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THREE-YEAR REPORT

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- I. Diplontic Selection and its Modification by X-Irradiation in a Colchicine-Treated Grass Hybrid.
- II. Comparative Radiosensitivity Studies in the Gramineae.

Summary

Introduction

This investigation began with an attempt to control ploidy levels in colchicine-treated meristems by interaction with gamma radiation. The production of polyploids through colchicine treatment has from the beginning been an empirical and unpredictable procedure. It occurred to us that in view of the expected radio-resistance of polyploid tissue (McCormick and Rushing, 1964; Sparrow, 1965; Bhaskaran and Swaminathan, 1965; many others) it might be possible to inhibit the growth of normal tissue in a chimeric meristem and permit the doubled tissue, produced by colchicine, to form new and fertile shoots in a predictable fashion. We have not yet succeeded in this effort.

Important advancements toward an understanding of radiosensitivity and its prediction have been very recently made, especially by Sparrow and his associates (Sparrow, 1965; Sparrow, Sparrow, Thompson and Schairer, 1965). In their view the higher radioresistance shown by some polyploids is a manifestation of their smaller interphase chromosome volume and does not have as a basis the presumed protective effect of genetic redundancy. In a comparison of energy absorbed per chromosome at lethal exposures between diploid and polyploid species they found no statistically significant difference.

The importance of this new interpretation to radiobiology in general justifies extensive testing of the hypothesis. Accordingly, we expanded our program of investigation and a unique series of representatives of the Gramineae ranging from diploid to octoploid levels were assembled and a preliminary investigation made. An enlarged program is envisioned for next year. We believe that our past experience as well as the data and materials we've developed may permit us to make a significant contribution

toward what effects polyploidy and hybridity have on sensitivity to ionizing radiation.

Main Research Accomplishments

I. Preliminary investigations.

The initial experimental material was a sterile, triploid hybrid between Agropyron cristatum and A. subsecundum. We established that:

1. The lethal level of radiation for this plant was approximately 4000R.
2. The average interphase chromosome volume (14.0 cubic microns) is a valid index of radiosensitivity for this plant.
3. The sterility of the hybrid is due to failure of synapsis. No homologous pairs are present; most of the 185 PMC interpreted at diakinesis or metaphase I had 21 clear univalents.

II. Colchicine and Radiation Interaction.

In successive years, 3 different combinations of colchicine and radiation treatments were tried on populations of the hybrid described above in an attempt to control diplontic selection in a chimeric meristem:

1964-1965: Recurrent and alternating treatments of colchicine and gamma irradiation. Colchicine treatment (0.2%) via the roots and by external spray on crown was given at 5 weekly intervals. Gamma irradiations (1000R, 500R) were applied at 5 weekly intervals following each colchicine treatment.

1965-1966: Clones immersed to top of crown in aerated 0.2% colchicine for 12 hours, then in aerated tap water for 12 hours, alternately, for 4 days. Three days later these were separated into lots and radiated with 2000R, 3000R, or 4000R of gamma radiation, with controls.

1966-1967: Exceptionally large, vigorous clones were given a cotton-pack colchicine treatment in crown and base of culms for 5 days. These were divided into lots and subjected to 500R, 1000R, or 1500R of X-irradiation two weeks later.

The first year we concluded that the colchicine treatments were given too late. Histological studies showed that spike initiation began in very early spring. The second year we concluded that the gamma dosages employed were too heavy; spike production was profoundly limited. The third year we concluded that we were simply not getting enough hexaploid tissue in the meristem to test the hypothesis. This plant is extraordinarily resistant to the usual effects of colchicine.

A great deal of data was collected (summarized in Annual Reports). However, the experiments can be quickly and honestly summarized as follows: Our attempts to produce a fertile hexaploid from a sterile triploid through inter-action of colchicine and ionizing radiation were an unequivocal failure.

III. Comparative radiosensitivity studies in the Gramineae.

The third year we began to turn our attention to the above subject.

A. Influence of types of polyploidy and hybridity on radiosensitivity.

In view of the success with which Sparrow and his associates (cited earlier) have had with average interphase chromosome volume

as an index of sensitivity we obtained this information for the polyploids and hybrids involved, summarized in Table 1.

These species and hybrids were subjected to various levels of acute X-irradiation during their vigorous spring growth. The data is summarized in Table 2 and Figure 1-7.

