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Technical Progress Report, 1965-66

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TECHNICAL PROGRESS REPORT for 1965-66, AEC Contract No. AT(11-1)-1400

Much new information has been obtained this past year concerning the normal pattern of floral bud development in 02 hybrid Tradescantia plants. Receiving special attention are changes induced by aging of the inflorescence, and the degree to which developmental rate is subject to environmental modification. Methods used have included detailed dissections of complete inflorescences, the compiling of daily flowering records, and the induction of ontogenetic mutant markers by acute irradiation. Part of the data from these studies are summarized in Mericle and Mericle (1966a, 1966b), preprint copies of which are enclosed with this report. Additional results in more detail are being readied for publication elsewhere.

In Table 1 is shown a typical record obtained from the dissection of a young bud cluster. Below the dissection record is the daily flowering sequence for a similarly aged cluster growing under the same environmental conditions. The flowering record extends through one complete bud cycle (the time in days from bud initiation to anthesis) and in this case represents the first complete cycle by the cluster since the onset of flowering.

In a number of cases, complete flowering records have been kept for individual inflorescences throughout their entire period of floral activity. In the intact plant, buds are continually initiated throughout the five or more months of floral activity. As many as 140 or more flowers may be produced by a cluster which never contains at any one time more than 17 buds in each of its two "cymes" (Mericle and Mericle, 1966b). This continuation of bud initiation over long periods of time is in contrast to the general "blooming-out" of preformed buds only, so often seen following

irradiation of unrooted Tradescantia cuttings (Sparrow and co-workers, unpublished). When senescence of the inflorescence finally does occur, it is not due to termination of bud initiation. Even though the rate of new bud formation does decline with cluster age (Mericle and Mericle, 1966b), at the time of last anthesis as many as five or more incompletely developed buds per "cyme" may still remain.

As shown in Fig. 7,8 of Mericle and Mericle (1966b), even though both bud number and flowering interval undergo large changes during aging of the inflorescence, the length of the bud cycle remains nearly constant. Age associated differences in the time of appearance of the various developmental stages within the cycle can, however, help to explain the mutation rate differences observed in clusters of different ages (Mericle and Mericle, 1966b).

Use of acute radiation data to predict age response to chronic radiation:

During this past year we have made considerable use of somatic mutations induced by acute irradiation to investigate individual bud development. The chief advantage of this method is non-destruction of the material being studied; such is not the case when the cluster is dissected. In the course of determining the effects of cluster age upon the size of the mutative cell population, the time in development of maximum mutability, and the duration of the effective period for mutation production, some valuable new insights have also been gained into the relationship between an organism's response to acute vs. chronic radiation.

Both mitotic cycle time and nuclear factors such as chromosome number, nuclear volume, and more recently, interphase chromosome volume, have been implicated by Sparrow and co-workers (Sparrow et.al., 1965) as important

determinants in the response of an organism to ionizing radiation. In the 02 clone of Tradescantia other factors such as inflorescence age can apparently also play contributory roles (Mericle and Mericle, 1965a, 1965b, 1966a, 1966b).

In the response shown by bud clusters of different ages to a single acute exposure of 60R X-radiation, the maximal mutation yield is far lower in the very old clusters than in young ones. Between mid-aged and young clusters, however, there is virtually no difference in maximum response which suggests that neither the size of the mutative cell population nor individual cell sensitivity differs. There is a possibility though that this lack of difference might in itself be radiation-induced if the cells of the young clusters originally possessed a higher original cell sensitivity. Radiation induced alterations in developmental rate may also be the cause of the shortening of bud cycle time observed in irradiated young clusters, the length of the cycle being below that typical for the environment in which the plants are growing. This point is being investigated in additional studies using lower amounts of radiation.

Another aging factor of importance especially under chronic radiation conditions, is the length of the period in development during which mutability remains high. Data from the acute X-radiation study indicate that the period in which the expressed mutation rate is 50 percent or more of maximum varies depending upon cluster age, being shortest in the very old and longest in the youngest clusters (Mericle and Mericle, 1966a, 1966b). If these results are used to simulate a chronic two-week exposure, the results shown in Fig. 1 are obtained. Note that even in the absence of any

difference in size of the mutable cell population, the youngest clusters could be expected to plateau somewhat later and at a higher level solely because of the longer effective period for mutation induction. Furthermore, if radiation effects were themselves a factor in the acute X-radiation results, their minimization in the case of low level chronic radiation should intensify the cluster age differential in mutation rate.

