STUDIES ON THE RADIATION BREEDING IN THE GENUS *MENTHA*

(VI) DOSE-RESPONSE CURVE FOR ROOT GROWTH AND INTERSPECIFIC DIFFERENCE IN RADIOSENSITIVITY

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Introduction

It is a well known fact in radiobiology that different organism shows different degree of sensitivity to radiation. Moreover, since the early studies by JOHNSOH (1933), SMITH (1942) and DAVIES (1962a, 1962b), it has been reported that there is also a marked difference in radiosensitivity between varieties or lines of the same species. Studies on interspecific difference in radiosensitivity not only provide valuable informations in radiobiology but also are required from the practical point of view in radiation breeding.

A series of experiments has been carried out by us to clarify the radiobiological and genetical aspects of interspecific differences in radiosensitivity in the genus *Mentha*. In the present report, effects of gamma rays on root growth will be presented with special reference to the dose-response curves and the interspecific difference in sensitivity.

Materials and Methods

5KR to 60KR of ^{60}Co gamma rays were given to dormant seeds of twenty of species or lines in the genus *Mentha* at a dose rate of 96R/min. That is, a sample of two petri dishes, with 50 seeds in each, was irradiated with ^{60}Co gamma rays of 5KR, 10KR, 15KR, 20KR, 40KR and 60KR. Prior to iradiation, the seeds were kept in a desiccator over saturated $NaClO_3$, solution for more than two weeks, during which all the material had the same moisture contents of about 17～20%. Immediately after irradiation, the seeds were immersed in usuplun sterilizing solution for five hours and washed with distilled water, and allowed to germinate on water soaked blotting paper in petri dishes. The dishes were placed under the daily alternations of temperature, especially of 15°C for 16 hours and 30°C for 8 hours. The non-irradiated control seeds were treated in the same way as the irradiated ones. Measurement was taken on the length of the seminal roots 20 days after irradiation.

Results

The mean root length of each irradiated plot was expressed as a ratio to the corresponding control value. This proportion will be indicated by "Ar". Fig. 1 shows the dose-response curves for 8 species out of 20 species or lines. From this figure the following features can be drawn :

(1) The eight species tested revealed different sensitivity. *M. arvensis* L. var. *piperascens* ($2n=96$) was most resistant and *M. arvensis* L. var. *agrestis* ($2n=72$) most sensitive.

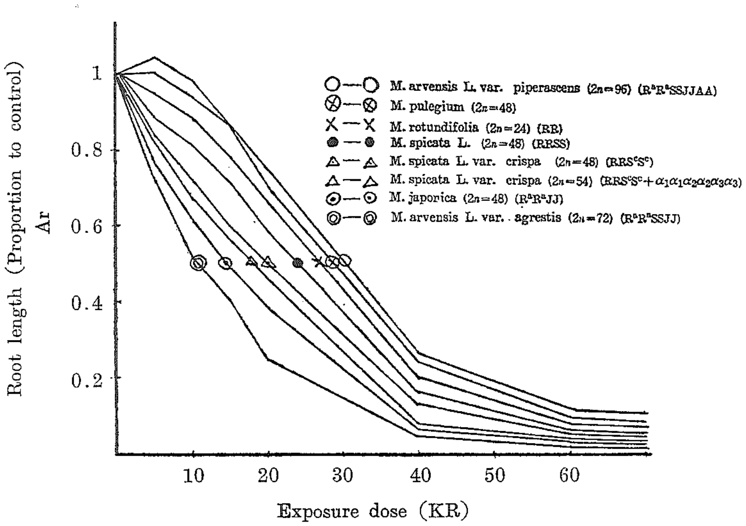

Fig. 1　Dose-response curves for root growth 20 days after irradiation for eight species in the genus *Mentha*.

(2) Dose-response curves are of sigmoid type for all the species tested and the more sensitive species have curves with steeper slope.

(3) By comparing the corresponding doses to a given value of Ar for any two species, it can be found that the ratio between two doses is almost constant.

(4) After irradiation of 5KR, a slight increase in root length was observed in some species.

(5) Root length was never reduced to zero even at the highest dose.

The length of root emerged at the highest dose is thought to be independent of radiation effect. For further comparison of dose-response curves between species, this length was subtracted from the mean of the irradiated plots and from that of the control plots, and the ratio between the residual values was calculated. This ratio will be referred to as "Ar" hereafter. Then in view of the above results (3), Ar were plotted against dose D divided by D_{50} (the dose at which Ar is 0.5) for each species in Fig. 2.

