Theor. Appl. Genet. 50, 53-56 (1977)

The Influence of Paternal Species on the Origin of Callus in Anther Culture of *Solanum* Hybrids*

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Summary. Stamen culture of several *Solanum* species and interspecific hybrids was performed and each stamen was scored for presence and origin of callus. Each species and hybrid has a characteristic callus growth pattern - either no growth (0), callus growth from the filament (F), or callus growth from the anther (A). Characteristic growth types of the interspecific hybrids indicate that callus growth takes precedence over no growth. Hybrids between F and A species exhibit mainly paternal passage of either characteristic callus type. Possible explanations for this pattern of inheritance are male plastome factors or imprinting of paternal genes. The latter explanation is presently preferred.

Key words: *Solanum* Hybrids - Paternal - Genes - Callus - Anther Culture - Imprinting

Introduction

Genetic analysis of plant tissue culture characteristics can be separated into two categories: tissue culture can be used either to obtain genetic variants or to score pre-existing variation. To obtain genetic variants with tissue culture, mutations are induced in culture and verified, after differentiation of the tissue and sexual reproduction, to be true genetic mutants (Carlson 1970; Maliga et al. 1973), and not acclimated physiological types (Mok et al. 1976), or variants of the normal gene dosage via aneuploidy, polyploidy, or chromosome substitution (Heinz and Mee 1971; Horak 1972). To score preexisting variation with tissue culture, explants of related plants are cultured and the mode of inheritance of a trait manifest in culture is decided by a comparison of the known genetic relationships with the observed pattern of the tissue culture characteristic.

This investigation deals with the latter type of analysis in *Solanum* "anther" culture, or more appropriately, stamen culture, since a portion of the filament is retained by the anther. The trait in question is the origin of callus growth, i.e. whether callus is derived from the anther or the filament.

Materials and Methods

The *Solanum* species and interspecific hybrids used were grown from true seed obtained from the collection of the Interregional Potato Introduction Station, Sturgeon Bay, Wisconsin. Plants were grown in the field during the summers of 1974, 1975, and 1976.

Stamens were cultured from buds of five to twenty plants of each species or hybrid in July and August. Buds used were primarily at the late meiosis or early microspore stage of microgametogenesis. Three, or sometimes two, stamens from each of five to fifty buds were cultured for each species or hybrid, with each stamen from a single bud cultured on a different hormonal variant of the basal medium (Murashige and Skoog 1962 or Miller 1963). Hormonal adjuncts ranged from two to four ppm IAA or 2, 4-D, and kinetin or benzyladenine. One stamen of each bud was fixed in ethanol-acetic acid (3:1) to estimate the meiotic stage of the bud.

After five to six weeks of culture in the dark at room temperature, callus growth for each stamen was categorized as non-existent (0), originating from the filament (F), or originating from the anther (A) usually in the region of the pore.

Results

Table 1 tabulates the occurrence and source of callus growth in stamen culture of *Solanum* species and their hybrids. The types of hormones and hormone levels

* Paper No. 2084 from the Laboratory of Genetics. Research supported by the College of Agricultural and Life Sciences, a gift from Frito-Lay, Inc., and grants from NIH (GM15422) and the International Potato Center.

Table 1. Relative success and source of callus growth in stamen culture of *Solanum* species and their hybrids over three years