Visible and ultra-violet light as environmental modifiers:

During the Colorado studies on the biological effects of natural terrestrial radiation, strong correlations were observed between the occurrence of high somatic mutation rates in O2 Tradescantia and the presence of clear, dry, sunny weather with its high UV and visible light intensities and large diurnal temperature variations (Mericle and Mericle, 1965b). In a confirming growth chamber study wherein ⁶⁰Co was used to augment the level of normal background radiation, reduction in light level alone produced a decrease in mutation rate (Mericle and Mericle, 1965a, 1965b).

In the past two years we have conducted several experiments aimed at separating the contributions made by various environmental components to mutation rate, aging, and rate of floral bud development. Some of the earlier results are presented in Mericle and Mericle (1966b).

In a study just recently completed while we were at Brookhaven National Laboratory, the effects of difference in light level alone were investigated (no ionizing radiation was added). During these studies diurnal temperature variation was held to only 4 F-degrees. The comparative effects of 2800 and 1000 foot-candles on spontaneous somatic mutation rate in petals and the changes in mutation rate which accompany aging are shown

in Fig. 2. Just as in the presence of augmented radiation levels, spontaneous mutation rate is significantly greater in the presence of higher visible light level. In addition, data from dissections of inflorescences and daily flowering records indicate that a higher light level alone can increase bud number and decrease flowering interval sufficiently so as to shorten the total bud cycle time. The direction of these changes is much the same as that noted in previous studies which involved a combination of high visible light intensity with high daytime temperature and large diurnal temperature variation. In contrast, however, age changes in somatic mutation rate during this latest study showed the same pattern under both high and low light levels as manifest before only in the high light - temperature regime. The results of these studies are being readied for publication in more detail.

Other experiments conducted at Brookhaven were designed to test the effect of UV light on somatic mutation rate in plants receiving low levels of chronic irradiation (^{60}Co gamma). The results of analyses on these data appear at this time to be inconclusive. While the studies did show that UV light supplementation in a growth chamber is practicable on a continuous daily basis for three weeks or longer, considerable difficulty has been encountered in trying to achieve a true balance of total light energy for the UV and non-UV treated materials.

The nature of the color mutant used in aging studies:

The occurrence of somatic color mutations in the flowers of the hybrid Tradescantia 02 clone has been or is being used in several studies other than this one, as a sensitive indicator of radiation level (Mericle and

Mericle, 1965a, 1965b) and to measure the modifying influence of and interactions with other environmental components (Mericle and Mericle, 1965b, 1966a, 1966b; Sparrow, 1966). Relatively little is known, however, concerning the real nature of the changes involved in the production of a red mutant cell(s) amidst the normal bluish-purple cells comprising the petals and stamen hairs of the hybrid.

It has been generally assumed by those working with the 02 clone that the hybrid is heterozygous for flower color and that the mutation process probably involves either a small loss of chromosomal material (deletion) or an intra-chromosomal change (true mutation). Certainly the obvious lack of any selective disadvantage on the part of the red cell (notching of the petal does not occur even when a mutant sector extends to the margin of the petal) argues against any large chromosomal loss. In our own studies, we have always been perturbed about how such a sensitive discrimination of small differences in radiation level, involving only low multiples of natural background (Mericle and Mericle, 1965a), could be due to chromosomal breakage events. Yet data of Sparrow (1962) show that the same low temperature conditions which are conducive to higher somatic mutation rate also increase the incidence of chromosomal aberrations. More recent data of Nayar (1966, unpublished) suggests an association between micronuclei formation (usually indicative of chromosomal fragmentation) and the occurrence of red mutant cells in the stamen hairs.

For some time now we have been collecting data on the occurrence of micronuclei in stamen hair cells within the mutated cells of a sector per se and within a distance of five cells to either side of a mutant sector.

