Table 1. Effects of α-ketoglutarate and other components on the tyrosine oxidase activity of a homogenate of nutsedge tubers.

Components added to homogenate	Activity $Q_{O_2}^{O_2}$ (N)
None	17
α-ketoglutarate (α-K)	26
α-K + folic acid	3
α-K + ascorbate	31
α-K + pyridoxal phosphate	36
α-K + glutathione	56
α-K + glutathione + ascorbate	37
α-K + glutathione + ascorbate + pyridoxal phosphate	68
None + glutathione + ascorbate + pyridoxal phosphate	31
α-K + glutathione + ascorbate + pyridoxal phosphate + folic acid	18
None + glutathione + ascorbate + pyridoxal phosphate + folic acid	30

7.0. Activity measurements were taken for 1 hr.

The final concentrations of L-tyrosine and α-ketoglutarate were $6.8 \times 10^{-3}M$ and $5 \times 10^{-2}M$, respectively, while the final concentration of the cofactors was: ascorbic acid, $5 \times 10^{-2}M$; pyridoxal phosphate, $5 \times 10^{-5}M$; reduced glutathione, $3.3 \times 10^{-3}M$; folic acid, $5 \times 10^{-4}M$.

Table 1 shows results of a typical experiment. Activity was stimulated by α-ketoglutarate alone, or in combination with either ascorbic acid, pyridoxal phosphate, or glutathione, but the greatest effects were obtained with a single combination of these. Folic acid with only α-ketoglutarate or in combination with the afore-mentioned cofactors markedly inhibited activity. These results are contrary to the stimulation by folic acid reported for this system in mammalian tissue (*3*) and the cockroach (*4*). Preliminary studies (*5*) indicate that folic acid is required for the oxidation of L-phenylalanine to L-tyrosine in the nutsedge homogenates.

It appears that the oxidation of L-tyrosine by homogenates of nutsedge follows a pathway in which tyrosine is deaminated by a transamination reaction with α-ketoglutarate, which requires pyridoxal phosphate (*6*) and yields *p*-hydroxyphenylpyruvic acid (Fig. 1). This acid is oxidized to homogentisic acid and carbon dioxide. This system in nutsedge evolved 115 $Q_{CO_2}^{O_2}$ (N) with the combination of cofactors in Table 1 that gave the greatest activity. The intermediate, 2,5-dihydroxyphenylpyruvic acid, is involved in the process which requires ascorbic acid and glutathione.

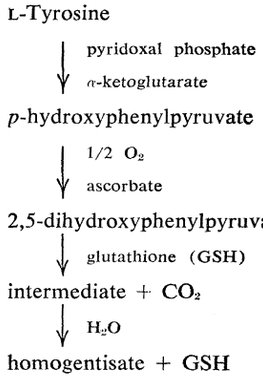

Fig. 1. A proposed scheme for the oxidation of L-tyrosine in *Cyperus rotundus* L.

The present study establishes that L-tyrosine is oxidized by a pathway similar to that reported in mammals and the cockroach (*7*).

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Correlation of Nuclear Volume and DNA Content with Higher Plant Tolerance to Chronic Radiation

Abstract. The causes of extreme differences in radiosensitivities of different plant (or animal) species have long puzzled radiobiologists. Our investigations show that, for diploid species, the larger the nuclear volume, the more radiosensitive the organism. Correspondingly, species with large nuclei have more deoxyribonucleic acid (DNA) per nucleus than those possessing small nuclei. Our data now make it possible to predict fairly accurately the tolerance of plant species to ionizing radiation on the basis of average nuclear volume and DNA content. The same correlations are expected to hold for some microorganisms and for animals and may explain differences in sensitivities of different cell types in many living organisms.

It has been noted in previous investigations (*1–3*) that the chromosome size characteristic of a species is related to the amount of radiation required to produce a given effect. In view of the tedious nature of accurately determining chromosome size or volume, no really quantitative data have been presented. We assumed that differences in chromosome size or volume in different species would be reflected in comparable differences in size or volume of interphase nuclei (*4*). On this basis, average nuclear volumes of a number of plant species of known radiation tolerance have been determined.