It can be seen in Fig. 2 that points for all the species used fall along the same line of sigmoid type. It is clear that all the dose-response curves can be approximated by one general function shown below :

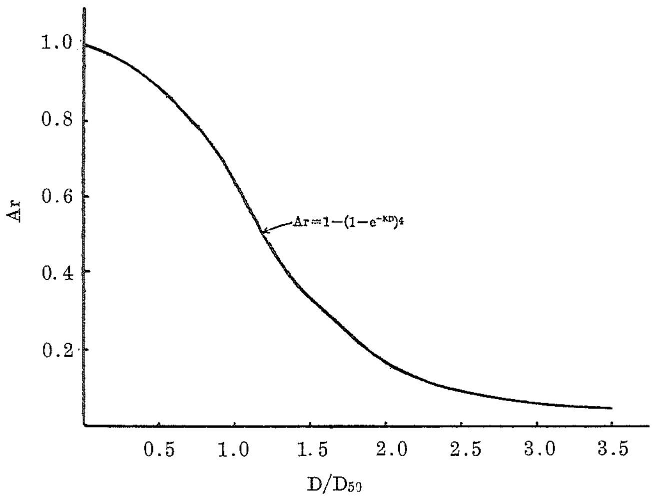

Fig. 2　Ar values plotted against dose D divided by D_{50} and an approximate curve to them

$$Ar = f\ (KD) \quad \cdots\cdots (1)$$

where K is a characteristic value for each species. Next, the function f was estimated according to the curve in Fig. 2 which takes the familiar sigmoid shape of the multi-target model in radiobiology. In order to indicate whether the following type of function fits the observed values with an appropriate value of n, integers from 1 to 12

$$Ar = 1-(1-e^{-X})^{n} \quad \cdots\cdots (2)$$

were substituted for n, where X is a variable proportional to D and n is an integer corresponding to the number of targets. It was found that function (2) shows the best fit for the observed values when n is 4. Two mathematical ways of estimation of n were tried and gave the same result. With n equals 4, X in (2) has the following relation to D:

$$X = 1.438D/D_{50} \quad \cdots\cdots (3)$$

Hence if we put

$$1.438/D_{50} = K \quad \cdots\cdots (4)$$

it can be shown that all the dose-response curves can be approximated by the following function irrespective of the sensitivity:

$$Ar = 1-(1-e^{-KD})^{4} \quad \cdots\cdots (5)$$

The above results were confirmed for the other species of mint.

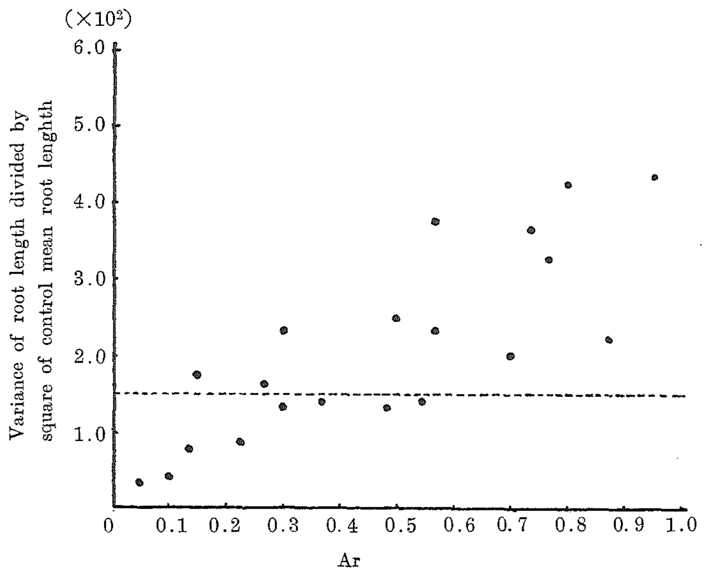

Fig. 3 Intra-interspecific variance of root length as a function of Ar calculated for the twenty *Mentha* species

Fig. 3 shows intra-interspecific variance of root length divided by the square of control mean root length for the twenty species or lines. It is clear that the variance follows almost the same pattern of curve inspite of interspecific difference in sensitivity when plotted against Ar instead of D. The variance decreases with the decrease of Ar showing no peak or plateau.

Frequency distribution of two subgenera of *Mentha* used in the second experiment for sensitivity has been presented in Fig. 4. These species belong to two subgenera of the genus *Mentha*, *Pulegium* subgenus and *Menthastrum* subgenus. A clear difference in the distribution among two subgenera was observed. The *Pulegium* subgenus were confined to high sensitivi-