For the most part, sectors consisting of only a single mutated cell have been utilized. All of our material for micronuclear detection is Feulgen-stained (rather than propriono-carmin-stained as in Nayar's material) because of the high specificity of the Feulgen reaction for nuclear components. Our results to date are shown in Table 2 and Fig. 3.

In acutely irradiated buds, the occurrence of micronuclei is much greater than in unirradiated material, their frequency following a single acute exposure of 60R X-radiation being approximately one micronucleus per sector (a few sectors had more than one, a few none at all). Within sectorized hairs of irradiated material, the micronuclei are located predominantly within the mutated cells proper or within the immediately adjacent cell, approximately 25 percent of all such cells containing a micronucleus. These results tend to substantiate those of Sparrow and Nayar.

In connection with spontaneous mutations, however, quite a different picture emerges. Even within a sectorized hair, the frequency of micronuclei is only about 1 for every 10 sectors (one-tenth of that in irradiated hairs). Also, in spontaneous mutations, the micronuclei, when they do occur, are restricted to only the mutated cells. These findings strongly suggest that there is more than one means by which somatic color mutation is being mediated, with chromosomal aberration perhaps the more usual method under conditions of high radiation level, but rarely involved in low level radiation or in the case of spontaneous mutations. Several alternatives concerning possible mechanisms for the latter are being considered with some tests soon to be carried out.

A few other points concerning the somatic mutation from purple to

red color and sectoring ability of the hybrid condition have come to light this past year. It has long been known that the sectoring ability of the O2 clone is somatically transmissible: asexual propagation from cuttings has been the usual method of maintaining the O2 stock. The sectoring ability is relatively stable in terms of presence or absence of occurrence, but may vary from cluster to cluster or plant to plant as to frequency or sensitivity of induction (Mericle and Mericle, 1965b). A few instances of wholly red flowering plants derived from the O2 stock have been obtained at Brookhaven National Laboratory, but unfortunately their history as to somatic or germinal origin is not known with certainty. During the past two years we have succeeded in getting a good yield of seed from artificial self-pollinations of the O2 hybrid, and in the last year have found a method of increasing seed germinability from less than 4 percent to more than 50 percent. Further efforts in this regard are still going on. Among the 59 offspring of the hybrid already blooming, red segregants have been obtained in 8 instances. These red segregants vary considerably in other morphological traits and amount of pigment present, but all have flowers which are completely red except for purple tips on the end of each stamen filament. Among the rest of the F-1 generation, non-sectoring occurs in somewhat more than one-third, the others being sectorers in much the same way as their hybrid parents. Thus, sectoring ability is also transmittable germinally. The segregation ratio for red : purple sectoring : purple non-sectoring flowers is at present just on the borderline of significance from a typical monohybrid ratio of 1:2:1 ($p = 0.05-0.10$). Lines are being selected from among the seedlings for further study of the genetic mechanisms involved

in the pigment formation. At the same time some of the more distinctive morphological variants such as extreme dwarfing, zig-zag non-tillier growth habit, red leaves, flower doubleness, etc. are being kept in the hope that someday these might provide a basis for genetic mapping of the Tradescantia chromosomes.

Cell population changes during embryogeny:

Last year barley embryo material was collected for cytological study of changes which occur during early embryogeny, in a search for the factors responsible for the radiosensitivity differences exhibited by the various stages. Most of this material has now been processed and is undergoing detailed study and analysis. The materials consist of Feulgen-stained sectioned and squash preparations of embryos given a single acute exposure of 450R X-radiation at one of several stages of proembryo development and sampled thereafter at specific time intervals. Determination of mitotic frequency, mitotic cycle time, and cell numbers in the control and irradiated samples are being carried out. Hopefully, some new information on nucleic acid and protein changes will also be obtained. Preliminary data concerning cell numbers in the non-irradiated controls is already available.

