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SOME FACTORS AFFECTING CALLUS PRODUCTION BY PEACH ANTHERS[1]

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Abstract. **Callusing of peach** (Prunus persica **L. Batsch) anthers occurred most frequently on a nutrient medium containing 2.0 ppm napthaleneacetic acid (NAA), 0.45 ppm 6-benzylamino purine (BA), 10 ppm ammonium nitrate (NH_4NO_3) and 1% sucrose. Callus was produced when anthers were explanted during early stages of spore development but not when anthers contained mature pollen grains. Haploid, diploid, polyploid and aneuploid cells were observed in callus from cultured anthers.**

Vigorous true-breeding diploid strains of commercial cultivars and breeding lines have been obtained by doubling the haploid chromosome complement in selected individuals of certain crops (3, 4, 7). Similar methods may be useful in obtaining desirable genotypes for biochemical and physiological studies (10) or for use in developing disease or pest resistant rootstocks in crops such as the peach.

Efficient use of haploid plants depends, however, upon their availability in large numbers (2, 10); they occur spontaneously at low frequencies among seedlings of many plants including the peach (5, 8, 9), but a more prolific source is desirable. Anther culture constitutes a rapid, practical means of obtaining haploid plants or haploid calluses in certain species. Calluses may be induced, in some of these species, to form buds or plantlets for subsequent development of mature plants (10).

Michellon *et al.* (6) observed haploid, diploid and triploid cells in calluses from cultured anthers of *P. persica*; Miller's medium with kinetin at 0.2 ppm, 2, 4-D (2, 4-dichlorophenoxyacetic acid) at 1.1 ppm and IAA (indoleacetic acid) at 0.1 ppm supported a higher percent of callusing (approx 22%) than did any of the other combinations of kinetin (0.2, 2.2 or 10.8 ppm), 2, 4-D (0.0, 1.1 or 11.1 ppm) and IAA (0.1 or 1.8 ppm). 2, 4-D was described as indispensable for callus induction.

The stages of spore development within cultured anthers were apparently examined by Michellon's group, but no mention was made of the developmental stages employed or of their relationships to anther callusing (6). The mitotic or pre-mitotic free-spore stages have been reported optimal for *in vitro* androgenesis in some but not all species (10). Neither embryoids from pollen grains nor plantlets from callus have yet been obtained by *in vitro* culture of peach anthers. The current report concerns effects of growth regulators, nitrogen, sucrose and stages of bud or pollen development upon callusing of peach anthers.

Materials and Methods

Growth factor study

Floral buds for these tests were excised from bud scales, measured for diameter, and then were surface sterilized by rinsing in 95% ethyl alcohol, immersing for 20 min in 1% sodium hypochlorite and rinsing 3 times in sterile distilled water. Some water was retained to keep excised buds moist until anthers could be explanted from them.

Our basic growth medium was a modification of Hoagland's inorganic nutrient solution (1) which had been supplemented with casein hydrolysate (500 ppm), adenine sulfate (25 ppm), inositol (100 ppm), nicotinic acid (0.5 ppm), thiamin HCl (0.5 ppm), benomyl (10 ppm) and ammonium succinate (0.3 ppm); pH was adjusted to 5.5 prior to heating for dissolution of agar (10 g/l). Media were distributed at the rate of 20 ml per 25 x 150 mm culture tube. The necessity for any particular member of the basic constituents was not examined. A factorial experiment was conducted, however, to determine the effects of sucrose (1, 5, or 10%), NAA (0.5 or 2.0 ppm), BA (0.045 or 0.45 ppm) and ammonium nitrate (0 or 10 ppm) upon 'Fla. 13-72' peach anthers, and the "best medium" was selected for use in subsequent studies. Two tubes each of 24 test media were inoculated with approximately 20 anthers from 'Fla 13-72' buds measuring 2.5 to 3.0 mm diameter. Anthers from similar buds contained the tetrad to free-spore stages of pollen development.

Developmental Study

Bud size and pollen development were studied in relation to callusing of 'Early Amber' and 'Sungold' anthers cultured on the "best medium" from the above tests. Size classes of buds for this study were (mm diameter): size 1, 2.4-2.9; size 2, 3.0-3.5; size 3, 3.6-4.5; size 4, 4.6-5.5; size 5, 5.8-7.0.

Five buds were sampled at random from each bud-size group of each cultivar, and 5 anthers from each of these buds were examined microscopically to determine the stages of pollen development which each contained. Three replicates of 'Early Amber' anthers (3 tubes per replicate; average 23 anthers per tube) were prepared from bud sizes 2 to 5.

One replicate (3 tubes; average 28 anthers per tube) was prepared with 'Sungold' anthers from newly harvested buds of each class; two additional replicates were constructed a day later from similar buds stored overnight at 4° C in moist petri dishes.

