Microspore Developmental Stage and Anther Length Influence the Induction of Tomato Anther Callus

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Abstract. Anthers of L-680A¹, 'Licato', and 'Ailsa Craig' tomato (*Lycopersicon esculentum* Mill.) were plated on Doy's basal medium 1 to determine whether microspore developmental stage and anther length influence anther callus production. Although calli were induced at all stages of anther development, anthers containing prophase I-stage microspores produced the highest frequency of calli. Fewer calli were produced as microspores approached the uninucleate and binucleate pollen stage. Callus diameter also decreased as anther development progressed. Significantly larger calli were produced from prophase I than later-stage anthers. Time of anther harvest (morning vs. afternoon) did not significantly affect callus number or diameter. Anther and flower bud length both were significantly correlated with anther developmental stage, the number of anthers producing calli, and mean calli diameter. In each case, anther length exhibited a significantly better correlation than bud length.

Efficient production of haploid or dihaploid tomato plants from microspores could provide plant breeders with the ability to produce large numbers of homozygous lines from an F₁ plant in just one generation. Although the development of haploid or dihaploid plants from cultured anthers have been demonstrated in several plant species, these techniques have not been widely adapted in tomato, because, until recently, callus production and plantlet formation rates were <0.7% (Zamir et al., 1980). However, selection of either an optimum medium or solidification agent can increase the frequency of callus formation to 13% and 18%, respectively. Callus induction frequency increases to 42% when these factors are combined (Jaramillo and Summers, 1990).

When immature anthers are excised and placed on a culture medium, typical gametophytic differentiation is inhibited, and cell division, callus formation, and plantlet regeneration can occur in a portion of the microspores (Dunwell, 1986). The degree of androgenetic response depends on the stage

of anther development and the species (Dunwell, 1985). Studies have shown that the stage of tomato-anther development influences whether calli or embryos will be obtained. Callus was produced by meiotic-stage anthers, whereas embryogenesis occurred from cells in the tetrad stage or beyond (Dao and Shammina, 1978). Researchers differ on the optimum stage of tomato anther develop-

ment required to produce the most anther callus. Gresshoff and Doy (1972b) found that tomato anthers in early meiosis were best, but anthers in late meiosis produced more callus for Zagorska et al. (1982). In contrast, tetrads (Zamir et al., 1980) or uninucleate microspores (Gulshan et al., 1981) were found to be best for generating calli.

In *Nicotiana*, a good relationship exists between the stage of anther development and flower bud length (Kasperbauer and Wilson, 1979). Therefore, correlating microspore development with bud or anther length might provide a way to select anthers more rapidly with high callus induction and growth potential. Although bud length criteria would be easy to use since no dissection of the bud would be required before selection, reports differ on which size bud contains microspores at an early stage of meiosis (Gresshoff and Doy, 1972b, 1974; Sharp et al., 1972). Part of this difference may arise due to a poor correlation between bud length and anther length. Direct measurement of anther length may prove to be a better criterion because anthers are initiated simultaneously, with few differences in growth observed through a length of 0.7 mm (Chandra et al., 1984; Greyson and Sawhney, 1972).

The objectives of this experiment were to: 1) determine whether anther developmental stage influences callus production and growth, 2) determine whether bud length or anther length was a better predictor of the number of anthers that form callus and the mean diameter of calli produced, and 3) determine whether the time of bud harvest (morning or afternoon) significantly affects the number and diameter of calli produced.

Three tomato cultivars were chosen for this study. 'L-680A' was selected because 60% to 80% of its anthers had produced calli in

Fig. 1. Microspore developmental stage and cultivar affect the number of tomato anthers per plate of five that produced calli. Bars represent means of 24 plates (120 anthers) each. SE = 0.21.

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Fig. 2. Mean diameter of tomato anther calli is affected by microspore developmental stage and cultivar. Bars represent means of calli derived from 24 plates (120 anthers.) SE = 0.19.

Table 1. Pollen mother cell meiotic development compared with anther and bud length in three cultivars of tomato.