In the early days of our barley work (1953) we arbitrarily divided barley embryogeny into seven stages (a - g) of proembryo (pre-differentiation) development, and six stages (1 - 6) of the differentiating embryo (Mericle and Mericle, 1957, 1961). For some purposes, stage 6 has been further subdivided into three stages (6a - 6c) of dormancy onset (Chang, 1957). In the present material, cell number tabulations among some of the control samples confirm that the number of cell divisions ensuing between the zygote and

late proembryo stages produces the same 1 - 1000 increase in cell number under the controlled growth chamber conditions now being used as occurred previously in greenhouse grown materials. The chief difference between the two sets of data lies in the shorter time encompassed by proembryo development under the growth chamber conditions.

As shown in Fig. 4, cell number doubles between stage a and b, and between b and c, then increases four-fold for each stage through g, the late proembryo. At the same time, the mean nuclear volume per cell decreases (Mericle and Mericle, 1961), initially more than two-fold, then 25 percent at each stage during the rest of the proembryo period. If the total volume occupied by all of the nuclei within the embryo is calculated as the product of the cell number times the cell nuclear volume, the results seen in Fig. 4 are obtained. Most notable is the sharp discontinuity of change in total nuclear volume of the embryo between stage intervals a - c vs. c - g. These data indicate that there is essentially little synthesis of nuclear material from the zygote through the four-celled stage and, in agreement with the mutation data (Mericle and Mericle, 1966b), suggest that the first few divisions after fertilization involve mostly a sorting out into several cells that which was originally contained in one.

Multi-partiteness of proembryo chromosomes:

The results of new analyses using recessive lethality and complete R-1 head sterility to determine the number of cell initials responsible for formation of the R-1 generative tissue in barley are reviewed in the attached manuscript by Mericle and Mericle (1966b). As set forth therein, large isomutant-carrying sectors are possible and do occur following irradiation

of the one and two-celled stages of embryo development. Sectors encompassing as many as five or more heads are twice as frequent as after mid-proembryo irradiation; yet the probability that mutations segregating within the main R-1 head will also be shared by at least a second head is only 50 percent, the same as for mid-proembryos. Thus the likelihood that the chromosomes of the early proembryo are highly multi-partite, becomes greater and statements such as the following by Gaul (1964) the more fallacious: "... zygotic and proembryo irradiation plainly lead to advantages: the whole new organism is genetically uniform, i.e., is not a chimera."

Despite the fact that the apparently high degree of multi-partiteness reduces the occurrence of extremely large isomutant R-2 populations to a somewhat lower frequency than we had originally anticipated, the proembryo method of irradiation for mutation induction still has the decided advantage of much larger sector size and concomitantly lower sterility than the dry grain method. The case for proembryo vs. mature grain irradiation has already been set forth clearly by Mericle and Mericle (1962, 1963), but new evidence concerning the efficiency of irradiating specific proembryo stages is presented for the first time in Mericle and Mericle (1966b).

Depth dose in proembryos:

For some time now, ⁶⁰Co has been our choice of radiation sources chiefly because of the ease with which a number of barley heads or Tradescantia inflorescences can be irradiated at one time for either an acute or a chronic exposure, and the possibility of carrying out long term chronic irradiation under controlled environment with essentially the same energy spectrum as that used for the acute application. In fact, any use

of X-radiation in this contract research during the past two years has only been because of delays in getting the new ^{60}Co gamma source at Michigan State University operable.

The high energy level of the gamma emissions from ^{60}Co (1.3 Mev) provide for deep penetrability of biological structures and little fall-off with increasing tissue depth. Dose build-up within the first few millimeters below the surface can present a problem, however, in the case of shallowly situated structures. At the surface the tissue receives only 37 percent of the maximum dose, the latter not being achieved until a depth of 3-5 mm is reached (Watson, et al., 1954). The fact that young barley embryos are situated in the ovary within the critical 0-5mm depth of tissue suggested to us earlier (Mericle and Mericle, 1961) that reduced dosage might explain to a large extent the seemingly low efficiency of ^{60}Co gamma irradiation of young proembryos as compared with 250 Kev X-irradiation.

This past year, after the ^{60}Co source at Michigan State University became operable, an experiment was conducted to test whether the theoretical differences in radiation dose in the first 3-5mm of tissue depth were indeed great enough to produce detectable differentials in biological effects. Barley heads containing embryos in one of several stages of proembryo development (from zygote through mid-proembryo) were taped to the front and back sides of quarter-inch lucite (to serve as a tissue equivalent for those embryos behind the lucite), then given a single acute exposure of 800R from ^{60}Co . Following the irradiation, the embryos continued development to maturity under controlled environment. Data on germination and early seedling growth have been obtained from the irradiated and control materials.

