck'	10	-	-	-
	17	+	-	-
	45	++	+	+
<u>C. maxima</u> 'Improved Hubbard'	10	-	-	-
	17	+	-	-
	45	++	-	+

^z(-) equals no growth or differentiation while (+) and (++) indicate increasing degrees of callus vigor and tissue organogenesis.

Table 2. Callus development and organogenesis of 45 DAP seed explants among four Cucurbita genotypes.

<u>Genotype</u>	<u># of Explants</u>	<u>% with Callus</u>	<u>Vigor of Callus</u>	<u>% with shoots</u>	<u>Vigor of shoots^z</u>	<u>% with roots</u>	<u>Vigor of roots^z</u>
<u>C. pepo</u> 'Black Beauty'	64	100	3	20	3	88	3
<u>C. pepo</u> 'Table Queen'	76	99	2	5	1	13	2
<u>C. pepo</u> 'Early Prolific Straight Neck'	80	99	2	11	28	38	2
<u>C. maxima</u> 'Improved Hubbard'	90	100	2	0	0	58	2

^zSubjective evaluation for vigor where 0 equals no observed growth or development and 3 equals the maximum.