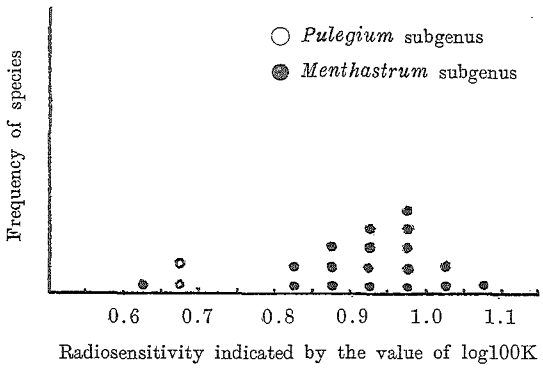

Fig. 4 Frequency distribution of twenty species of *Mentha* belonging to two subgenera for radiosensitivity expressed as log100K. Each mark represents one species

ty while the *Menthastrum* subgenus showed middle or low sensitivity as far as this experiment was concerned. There was a wide variation in radiosensitivity in the species of the *Menthastrum* subgenus. *M. arvensis* L. var. *agrestis* ($2n=72$) was most sensitive among the latter subgenus while *M. arvensis* L. var. *piperascens* ($2n=96$) was most resistant. The former species is about four times as sensitive as the latter species. The values of D and K for some respective species have been shown in Table 1.

Table 1. Interspecific difference in radiosensitivity of root growth calculated for eight species

Species	No. of chromosome ($2n$)	Genome	※ D_{50}	※※ K
M. arvensis L.	72	R^aR^aSSJJ	12.1^{KR}	11.94
M. japonica	48	R^aR^aJJ	13.4	9.24
M. spicata L. var. *crispa*	54	$RRS^cS^c+\alpha_1\alpha_1\alpha_2\alpha_2\alpha_3\alpha_3$	18.3	8.13
M. spicata L. var. *crispa*	48	RRS^cS^c	19.8	7.28
M. spicata L.	48	RRSS	23.4	6.06
M. rotundifolia	24	RR	25.1	5.27
M. Pulegium	48	—	25.9	4.46
M. arvenis L. var. *piperascens*	96	$R^aR^aSSJJAA$	30.3	2.99

※ Dose at which Ar value is 0.5
※※ Coefficient of sensitivity

Discussion

The present study indicated that there was a large difference in radiosensitivity between species of *Mentha*. There was also differential distribution for sensitivity between two subgenera. From these facts, it is thought to be necessary to consider interspecific difference in determining doses to be used in practical radiation breeding. It is also erquired to see if such large interspecific difference in sensitivity exists for other radiation effects important from a practical and radiobiological point of view.

Dose-response curves showed the same shape of sigmoid type. Interspecific differences in the dose-response curves may be divided into two types by existence and non-existence of the general function shown in (1). In order to simplify terminology of this phenomenon, we shall distinguish these two types as Common Response (CR) type and Difference Response (DR) type. The CR-type of interspecific difference afford favorable points for expressing the relative sensitivity. The radiosensitivity of a species can be expressed only by the value K in equation (1) irrespective of dose used. The fact that the value K is proportional to the inverse of D_{50} is clear from equation (4), indicating that K represents quantitatively the level of radiosensitivity. In other words, we can express the quantitative difference between species only by calculating the ratio between their K values. In a statistical analysis where additivity is needed, the value of log100K is a better expression than K itself. To avoid negative logarithmic cases, K is multiplied by 100. We shall hearafter call K, coefficient of sensitivity. In the CR-type, the degree of effect on root growth "Ar" can be predicated at an arbitrary dose, if the best approximate function is determined and the value of K is known for the considered species.

The CR-type of interspecific difference which was obtained in the genus *Mentha* suggests that the primary events induced by radiation at cell level may be identical for all the species investigated inspite of the marked difference in their sensitivity. The differential radiosensitivity may be explained by the difference in the probability of occurence of the primary events leading to depression of root growth at a unit dose of irradiation.

Though it was found that dose-response curves could be approximated by one general function for the multi-target model, further investigations about the primary effects at cell level are required in order to apply the target theory to clarify the present results since the reduction in root length is just final output of a chain of radiation effects.

Literature Cited

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放射線によるハッカ属植物の育種学的基礎研究
（第6報）ハッカの根長における線量反応曲線と種間差異

小　野　清　六

要　　　約

ハッカの異なる種の乾燥種子に種々の線量の γ 線（^{60}Co）を照射し後直ちに，これをペトリ皿上に播種して発根をうながし，20日後の種子根長に対する照射効果を調べた結果，根長抑制程度に著しい種間差異が認められた．また，その線量反応曲線は次の関数できわめてよく近似できることがわかった．

$$Ar=1-(1-e^{-KD})^4$$

ここに Ar は対照区平均に対する比で示した各線量区の根長平均，Dは線量，そしてKは種に固有の定数である．種の放射線感受性は線量に関係なく，このKの値だけで表示でき，かつ表示された値は厳密に量的な意味をもつ．20の種あるいは系統について放射線感受性を推定した結果，最大約4倍の種間差異が見られ，また，ハッカ属の第1亜属と第2亜属間で感受性の分布が異なっていた．

線量反応曲線の型から感受性の種間差異の機構について若干の考察を試みた．