A summary of preliminary information concerning the tolerance of higher plants to chronic gamma irradiation from cobalt-60 was given by Sparrow and Christensen (*2*). In a later report 79 species of plants were grouped according to daily dose rates required to produce a comparable degree of morphological deformity or growth inhibition. The dose rates required varied from 30 to 6000 r/day. The most resistant plants were polyploid (*3*). In most of our tolerance experiments accurate measurements of growth inhibition were not made, but the row (or group) of plants which appeared to have shown not more than about 10 to 20 percent of the normal growth was chosen as the critical dosage level. Thus, the end point used was an approximation which can be assumed to be the daily dose rate which would essentially stop further cell division. In our studies the critical dosage level as determined above seems sufficiently valid because the possible error for each species is relatively small compared to the variation between species. By using the degree of growth inhibition as a measure of radiation damage, we have found that plant species ranging from algae to higher plants differ in their tolerance by a factor of at least 5000. The tolerance difference was still at least 100-fold when algae and known polyploid species were eliminated.

Collections for nuclear volume studies were made from growing plants before floral transition. Shoot apices were killed, fixed in Craf III, dehydrated, and infiltrated with paraffin by the use of a tertiary butyl alcohol series. Sections were cut at 10 μ and stained with safranin-fast green. The diameters of interphase nuclei in the tunica layer(s) and outer corpus of the vegetative meristems were measured with a Zeiss ocular micrometer. Ten nuclei on each of two slides were measured for each species, and average nuclear volumes were calculated. The deoxyribonucleic acid (DNA) was extracted from root-tip material with sodium chloride, a modification of the Schmidt-Thannhauser procedure (*5*), and the amount of DNA was estimated by the

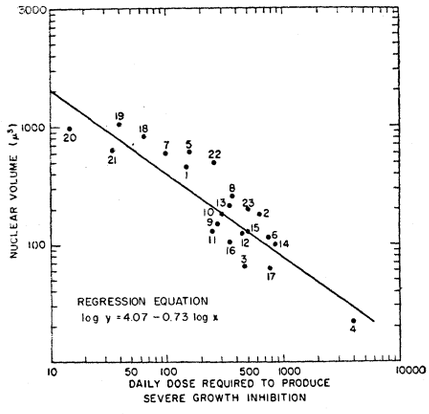

Fig. 1. Relationship between nuclear volume and radiosensitivity in 23 species of plants. 1, *Allium cepa*. 2, *Anethum graveolens*. 3, *Antirrhinum majus*. 4, *Arabidopsis thaliana*. 5, *Brodiaea bridgesii*. 6, *Graptopetalum bartramii*. 7, *Haworthia attenuata*. 8, *Helianthus annuus*. 9, *Impatiens sultanii*. 10, *Luzula purpurea*. 11, *Nicotiana glauca*. 12, *Oxalis stricta*. 13, *Pisum sativum*. 14, *Raphanus sativus*. 15, *Ricinus communis*. 16, *Saintpaulia ionantha*. 17, *Sedum oryzifolium*. 18, *Tradescantia ohiensis*. 19, *Tradescantia paludosa*. 20, *Trillium grandiflorum*. 21, *Tulbaghia violacea*. 22, *Vicia faba*. 23, *Zea mays*.

diphenylamine reaction (*6*). The nuclear volumes of the root and shoot meristem cell nuclei are approximately the same in the plants studied.

The average volumes of shoot meristem nuclei from 23 diploid species were plotted against the chronic daily dose required to produce severe inhibition of growth. The resultant graph (Fig. 1) shows a clear relationship between the two variables—that is, in general, the larger the nuclear volume the more sensitive the cells (or meristems) of that species. The population regression line has a negative slope of mean value −0.73 with 95-percent confidence limits of −0.882 to −0.582. While the slope of the curve relating nuclear volume and radiosensitivity is less than −1, this does not rule out the possibility that it may approach a slope of −1 when more extensive data are available. A slope of −1 would be of considerable interest since it would mean that the product of the two variables (log dose × log nuclear volume) would be a constant. Since dose can also be expressed as ionizations per unit volume, we can deduce that a reasonably constant number of ionizations is required to inhibit multiplication of somatic diploid cells regardless of the average size of the nucleus (*7*).