The following conclusions were drawn from this preliminary experiment:

1. The sterile 4n hybrid between A. trachycaulum and H. jubatum was clearly the most radioresistant of the plants tested. Figures 1-7 portray only differences in height of culms, and not luxuriance of growth, number of culms, spike production, etc. On the basis of all observations there was no doubt that this plant was the least sensitive.
2. A. cristatum (2n) ranked next to the 4n hybrid above in radioresistance.
3. The most radiosensitive plants were H. jubatum and the 8n fertile hybrid between A. trachycaulum and H. jubatum.
4. Ploidy level per se appears to be relatively unimportant as a factor in radiosensitivity.
5. No marked correlation between ICV and sensitivity is evident. A reason for this may be conclusion No. 6 below.
6. A serious criticism of this study is the fact that lethal levels were not reached (excepting the 8n A. trachycaulum X H. jubatum). This was due to lack of filtration in the X-ray apparatus which resulted in large quantities of weakly-penetrating, soft X-rays in the dosages measured. This will

be corrected by the use of a 1 mm aluminum filter in the expanded experiment next year.

Marked contrast in the effects of heavy vs. light dosages was evident at 6-8 weeks (Figures 1-7). This difference greatly diminished by 12 weeks. The initial contrast was clearly due to marked delay in growth of flowering culms in plants receiving the heavier dosages.

Growth stimulation at lighter dosages, resulting in plant height exceeding the controls, was particularly evident in diploid and tetraploid A. cristatum. Auxin imbalance (Sax, 1963) and precocious maturation (Van't Hof and Sparrow, 1965) have been suggested as explanations for this effect in other plants.

It is of interest to note that while the sterile tetraploid hybrid between A. trachycaulum and H. jubatum was the most radio-resistant of the plants tested, the fertile, colchicine-induced octoploid derivative of this hybrid was one of the most sensitive. Meiselman, Sparrow and Gunkel (1961) reported a hybrid *Nicotiana* more radiosensitive than either parent. Pershad and Bowen (1961) found hybrids between distantly related species of *Lilium* to be more sensitive than the species themselves. The effects of hybridity on radiosensitivity deserve further study.

B. Attempted hybridization between A. subsecundum and A. cristatum.

This hybrid occurs naturally in the field but has not yet been synthesized. That its progenitors are those species suggested above is virtually certain (Boyle and Holmgren, in press). However, unequivocal proof is desirable since in our studies on comparative radiosensitivity we especially value hybrids and polyploids of known origin.

Extensive attempts to synthesize this hybrid were made the last two years, and are described in the Annual Reports. Unfortunately we were unsuccessful. It is not surprising that difficulties are encountered in crossing species separated since perhaps Tertiary time.

C. Karyotype analysis as an index of radiosensitivity.

Braegger (1966) established that total chromosome length, measured at mitotic metaphase, is unrelated to radiosensitivity. However, if this data is converted into average chromosome length (suggested by A. H. Sparrow) an inverse relationship between average chromosome length and radioresistance becomes apparent (Table 3), except for Crepis capillaris. Chromosome length of this species should be checked from fresh material since our measurements were extrapolated from camera lucida drawings (Hollingshead, 1930) and do not represent direct measurements.

Proposed research: Comparative Radiosensitivity Studies in the Gramineae.

Objectives for 1967-1968:

1. To determine what influence autopolyploidy and allopolyploidy have on radiosensitivity.

The preliminary experiment performed last year indicated that polyploidy does not have an important influence except as it may be related to average interphase chromosome volume. However, the absence of a clear-cut relationship between sensitivity and interphase chromosome volume in this preliminary experiment clouds the issue and a more intensive study needs to be made with an expanded population.

2. To determine what effect hybridity has on radiosensitivity.

The preliminary experiment performed last year on an F_1 sterile hybrid between Agropyron trachycaulum and Hordeum jubatum indicated that this hybrid is more radioresistant than the parents. Other investigators (Meiselman, Sparrow and Gunkel, 1961; Pershad and Bowen, 1961) in contrast, found hybrids to be more sensitive than the parents. Populations of 4 hybrids and their parents have been assembled, permitting an expanded analysis.

A significant feature of the above investigations is the employment of experimental materials whose origin and cytology is well understood (Boyle, 1963; Boyle and Holmgren, 1955; Ashman and Boyle, 1955; Dewey, 1961).