Cultures prepared for tests of growth factors were incu-

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bated at room temperature on laboratory benches with 16 hr of light from cool white fluorescent lamps; developmental studies were conducted, during the following spring; in a specially constructed growth chamber which provided fluorescent light at 180 microeinsteins/cm^2. Temperatures outside culture tubes in the latter tests were 23 ± 2°C just before lights were turned on and they gradually rose to 37 ± 2°C 11 hrs later. The higher temperature range was maintained for the remaining 5 hrs of each 16 hr light period.

The number of anthers producing calluses from locular regions was recorded. Data were subjected to analysis of variance after application of the arcsin $\sqrt{\text{percentage}}$ transformation. Duncan's New Multiple Range Test at the .05 level of significance was used for separation of means after F-tests indicated that significant differences existed.

Results and Discussion

Growth factors:

The percent of callusing was greater for 'Fla. 13-72' anthers when media contained 2.0 ppm NAA, 0.45 ppm BA, 10 ppm NH_4NO_3, and 1% sucrose than when other levels of these variables were employed (Table 1). Fortyfive percent of anthers callused when the above levels of each variable were combined in a single medium. Callusing rates observed on this medium in subsequent studies verify its efficacy for culturing peach anthers.

Table 1. Effects of growth factors upon callusing of 'Fla. 13-72' anthers.

Factor	Level (concn)	No. of observations[z]	Mean % of anthers callused	F[y]
Sucrose (%)	1	16	11.87	6.27*
	5	16	2.81	
	10	16	0.94	
NH_4NO_3 (ppm)	0	24	2.92	2.04 n.s.
	10	24	7.50	
BA (ppm)	0.045	24	1.88	7.07*
	0.450	24	8.54	
NAA (ppm)	0.5	24	1.67	6.33*
	2.0	24	8.75	

[z]Each observation represents approx 20 cultured anthers.
[y]*indicates significant value of F at .05 level; n.s. indicates non-significant F at .05 level.

Previous work indicated that 3% was better than 2% sucrose (6) but our percent of callused anthers was greater on 1% than on either 5% of 10% sucrose (Table 1). These conflicting results indicate a need for additional research with smaller increments of change over a wide range of sucrose concentrations.

Anthers callused more often when both NAA and BA were employed at their higher concentrations (Table 1). Additional experiments should be conducted to compare the kinds and levels of growth regulators used in our work to those of other workers (6); the data indicate, however, that 2, 4-D is not indispensable for induction of peach anther callusing.

Developmental effects

'Early Amber' anthers callused at a negligible rate (3/828 or 0.4%), while 'Sungold' anthers callused at a significantly higher rate (113/1298 or 8.7%). Differential cultivar responses may be most readily attributed to maturity differences in cultured anthers. Pollen maturation was attended by reduced propensity of anthers to proliferate (Table 2); neither microspore-mother-cells nor tetrads were present in 'Early Amber' anthers while these immature stages were associated with the highest callusing rate for 'Sungold' cultures. It should be noted, however, that 'Sungold' is a glabrous peach, i.e., a nectarine, 'Early Amber' is homozygous dominant for pubescence and 'Fla. 13-72' is heterozygeous for this trait. 'Sungold' has the higher chilling requirement (550 hr), while 'Early Amber' has 350 and 'Fla. 13-72' has 200 hr requirements. The cause of this apparent cultivar effect should be further examined.

Table 2. Effects of bud-size and refrigerated storage of excised buds upon callusing of 'Sungold' in 1973.

Replicate	Percent[z] of callused anthers/per bud-size 1	2	3	4	5
1 (cultured immediately)	36.6c	22.2b	14.0b	2.7a	0.0a
2 (refrigerated overnight)	35.6c	1.0a	5.3a	0.0a	0.0a
3 (refrigerated overnight)	13.8b	0.0a	0.0a	0.0a	0.0a

[z]Percent represents the mean of non-transformed data from 3 tubes (approx 28 anthers per tube) per replication of each bud-size group; means accompanied by similar letters are not significantly different at the .05 level in Duncan's NMR test of the transformed data.

Callusing was most frequent (28.6%) in 'Sungold' anthers from size 1 buds which contained the microspore-mother-cell, tetraspore, or uninucleate freespore stages of development (Table 2). It was significantly less frequent in buds of size 2 (7.7%) which contained uninucleate to immature-binucleate pollen grains and in buds of size 3 (6.4%) which contained immature-binucleate to mature pollen grains. A negligible percentage of callusing (1.2%) was obtained from anthers of size 4 buds, and no callusing was obtained in size 5 buds. Anthers of the latter 2 bud-sizes contained only mature binucleate pollen grains. Relationships between callusing and particular stages of pollen development were obscured by overlap of stages in microsporogenesis within bud-size classes and within individual anthers.