	Culture Success*			Predominance of callus from the anther**		
	1974	1975	1976	1974	1975	1976
Species exhibiting primarily no callus growth (0)						
med (*S.medians* PI 265872)	0(18***)	-	-	-	-	-
mlt (*S.multidissectum* PI 210043)	.04(47)	-	-	.50	-	-
Species exhibiting primarily anther callus (A)						
brd (*S.brevidens* PI 218228)	.39(19)	.57(68)	.73(136)	.75	.92	.85
sct (*S.sanctae-rosae* PI 283089)	.42(12)	.29(128)	-	1.00	.86	-
spg (*S.spegazzinii* PI 205394)	.46(13)	.58(34)	.43(95)	1.00	.80	.73
ver (*S.verrucosum* PI 160228)	.48(31)	.41(28)	-	.80	.83	-
Species exhibiting primarily filament callus (F)						
bcp (*S.brachycarpum* PI 243344)	.38(16)	.55(12)	-	0	.43	-
clr (*S.clarum* PI 243355)	.67(14)	-	-	0	-	-
frn (*S.fernandezianum* PI 320270)	.71(7)	-	.22(109)	0	-	.16
phu (*S.phureja* PI 225683)	.36(11)	-	.28(110)	0	-	.16
Interspecific hybrids of 0 × 0						
med × mlt (WRF156)	-	.02(48)	-	-	0	-
mlt × med (WRF156)	-	0(36)	-	-	-	-
Interspecific hybrids of 0 × A and A × 0						
ver × mlt (WRF199)	-	.17(35)	-	-	.83	-
mlt × ver (WRF199)	-	.33(30)	-	-	.80	-
Interspecific hybrids of A × A						
ver × spg (WRF240)	-	.50(94)	-	-	.87	-
ver × sct (WRF233)	-	.82(11)	-	-	.89	-
Interspecific hybrids of A × F						
ver × clr (WRF88)	-	.33(9)	-	-	.33	-
spg × phu (WRF212)	-	.37(108)	.60(124)	-	.48	.16
brd × frn (WRF42)	-	-	.79(114)	-	-	.20
Interspecific hybrids of F × A						
phu × spg (WRF212)	-	.67(33)	.40(114)	-	.86	.74
frn × brd (WRF42)	-	-	.79(89)	-	-	.73

* Culture success = $\frac{\text{Number of stamens exhibiting callus growth}}{\text{Number of stamens cultured}}$

** Predominance of callus from the anther = $\frac{\text{Number of stamens exhibiting anther callus}}{\text{Number of stamens exhibiting anther callus or filament callus}}$

*** (number of stamens cultured)

were not related to the type of growth expressed. Further, most buds cultured were in the early microspore stage so that the effect of these two variables on the results was minor and is not indicated here.

The probability of finding callus growth from either the filament or the anther in culture ranges between 22 % and 73 % for all species except *S.medians* and *S.multidissectum*, where at most 4 % success is detected. Thus, these two species, wherein callus growth for individual stamens tends to be 0, are denoted 0 species. All interspecific hybrids between species not in the 0 category exhibit a range of culture success similar to the parent species, whereas hybrids between 0 species have at most 2 % success. Hybrids between *S.verrucosum*, a successful species in stamen culture, and the 0 species, *S.multidissectum*, result in an intermediate to good level of success.

Where callus is formed regularly, it tends to originate from either the filament or from the anther for individual stamens of each species and this tendency recurs from year to year. These are denoted F species and A species, respectively. Of the stamens forming callus, callus arises from the anthers of F species 0% to 43% of the time whereas A species produce callus from the anther 74% to 100% of the time, depending on the year and species.

Stamens of interspecific hybrids within and between F and A species also tend to form either F callus or A callus. A × A, 0 × A, and A × 0 hybrids produce A callus as routinely as do A species. Surprisingly, F × A hybrids produce callus from the anther as regularly as A species while A × F hybrids exhibit callus growth from the filament nearly as frequently as F species.

Individual plants within a species or hybrid reflect the general trend of that species or hybrid rather than segregate into plants with stamens producing primarily no callus, A callus, or F callus. This was determined in 1975 by recording the origin of buds by plant (unpublished data).

Discussion

The effect of various deficiency aneuploids upon the success of wheat anther culture indicates the presence of a dominant genetic factor on chromosome 4A which prevents callus formation in aneuploids (Shimada and Makino 1975). In the tuber-bearing Solanums, conversely, the control of success in stamen culture appears to be dominant to, possibly incompletely, the inability for callus to arise, as evidenced by the A × 0 and 0 × A hybrids.