3. To further explore the usefulness of average chromosome volume at mitotic metaphase as an index of radiosensitivity.

The information gained last year suggests that this has considerable promise. The usefulness of average chromosome length

leads us to think that average metaphase chromosome volume will be even more valuable as an index of radiosensitivity. Wakong-Vaartaja (1963) found a close correlation between total chromosome volume per cell and frequency of chromosomal aberrations in X-irradiated plants. Using pairs of closely related species having the same chromosome number but a large difference in chromosome size she found that the species with a much greater volume of chromosomes also had a much higher percentage of chromosomal aberrations.

Sparrow and Evans (1961) chose not to use chromosome size at metaphase as a source of data for radiosensitivity studies because it is slow and tedious data to gather, and because metaphase chromosome size and interphase nuclear volume appeared to be highly correlated. We think it quite possible that chromosome volume determinations from materials uniformly treated with colchicine, using root tip or coleoptile squash preparations, may be less difficult (at least for the Gramineae) than dissection of meristems, preparations of slides by the paraffin method, and selecting nuclei for measurement. Furthermore, the complications of floral apices and fluctuations in nuclear volumes are avoided. Lastly, this method would have the advantage of direct measurement of a real entity.

There are of course difficulties in calculating metaphase volume. Although chromosomes approximate a cylinder and the formula for volume can be applied, the problems of constrictions, satellites, accentuated chromatids, curved chromosomes, and heterochromatic segments present difficulties. We are experimenting with measurement of chromosome mass by photo-electric cell and scanning devices which may offer a rapid index of volume.

<u>Species or Hybrid</u>	<u>Average Nuclear Volume (microns)</u>	<u>Somatic Chromosome No.</u>	<u>Average Interphase Chromosome Volume</u>
<u>Agropyron cristatum</u> , 2n	299.3	14	21.3
<u>A. subsecundum</u> , 4n	436.4	28	15.5
<u>Hordeum jubatum</u> , 4n	412.1	28	14.7
<u>A. trachycaulum</u> X <u>H. jubatum</u> , 8n	815.5	56	14.5
<u>A. trachycaulum</u> X <u>H. jubatum</u> , 4n	351.1	28	12.5
<u>A. cristatum</u> , 4n	309.6	28	11.0
<u>A. trachycaulum</u> , 4n	265.1	28	9.4

Table 1. Summary of nuclear and average interphase
chromosome volumes of species and hybrids involved.

<u>Plant</u>	<u>Av. Interphase Chrom. Volume</u>	<u>Chromosome Number</u>	<u>% Normal Growth</u>
<u>A. cristatum</u> , 2n	21.3	14 (diploid)	38.4
<u>A. subsecundum</u> , 4n	15.5	28 (allotetraploid)	35.7
<u>H. jubatum</u> , 4n	14.7	28 (allotetraploid)	22.7
<u>A. trachycaulum</u> X <u>H. jubatum</u> , 8n	14.5	56 (auto-allo- octoploid)	36.8
<u>A. trachycaulum</u> X <u>H. jubatum</u> , 4n	12.5	28 (allotetraploid)	60.3
<u>A. cristatum</u> , 4n	11.0	28 (autotetraploid)	40.5
<u>A. trachycaulum</u> , 4n	9.4	28 (allotetraploid)	36.3

Table 2. Effects of 8000R on growth six weeks after irradiation. Average interphase chromosome volumes and ploidy levels are compared.

<u>Plant</u>	<u>Average Chromosome Length at Metaphase</u>	<u>Lethal Level</u>
<u>Hordeum vulgare</u>	6.7	4348R
<u>Triticum aestivum</u>	8.4	4022R
<u>Agropyron cristatum</u> X <u>A. subsecundum</u>	8.5	4000R
<u>Vicia faba</u>	11.4	1000R
<u>Crepis capillaris</u>	13.6	3750R

Table 3. Comparison of lethal levels of radiation with average metaphase chromosome lengths in root tip cells for four species and a hybrid.

(Lethal levels of H. vulgare and T. aestivum predicted by Sparrow, Thompson and Schairer, 1965, and those of C. capillaris and V. faba experimentally determined, same publication. Chromosome length of C. capillaris from Hollingshead, 1930; T. aestivum and H. vulgare from Bhaskaran and Swaminathan, 1961.)

Figure 1. *Agropyron cristatum*, 2n. Comparative growth rates of irradiated and control plants.