Replications which were constructed after refrigerated storage of excised buds yielded lower percents of callused anthers than did those which were explanted immediately (Table 2). The latter effect was more pronounced in size group 2 (22.2% vs. 0.5%) and 3 (14.0% vs. 2.6%) than in the smaller diameter buds of size group 1 (36.6% vs. 24.7%). Reduction in percents of callused anthers may have been due to continued maturation of, or low temperature injury to anther tissues, to microspore-mother-cells, or to spores in refrigerated buds. Additional work would be required to determine the true cause of this effect.

Forty-three haploid cells (n=8), 180 diploid cells, 5 aneuploid cells (n+1, 2 cells; n+3, 2 cells; n+4, 1 cell), and 4 polyploid (triploid or higher) cells were observed among 232 mitotic cells in squashed samples of 25 'Sungold' anther calluses. Haploid cells thus represented over 18% of all cells counted; variant ploidy levels were not noticeably restricted to calluses from anthers of any particular bud-size group.

Haploid, diploid and polyploid, but no aneuploid cells have been previously reported in peach anther callus (8). Additional research will be required to confirm our observations of aneuploid cells and to determine whether

similar cells occur more frequently under other experimental conditions or with other cultivars.

Aneuploid and diploid cells may have originated in atypical sporads, i.e., diads, hexads, octads, and decads, which were evident in squashed 'Sungold' anthers. Diploid and polyploid cells may have originated in either somatic tissues or spores, but haploid cells probably originated in developing microspores; more extensive studies would be required to verify origins of each cell type. Haploid, diploid, aneuploid, and polyploid cells in anther calluses are potential sources of peach plants with corresponding ploidy levels. Realization of this potentiality will depend upon development of systems in which peach plants can be regenerated from these cells or calluses.

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ORIGIN AND DESCRIPTION OF 'DORSETT GOLDEN' APPLE

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Abstract. **The introduction of 'Dorsett Golden' apple into Florida and its possible origin are discussed. This cultivar has been shown to be a pollinator for 'Anna'. It is an excellent eating quality fruit in its own right. A description of 'Dorsett Golden' is given because of confusion with other apples of the same or similar name.**

The 'Dorsett Golden' apple is growing in popularity because it serves as a pollinator for 'Anna', and is a delicious fruit in its own right. The increased interest in home gardening and local market sales has resulted in about one half-million apple trees being produced and sold in Florida between 1971 and 1981. About one-third of these were 'Dorsett Golden', despite there being no knowledge about its performance until 1976.

Origin

'Dorsett Golden' originated from a seed planted in 1953 by Mrs. I. Dorsett of Nassau, New Providence Islands, Bahamas (1). It is said to be a chance seedling of 'Golden Delicious', however this is doubtful because of its low chilling requirement. For example, chilling is known to be controlled by many genes, perhaps 10 to 20. We have observed thousands of Golden Delicious seedlings without finding any that were near the extreme low chilling requirement of 'Dorsett Golden'. For low chilling to occur in so many genes simultaneously and produce 'Dorsett Golden' would be very unlikely. Based on acceptable single gene mutation rates of one in ten thousand to one in every hundred thousand, the probability of 2 genes mutating together at the low mutation rate would be one per hundred million. For 5 genes to mutate simultaneously the chance would be one per hundred quintrillion or $(1/10{,}000)^5$.

Consider the possible origin of 'Dorsett Golden' not arising from a seed of 'Golden Delicious'. A strong case can be made that it originated from the germplasm of Mr. Stein at the 'Ein Shemer' Kubutz (1) or from another breeding program in Israel (5). Extremely low chilling apples are unknown outside the local Arab types available in northern Egypt and Israel (5, 7, 8). The 'Golden Delicious' cultivar was used in apple breeding by Stein and Oppenheimer in Israel and would account for 'Dorsett Golden's' resemblance of 'Golden Delicious' in shape and color. It is our speculation that a world traveler obtained fruit of this germplasm, probably as a result of visiting in Israel, and brought seed to Nassau where it was planted. 'Dorsett Golden' was assumed to be a seedling of 'Golden Delicious' either because the fruit from which the seed was taken was mistaken for 'Golden Delicious', or because the fruit from the seedling so resembled 'Golden Delicious'. While this is pure speculation, the odds of this happening appear to be far greater than chance mutation simultaneously of many chilling requirement genes.

'Dorsett Golden' was introduced into the United States in 1961 and about 2000 to 3000 trees were propagated in the Miami area during the 1960's by Newcomb Nursery. It was obtained for testing in Gainesville in 1973 from B. C. Bowker of Miami and in 1974 from Dr. R. J. Knight of the Plant Introduction Station at Miami. It should be noted that several clones with the same or a similar name have been distributed in Florida (4, 6), but that most 'Dorsett Golden' plants sold since 1977 have been traced to budwood distributed by the Fruit Crops Department of the University of Florida.

Description

Fruiting of 'Dorsett Golden' was seen on 2 grafted trees at the University Horticulture Farm in 1975. Its low chilling requirement and high fruit quality were immediately recognized and test plantings were established for additional observations. 'Dorsett Golden' has bloomed con-

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