Developmental stage	Anther	Length (mm)*		
		Bud		
		A. Craig	L-680A	Licato
Prophase I (leptotene)	<1.6	<3.0	<3.5	<3.5
Zygotene to diakinesis	1.6–2.0	3.0–3.5	3.5–4.0	3.5–4.5
Metaphase I to telophase II	2.0–2.8	3.5–4.5	4.0–6.5	4.5–7.0
Uninucleate	2.8–3.5	4.5–6.0	6.5–8.0	7.0–9.0
Binucleate	3.5–4.0	>6.0	>8.0	>9.0

*Anther length ranges represent values from 72 flowers. Bud length ranges represent values from 24 flower buds.

previous studies. 'Licato' and 'Ailsa Craig' were chosen to represent cultivars that respond poorly to anther culture (Jaramillo and Summers, 1990, 1991). Greenhouse-grown plants were watered as needed and fertilized weekly with 300 ppm of 20N-20P-20K + microelements (Peters Professional; W.R. Grace & Co., Fogelsville, Pa.). To accommodate the cytological examination and anther plating tasks, 10 buds per cultivar per AM/PM treatment were harvested on each of 6 days. Flower buds were collected at 10:00 AM and 3:00 PM each day and their length recorded. Buds were surface-sterilized for 15 to 20 sec in 70% ethanol, immersed in 0.5% sodium hypochlorite for 5 min, and rinsed four times with sterilized Type I water (ASTM, 1988). Individual buds were assigned to one of five meiotic stages based on cytological examination of one acetocarmine-stained anther per flower. The five meiotic stages were chosen to represent a range of anther development from prophase I- (leptotene) stage cells to binucleate pollen (Table 1).

Five remaining anthers from each bud were dissected, measured, and plated on a petri dish (Fisher 6 × 1.5 cm) containing 10 ml of DBM₁ medium, pH 5.8 (Gresshoff and

Doy, 1972a, 1972b), supplemented with 2 mg 1-naphthaleneacetic acid (NAA)/liter, 5 mg *N*-(2-furanylmethyl)-1*H*-purin-6-amine (kinetin)/liter, and 7 g Noble agar/liter. The dishes were wrapped with parafilm and placed in a dark growth chamber at 26 ± 1.5°C for 4 weeks. After the dark treatment, each plate was exposed to 4 weeks of 16-h photoperiod (88 μm·m⁻²·s⁻¹) provided by cool-white fluorescent lamps. Culture dishes were distributed at random in a growth chamber using a split-plot design with time of bud harvest (morning or afternoon) as main lots. Within main plots, plates representing three cultivars, five developmental stages, six harvest dates, and two replications were distributed at random. We recorded the number of anthers with calli and calli diameters 8 weeks after each harvest date. The diameter of callus pieces was determined by taking the mean of the polar and equatorial diameters observed in a reticle-equipped Nikon (Tokyo) SMZ-10 stereoscopic microscope. Data were tested by analysis of variance (ANOVA) techniques. Sources of variability that were biologically significant are presented in Table 2. Homogeneity tests between selected bud length and anther length correlations were calculated using the Z transformation (Steel

and Torrie, 1980). Even though anther development was similar for the three tomato cultivars (Table 1), we observed a range of developmental stages within each flower and within anthers from the same flower. In this study, anthers were smaller than those observed by Aung and Byrne (1977) and Gulshan et al. (1981), but similar to the values reported by Dao and Shamina (1978). Within each developmental stage, 'Ailsa Craig' produced shorter anthers than did either 'L-680A' or 'Licato'.

Since neither the time nor the date of bud harvest significantly affected the number of anthers that produced callus or callus diameter, anthers may be harvested at different times during the day with little effect on callus initiation and growth (Table 2). Anthers from various cultivars may exhibit a wide range of callus initiation and growth rates. In this study, 42% of the 'L-680A' anthers produced callus compared with 30% of 'Licato' or 28% of 'Ailsa Craig' anthers. Calli produced by 'L-680A' anthers grew almost twice as fast as 'Ailsa Craig' callus and a third faster than 'Licato' (data not shown).

As anther development progressed from leptotene to uninucleate, callus induction and growth significantly decreased for each cultivar (Figs. 1 and 2). Eighty-four percent of leptotene stage anthers produced calli while only 4% of the uninucleate stage anthers formed callus. No additional decline in callus production was noted as anthers developed from the uninucleate to binucleate stage. Callus diameter exhibited a similar pattern. Larger calli (2.2 mm) were produced by leptotene stage than by uninucleate or binucleate stage anthers (0.2 mm).