Irikura (1975) reports comparable research distinguishing between callus origin from the filament and anther in several tuber-bearing *Solanum* species included here and in unpublished work by us. Although callus from the filament is much more frequent in Irikura's work, a rough division of the data into 0, F, and A callus tendencies indicates an agreement in nine of fourteen species. This reinforces the categorization presented. Another reinforcement is demonstrated by the data wherein variation in culture success between years for species within each category often appears to be large and random, whereas the proportion of callus arising from the anther is relatively stable between years. Thus, the type of growth is independent of the culture success.

The proportion of anther-derived callus in hybrids is generally in the same range as in either A or F species, so that the hybrids apparently express the phenotype of only one parental species rather than an intermediate phenotype. Also, the parent species characteristic expressed in hybrids of A and F species is not either A for all hybrids or F for all hybrids (as would be expected in a system of dominance), nor is it that of the maternal species. Instead it is the phenotype of the paternal species which is expressed in these hybrids. The genetic analyses of pre-existing variation expressed in tissue cultures of somatic tissue from genetically variable stocks have exhibited typical nuclear control of albinism (Venketeswaran and Mahlberg 1962), ontogeny (Buiatti et al. 1974), and chimerism (Sree Ramulu et al. 1976) whereas callus formation appears to be controlled by both nuclear and maternal influences in maize endosperm (Tabata and Motoyoshi 1965). Therefore, the genetic system of callus origin in *Solanum* species requires further interpretation.

Inheritance of the type demonstrated here can be attributed to one of two general modes. First, since the male plastome is not yet eliminated in uninucleate pollen (Nilsson-Tillgren and von Wettstein-Knowles 1970) it could be due to exclusive male transmission of a cytoplasmic factor in the hybrids via preferential proliferation of this factor like some plastome mutants in *Oenothera* (Kutzelnigg and Stubbe 1974), or nonrandom sorting out of this factor to male organs of the F_1, or simply a lack of this factor in the egg cytoplasm, for whatever reason. Second, this pattern of inheritance could also be due to imprinting of paternal genes, possibly those near the locus conditioning andric expression, since this locus is obviously expressed differentially in the stamen and the pistil. Examples of the second category exist in *Sciara* (Crouse 1960) and maize (Kermicle 1975), but for maternal chromosomes. The apparent lack of segregation of individual plants within a species or hybrid to types not representative of that species or hybrid would favor either imprinting or paternal inheritance because both are whole plant traits. However, the paucity of examples of paternal inheritance in *Oenothera* reduces its credibility as a viable alternative since this appears to be a general phenomenon in the *Solanum* species hybrids included, not a rare occurrence in an otherwise reversed (maternal) mode of inheritance.

A discriminating test of these alternatives is paired backcrosses of A × (A × F) and F × (F × A) where paternal inheritance would result in all F or all A plants, respectively, while imprinting, assuming a random activation of the A and F factors of the male parent, will give equal numbers of F and A plants in both crosses. The reciprocals of these backcrosses, (A × F) × A and (F × A) × F, would result in all A plants and all F plants, respectively, with either alternative, but would measure any maternal influence.

The amount and origin of callus in stamen culture of *Solanum* species and their hybrids appears to be a property of each species or hybrid. These traits are amenable to analysis and a substantial paternal influence is detected. Histological examination indicates anther callus to be gametophytic in origin (unpublished data). If this is confirmed, it has practical implications concerning the use of different species in stamen culture for obtaining somatic or sporogenous callus (from F and A species, respectively). Or of more fundamental interest, these two types of species may represent a preferential activation of sporophytic or gametophytic cells in F and A species. Differentiation of anther callus is necessary to expand upon either implication.

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Received February 11, 1977
Communicated by H.F. Linskens

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