Table 1. DNA content and average volume of root-tip nuclei and radiosensitivity of six species of plants. The chromosome number of the species is shown in parentheses. Determinations: P, photometric; E, chemical extraction.

Av. vol. of root-tip nuclei (μ^3)	DNA content ($\mu\mu$g) per Nucleus P*	Nucleus E	Chromosome E	Radioresistance (r/day)†
	Lilium henryi (24)			
1100	100	100	4.1	80‡
	Tradescantia paludosa (12)			
640	52	59.4	5.0	40
	Allium cepa (16)			
570	40	54.3	3.4	150
	Vicia faba (12)			
490	18	38.4	3.2	120
	Zea mays (20)			
280	4	14.1	0.7	375§
	Glycine max (40)			
150		6.5	0.16	400

*As determined by Rasch and Woodard. †Daily dose required to produce severe growth inhibition (in gamma greenhouse). ‡Estimated value based on data for *Lilium longiflorum*. §Estimated value based on field experiment.

It has been reported that nuclear volume and the amount of DNA per nucleus may be closely correlated (*8*). We are, therefore, attempting to extend the correlation by making DNA measurements on species of known nuclear volume and radiosensitivity. From Table 1 and Fig. 2 it can be seen that our preliminary results indicate that the amount of DNA per nucleus is related to nuclear volume and radiosensitivity and that average DNA content per chromosome is also related to radiosensitivity in the species studied. Published values of DNA content (*9*, see Table 1) also show a reasonable correlation with average nuclear volume as determined in our laboratory. With decreasing nuclear volumes, that is, from the larger to the smaller volumes, there is a corresponding reduction in DNA content and in radiosensitivity. Much more extensive data are needed to establish the degree of correlation, but it seems highly probable that a general relationship may exist between average nuclear volume and average DNA content. There are certain exceptions—nuclear size and DNA content of a tissue or of a species do not always vary together (*10*)—but this should not invalidate the general concept.

The above correlations have been worked out with plant material. However, since plant and animal nuclei have so much in common, it seems highly probable that the same correlations will hold for some microorganisms and for some animal species as suggested by Puck (*11*). Certainly other factors are important, but DNA content and nuclear volume may also be correlated with differences in radiosensitivity of different cell types or different stages of development in animals as well as in plants (*12, 13*).

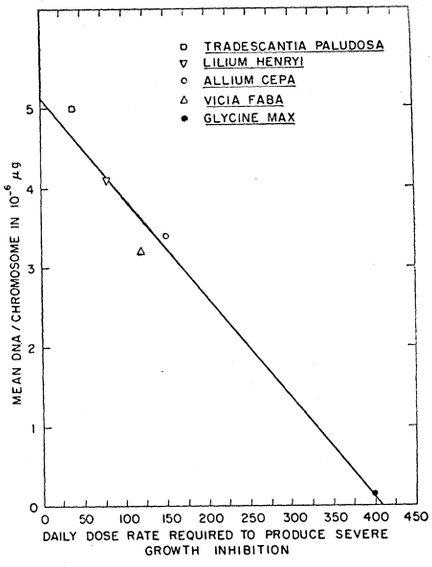

Fig. 2. Relationship between mean DNA values per chromosome and radiosensitivity in five species of plants.

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12. A more thorough discussion of the theoretical aspects of the nuclear volume correlation and other factors affecting the responses of plants to radiation exposures is in press (*7*).
13. A portion of this investigation was carried out during the tenure of a postdoctoral fellowship (CF-10,708) to one of us (J.P.M.) from the National Cancer Institute, U.S. Public Health Service. We are indebted to L. A. Schairer, Anne Rogers, and Agnes Smith for their excellent technical assistance. The research was carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.

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