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Culture Date and Germination Procedure Affects Success of Nectarine Ovule and Embryo Culture

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Abstract

Immature 'Sunlite' nectarine ovules were grown on Knop's ovule growth medium for 30 days and germinated following stratification either in vitro on a modified Knop's germination medium or in moist perlite. The minimum age at which any embryos were successfully ovule cultured and germinated was 53 days after full bloom. Germination percentage increased significantly at 60 and 67 days after full bloom. Germination percentage in perlite was not greatly different from that of ovules cultured in vitro on modified Knop's germination medium, but the 53 and 60 days after full bloom embryos germinated in perlite were visibly weaker.

Embryo culture is an important component of many classical breeding programs and has been used to rescue immature embryos from early ripening fruits, in fruits that exhibit embryo abortion, and in embryos from interspecific hybrids where embryo abortion occurs during development. Embryo culture is now being used by grape (*Vitis vinifera* L.) breeders to develop seedless table grape cultivars (2, 3, 8). Embryo (without seed coat) and ovule (with seed coat) culture techniques are also commonly used by *Prunus* breeders in the development of early ripening cultivars (7).

Embryo rescue is of particular importance in the peach (*Prunus persica* (L.) Batsch) breeding program at the University of Florida. The window for peach and nectarine production in Florida is early and narrow. Fruit must ripen within 100 days after flowering, ie. fruit development must occur between spring frost and the summer rainy season in early June. The emphasis on early-ripening enhances the need for embryo rescue so that both parents contribute to a short fruit development period in the hybrid progeny, thereby increasing the percentage of early ripening individuals from which selections can be made (5).

Embryo survival in vitro depends on the medium used and the stage of embryo development (7). The younger the embryo, the more complex the media required to insure embryo survival growth (6). These experiments

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were undertaken to investigate the minimum age or days after full bloom at which immature peach embryos could be cultured, and to determine if embryos that were ovule-cultured could then be germinated in moist perlite, bypassing the transfer to an in vitro germination medium.

Materials and Methods

Open-pollinated fruit of 'Sunlite' nectarine were harvested at 32, 39,46, 53, 60, and 67 days after full bloom, ovule cultured for 30 days on Knop's ovule growth medium and germinated following stratification either in vitro on a modified Knop's germination medium or in moist perlite. The criteria for full bloom was when 50 percent of the flower buds were open. Data were taken for two consecutive years and combined due to a lack of yearly differences. Whole fruit were sterilized in 2.6% sodium hypochlorite for 10 to 15 minutes. The ovules were excised under aseptic conditions and cultured in 25 x 95 mm vials containing 10 ml of Knops (9) ovule growth medium (Table 1). The pH was adjusted to 5.7 with NaOH. Approximately 130 ovules were cultured per germination treatment (moist perlite vs. in vitro reculture) at each of the 6 culture dates. These ovules were incubated at about 26C in the dark for 30 days. After the initial culture period of 30 days, the ovules were removed from Knop's ovule growth medium and given stratification at 2C in either moist perlite or recultured in a modified Knops germination medium (4). The ovules were observed periodically between 30 and 60 days after reculture and radicle elongation of 1 cm was used as a criteria for germination. Data were subjected to an analysis of variance with years being the replications and a least significant difference test was performed.

Results and Discussion

Little germination either in vitro or in moist perlite was observed in ovules cultured at or prior to 53 days after full bloom in either of the two years. Germination rates in vitro (on Knop's germination medium) and in moist perlite from embryos at 60 and 67 days after full bloom were higher ($P = 0.05$) than the 53 day and earlier embryos. The germination increase from 53 to 60 days after full bloom visually coincided with the transition from the globular to heart stage of embryo development. There were no significant germination differences between 60 and 67 day embryos or between in vitro and perlite. However, embryos germinated in perlite were visibly weaker than those germinated in the Knops germination medium. There are

Table 1. Formulas of the ovule culture growth and germination media.

	Media	
	K-1[z]	K-2[y]
	Macronutrients (mg/l)	
KNO_3	4759	190
NH_4NO_3	412.5	—
$CaCl_2.2H_2O$	449	—
$Ca(NO_3)_2.4H_2O$	140	—
$MgSO_4.7H_2O$	370	370
KH_2PO_4	170	170
	Micronutrients (mg/l)	
KI	0.83	0.83
H_3BO_3	6.2	6.2
$MnSO_4.4H_2O$	22.3	22.3
$ZnSO_4.7H_2O$	8.6	8.6
$Na_2MoO_4.2H_2O$	0.25	0.25
$CuSO_4.5H_2O$	0.025	0.025
$CoCL_2.6H_2O$	0.025	0.025
Na_2EDTA	18.6	37.2
$FeSO_4.7H_2O$	14.0	27.9
	Organics (mg/l)	
Nicotinic Acid	0.5	0.5
Thiamine HCL	0.1	0.1
Pyridoxine HCL	0.5	0.5
Glycine	2	—
Myo-Inositol	100	100
	Carbohydrate (g/l)	
Sucrose	60	30

[z]Knops (Tabachnick and Kester, 1977) ovule growth medium.
[y]Knops (Lyrene, 1980) germination medium.

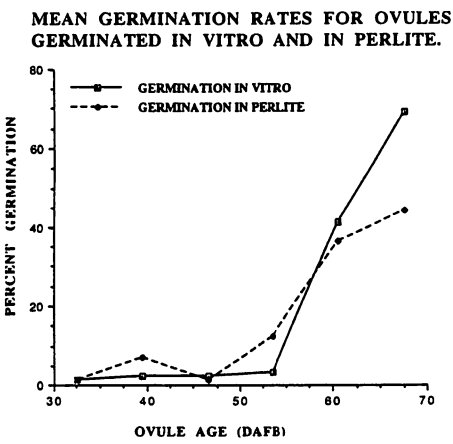

Figure 1. Germination at 33, 39, 47, 53, 60 and 67 days after full bloom (DAFB) of 'Sunlite' nectarine seed grown on Knop's ovule growth medium and transferred to either Knops germination medium or to moist perlite.

indications from recent literature and personal communication that ovule culture media may achieve best results with up to 10% sucrose and germination media down to 1% sucrose.

We did obtain 8 seedlings from ovules cultured at 39 days after full bloom that survived for 6 weeks, but these had very weak radicle growth and later died. This suggests that given more refined media and growth conditions, such ovules could probably be induced to produce viable plants.

In summary, the minimum date at which ovules were successfully cultured using these media and protocols is more than 53 days after full bloom. Germination of embryos younger than 67 days after full bloom in perlite is not recommended since the seedlings obtained tended to be weak. Further research in peach ovule culture should be oriented toward the improvement of embryo growth prior to germination. Abscisic acid is an excellent candidate for such research since it has been shown to play an important role in the normal development of zygotic embryos and promotes the accumulation of storage proteins in cotyledons (1).

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Peach and Nectarine Varieties—A Hypertex Index

This is a self contained computer program describing more than 600 varieties and their performance in southeastern United States. The information can be accessed by searching for any word or name. A master index of names and synonyms lists more than 6000 names used in the U.S., plus many foreign names. The index includes pedigree, origin, and a coded description. All North American Breeding programs are chronicled. The program is available for MS-DOS computers with an EGA or VGA monitor, and may be freely distributed. From Okie. 1993. *HortScience* 28:1186-1187.