Cursory examination of the germination data indicates that a decided difference in response is manifest by very young proembryos irradiated with and without the tissue equivalent. Detailed analyses of all the data will be carried out next year by means of the University's CDC 3600 computer.

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EXAMPLE OF A TYPICAL RECORD

Table 1. Dissection record of a young cluster which has been blooming for four days and produced a total of seven flowers. Both "cymes" flowered on the day of dissection.

Pigmentation		Stage	Bud	Number	Bud	Stage	Pigmentation	
Stamen	Petal	microsporo- genesis	size mm	bud positions	size mm	microsporo- genesis	Stamen	Petal
complete	complete	mature pollen 2-nucleate	7.0 ⁺	1	7.0 ⁺	mature pollen 2-nucleate	complete	complete
partial	partial	young pollen 2-nucleate ≠	7.0 ⁺	2	7.0	young pollen 2 nuclei =	onset	onset
		young pollen 2-nuc. + div.	7.0 ⁻	3	6.5	young pollen nuclear div.		
		young pollen 1-nucleate	6.5	4	6.0	young pollen 1-nucleate	no stamen hair div- isions stamen hair divisions	
no stamen hair divisions		young pollen 1-nucleate	6.0 ⁺	5	5.0 ⁺	young pollen 1-nucleate		
stamen hair div- isions		young pollen 1-nucleate	5.0 ⁺	6	5.0	young pollen 1-nucleate		
		tetrads, few dyads	4.5	7	4.0 ⁺	meiosis II, dyads, some tetrads		
		early prophase	3.5	8	3.5	early meiosis		
		leptotene-zygotene				leptotene		
		premeiosis	3.0 ⁺	9	2.5	premeiosis, stamen hairs present		
		stamen hairs present				premeiosis, no sta- men hairs present		
		premeiosis, stamen hair initiation	2.0	10	2.0			
2 bracts + 3 sepals + 3 petals + 6 stamens + single carpel			1.5	11	1.0 ⁺	2 bracts + 3 sepals + 3 petals + 6 stamens present + single carpel present		
2 bracts + 3 sepals + 3 petals + 6 stamens + carpel primord.			1.0	12	< 1.0	2 bracts + 3 sepals + 3 petals alternate ♂ sepals + 6 stamens alt. & opp. ♂ petals		
2 bracts + 3 sepals + 3 petals + 3 stamens + 3 stamen primord.			< 1.0	13		2 bracts + 3 sepals + 3 petals and 3 sta- men primordia both alternate ♂ sepals		
2 bracts + 3 sepals + 3 petals + 3 stamen primordia alt. ♂ sepals				14		2 bracts + 3 sepal primordia		
2 bracts + 3 sepal primordia				15		2 opposite bract. primordia		
2 opposite bract primordia				16		simple floral primordium		
simple floral primordium				17				

Daily flowering sequence for similarly aged bud cluster as that above.

1 2 2 2 2 2 3 2 1 2 1 1 1 2 2 0 2 1 1 2 1 1

Digits represent the number of flowers produced per day per inflorescence, during the first complete bud cycle of 22 days.

Table 2. Occurrence of micronuclei in association with mutant sectors in stamen hairs of irradiated and non-irradiated Tradescantia inflorescences.

Stamen hair history	# cells scored	# mutant cells	# mutant sectors	# micro-nuclei	Frequency $\mu n/cell$	Ratio $\mu n/sector$
Non-irradiated						
Non-sectored hairs	945	----	----	2 (+ 1?)	0.002	----
Spontaneously sectoring hairs	425	22	21	2 (+ 2?)	0.005	1/10.5
X-ray induced (60R acute) sectoring hairs	260	27	16	14 (+ 1?)	0.054	1/1.1

Fig. 3

LOCATION AND FREQUENCY OF MICRONUCLEI
IN PROXIMITY TO CELLS OF MUTANT SECTORS

Fig. 4

Fig. 4