Figure 2. *Agropyron cristatum*, 4n. Comparative growth rates of irradiated and control plants.

Figure 3. Agropyron trachycaulum X Hordeum jubatum, 4n.
Comparative growth rates of irradiated and control plants.

Figure 4. Agropyron trachycaulum X Hordeum jubatum, 8n.
Comparative growth rates of irradiated and control
plants.

Figure 5. Agropyron trachycaulum, 4n. Comparative growth rates of irradiated and control plants.

Figure 6. *Agropyron subsecundum*, 4n. Comparative growth rates of irradiated and control plants.

Figure 7. Hordeum jubatum, 4n. Comparative growth rates of irradiated and control lots.

Graduate students trained; degrees granted.

1. Paul J. Bottino. M.S. degree, Department of Botany, Utah State University, 1965. Radiosensitivity and cytogenetic studies on an interspecific grass hybrid. Mr. Bottino is now a doctoral candidate at Washington State University.
2. David R. Braegger. M.S. degree, Department of Botany, Utah State University, 1966. Karyotype analysis as an index of radiosensitivity. Mr. Braegger is now an instructor at the College of Southern Utah, Cedar City.

Publications

1. Bottino, P.J. 1965. Radiosensitivity studies on an interspecific grass hybrid. Jour. Hered. 56: 225-258.
2. Braegger, D.R. 1966. Karyotype analysis as an index of radiosensitivity of a grass hybrid. M.S. Thesis, Utah State University
3. Boyle, W.S. and A.H. Holmgren. 1967. A cytotaxonomic study of a hybrid between Agropyron cristatum and Agropyron subsecundum. Submitted to Madrono.

Appraisal of present status of knowledge in the
field of comparative radiosensitivity with reference
to growth inhibition and lethality.

For many years the conventional wisdom among investigators concerned with the biological effects of ionizing radiation has been that the prime basis of damage lies in chromosome fracture and subsequent loss of acentric fragments. This accounted also, it was believed, for the apparent radioresistance of polyploids since the genetic redundancy present should permit them to lose fragments without seriously impairing essential processes.

Major modifications of these generalizations and new concepts of the bases of radiosensitivity have very recently appeared in the literature. Evans (1965) presented evidence that chromosome breakage and loss is not the primary reason for reduction or cessation of growth in irradiated meristems of Vicia faba. Instead, he concluded that radiation blocks the mitotic process, and premature differentiation or cell death occurs. Although the loss of fragments can lead to cell death---certainly grossly deficient cells are inviable---this is probably not the most important factor in Evans view.

Sparrow and his associates (Sparrow, 1965; Sparrow, Sparrow, Thompson and Schairer, 1965) have supplied important evidence that the best index of radiosensitivity thus far is the average interphase chromosome volume, and more specifically, the amount of energy absorbed per chromosome. In their view the higher radioresistance shown by some polyploids is a manifestation of their smaller interphase chromosome volume and does not have as a basis the presumed protective effect of genetic redundancy. In fact, Sparrow (1964) has questioned whether the term "differential radiosensitivity"

is a useful one in the light of his evidence that the presumed difference is slight if comparisons are made on the basis of interphase chromosome volume and energy absorbed per chromosome. Convincing evidence is presented indicating that growth inhibition and lethality primarily result from injury to the genetic material. The conclusion is reached: "a precise relationship between DNA content and sensitivity, however, has not been established. Significant changes in sensitivity occur without a corresponding change in DNA content of nuclei."

Thus far we have not found ICV values to be as useful and reliable as have our much more experienced colleagues. ICV data is subject to considerable variation from fluctuation in growth cycles, floral meristem initiation, selection of nuclei for measurement, and is sometimes not closely reproducible in different laboratories.

Among the many problems of radiosensitivity one of the most interesting is the fact that woody plants are approximately twice as radiosensitive to acute exposures as would be predicted on the basis of ICV (Sparrow, 1964). The basis of the inheritantly greater sensitivity is not understood. The limited data on hybrids (Meiselman, Sparrow and Gunkel, 1961; Pershad and Bowen, 1961) suggests that they may also be more sensitive than predicted, although our data is contradictory.

The ever growing use of ionizing radiation in medicine, industry, studies on the fundamental nature of the hereditary material, plant breeding, and mutagenesis lends urgency to the need for a better understanding of the basis of radiosensitivity and its prediction.

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