Significant cultivar × developmental stage effects were observed. Much of the difference can be noted in the callus production of leptotene vs. zygotene to diakinesis stage anthers (Table 2). While the number of anthers producing calli declined to 38% and 46% for 'Ailsa Craig' and 'Licato', respectively, only 10% fewer 'L-680A' zygotene stage anthers produced calli. However, when the performance of prophase I anthers, leptotene and zygotene through diakinesis, were compared to dyad, uninucleate, and binucleate stage anthers, significantly fewer 'L-680A' anthers produced calli (74%) compared to 'Ailsa Craig' (59%) or 'Licato' (52%) (Fig. 1). Much of the difference in callus diameter can be attributed to differential performance of 'L-680A' vs. 'Ailsa Craig' and 'Licato' anthers when leptotene and zygotene stage anthers were compared to other anther stages (Table 2). Although 'L-680A' prophase I anthers as a group produced calli with a mean diameter of 2.8 mm, post-prophase I stage anthers produced calli of smaller diameter (0.6 mm). This difference (78%) in growth was greater than that (66%) observed for similar stage 'Licato' anthers (Fig. 2).

To address the question of which variable, flower bud length or anther length, is a better predictor of anther developmental stage, the number of tomato anthers that produce calli, or mean calli diameter, correlation coefficients were generated and found to be sig-

Table 2. Condensed analysis of variance and mean squares for the number and diameter of tomato anther-derived calli.

Source of variation ^a	df	Callus	
		No. ^y	Mean diam (mm) ^y
Time of bud harvest (T)	1	1.00	0.24
Error A [Rep (Time)]	2	0.67	1.04
Harvest date (H)	5	1.37	1.68
Cultivar (C)	2	17.17***	15.17***
J	(1)	34.23***	28.15***
K	(1)	0.10	2.19
Developmental Stage (D)	4	229.63***	56.80***
L	(1)	57.51***	6.98*
M	(1)	822.63***	206.27***
N	(1)	37.93***	13.50***
O	(1)	0.44	0.44
C × D	8	3.64***	2.74**
J × L	(1)	10.50**	1.12
J × M	(1)	14.58***	17.10***
J × N	(1)	0.29	0.44
J × O	(1)	0.88	0.00
K × L	(1)	0.51	0.12
K × M	(1)	2.10	2.69
K × N	(1)	0.22	0.04
K × O	(1)	0.00	0.34
Residual interactions	159	1.46	0.92
Error B	178	1.10	0.87

^aJ = 'L-680A' vs. ('Ailsa Craig' + 'Licato'); K = 'Ailsa Craig' vs. 'Licato'; L = leptotene vs. zygotene +; M = (leptotene + zygotene +) vs. [(metaphase I+) + uninucleate + binucleate]; N = (metaphase I+) vs. (uninucleate + binucleate); O = uninucleate vs. binucleate.

^yMean squares.

*,**,***Significant at $P = 0.05, 0.01, \text{ or } 0.001$, respectively.

Table 3. Tomato flower bud length and anther length correlations with cytological stage of development, the number of anthers producing callus, and mean diameter of the callus produced.

Variable	Bud length	Developmental stage	Callus (no.)	Mean diam
Bud length	---	0.83***	-0.67***	-0.50***
Anther length	0.87***	0.95***	-0.78***	-0.61***
Homogeneity test ^a	$P =$	<0.005	0.08	0.16

^aHomogeneity tests between selected correlations were calculated using the Z transformation (Steel and Torrie, 1980).

***Significant at $P = 0.001$.

nificant (Table 3). Correlations that included anther length were higher than similar correlations with bud length. These results suggest that anther length is the better predictor of callus induction and growth.

The present study has shown that tomato anther calli can be induced from any stage of anther development from prophase I (leptotene) to the binucleate stage microspores, although some cultivars may not produce calli after metaphase I. The optimum stage for callus induction and growth was prophase I, which corresponds to an anther length of <1.6mm. These results agreed with those found by Gresshoff and Doy (1972b), who suggested that prophase I was best for callus induction. However, our results showed that there was more callus induction from microspores at early prophase I (leptotene) than at late prophase I (zygotene). Also, fewer calli were produced as anther length increased and development proceeded, resulting in poor callus induction from uninucleate or binucleate microspores. These observations

do not agree with those of Gulshan et al. (1981) and Levenko et al. (1977), who found that only 3-mm-long tomato anthers containing uninucleate stage microspores produced callus.

In previous studies, where fertile vs. male-sterile anthers were compared for their ability to produce callus, 26% of the male-sterile anthers produced callus compared to 0.2% of fertile anthers (Zamir et al., 1980, 1981). In this study, 1940 fertile anthers were plated and 33% produced callus. These results suggested that the developmental process of an anther does not need to be genetically disrupted to achieve increased callus induction rates.

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