Studies on the Radiation Breeding in the genus *Mentha*

(VIII) Relation between the water content of mint seeds and their sensitivity to different dose of γ-rays

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Introduction

It is a well known fact in radiobiology that different organisms show different degrees of sensitivity to radiation. 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.

Since CALDECOTT (1955b) induced mutations in barley by X-ray, it has been accepted by numerous workers that a positive correlation exists between the water content of biological systems and their X-ray sensitivity.

However, EHRENBERG and NYBOM (1954) reported that, actually, an inverse relationship existed between the X-ray sensitivity and the water content of dormant barley seeds which had reached weight equilibrium at different relative humidities.

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, relationship between the water content of dormant seeds of genus *Mentha* and their sensitivity to γ-rays was studied.

Material and Method

Dry dormant seeds of four species in the genus *Mentha* were irradiated by γ-rays from ^{60}Co source in the γ-room of Institute of Radiation Breeding. That is, a sample of two petri dishes, with 200 seeds in each, was irradiated at a dose rate of 95R/min with ^{60}Co gamma rays of 5KR, 10KR, 20KR and 40KR.

Table 1. Chemicals used to control seeds water content

No. of desiccator	Stored in desiccator at room temperature over :		Expected relative humidity of air (%)	
1	H_2O	Satu.		100.0
2	$NH_4H_2PO_4$	〃	Ca	90.0
3	K_2CrO_4	〃	〃	85.0
4	NaCl	〃	〃	80.0
5	NH_4NO_3	〃	〃	65.0
6	$Ca(NO_3)\cdot 4H_2O$	〃	〃	55.0
7	K_2CO_3	〃	〃	45.0
8	$MgCl_2\cdot 6H_2O$	〃	〃	35.0
9	KCH_2COOH	〃	〃	25.0
10	P_2O_5	〃	〃	20.0

Prior to irradiation the water content of seeds of the species used was equilibrated by storage at different relative humidities (produced by substance contained in desiccators as shown in Table 1) until they reached weight equilibrium. To determine the water content of thes eeds, samples were weighed before and after drying in an electric oven for one day at 110℃.

Immediately after irradiation, the seeds were immersed in usuplun sterilizing solution for five hours and washed with distilled water, and allowed ot germinate on water soaked blotting paper in petri dishes. The dishes were placed under the daily alternations of temperature, especially of 15℃ for 16 hours and 30℃ for 8 hours. The irradiated seeds were sown 7 days after irradiation. Radiosensitivity were taken on measured and evaluated on the bases of the height of individual seedling and survial rate at about one month after germination when they had developed 2~3 leaves. The materials used in this experiment are shown in Table 2.

Table 2. Genome constitutions and chromosome numbers of the species used

Species	No. of chromosome ($2n$)	Genome
M. rotundifolia (L.) HUDS	24	RR
M. spicata L.	48	RRSS
M. arvensis L. var. *agrestis*	72	$R^a R^a$ SSJJ
M. arvensis L. var. *piperascens*	96	$R^a R^a$ SSJJAA

Result

M. rotundifolia (L.) HUDS ($2n=24$) Nearly identical results were obtained from both *M. rotundifolia* (lines 〔4〕 and 〔3〕) used. Data of results reported are, however, mainly on 〔4〕 *M. rotundifolia*. There was no difference in germination percentages among irradiated seeds fo various water content, except when the content was more than 20% or less than 11%. The highest survival percentage following any dose fo

Table 3. Relation between water content of mint seeds and seedling height after exposure to various doses of γ-rays (*M. rotundifolia*)

Water content of seed (%)	Exposure dose (KR)			
	5 ※	10	20	40
5	4.23%	15.69%	6.93%	1.21%
8	19.56	18.11	13.54	5.92
11	52.15	32.21	20.72	9.69
14	54.63	34.53	26.41	13.82
17	53.59	39.68	27.16	10.51
20	52.16	31.44	21.79	3.92
23	46.89	20.51	15.49	2.62
26	16.24	13.59	11.23	1.29

※ Index, control=100

irradiation was among seedlings which sprouted from seeds that contained betwen 11 and 20% water. No seedling was obtained from seeds that contained more than 23% or less than 5% water. Seedling height at 30 days after γ-rays irradiation is given in Table 3. It shows that the seedling height decreased with the increase in dosage of γ-rays. The greatest resistance of dormant seeds to γ-rays was observed at a water content of between 11 and 20%.

M. spicata L. ($2n=48$) In dormant seeds with 5 to 14% water, regardless of the dosage of γ-rays, there was a little difference in germination percentage. From the data on seedling height at 30 days after γ-rays irradiation, it seemed that the radiation resistance decreased at the dosage increased (Table 4). At 10KR and 20KR the

Table 4. Relation between water content of mint seeds and seedling height after exposure to various doses of γ-rays (*M. spicata* L.)

Water content of seed (%)	Exposure dose (KR)			
	5	10	20	40
5	5.21%	7.43%	7.15%	6.21%
8	18.23	18.41	17.91	8.32
11	64.93	45.05	33.78	10.15
14	64.25	45.23	36.52	17.03
17	63.52	45.51	36.16	16.51
20	63.04	42.52	35.03	9.12
23	14.95	13.91	11.19	6.92
26	4.87	12.78	2.61	1.13

seeds with water content of 11 to 20% were more resistant to irradiation than at other percentages, and at 40KR they were also somewhat more resistant with a water content of 14 to 17%.

M. arvensis L. ($2n=72$) Most dormant seeds with high water content, such as at 32%, failed to germinate, even with no irradiation. On the other hand, the seeds with a lower water content showed relatively good germination, especially when the content was 5 to 20%. The highest survival percentage at 30 days after γ-rays irradiation was in sesds with a water content of 3 to 18% for 5KR and 10KR, 8 to 20% for 20K and 8 to 17% for 40KR, respectively. Seedling height at 30 days after γ-rays irradiation is given in Table 5. From Table 5, the dormant seeds with a water content of 11 to 20% showed a high resistance to 5KR, 10KR and 20KR of γ-rays, and similarly dormant seeds with 8 to 20% water content showed a high resistance to 40KR.

M. arvensis L. var. *piperascens* ($2n=96$) In general, this species showed high resistance to radiation. Regardless of the dosage of γ-rays irradiation by ^{60}Co, dormant seeds of between 11 and 20% water content showed no difference in ability to germinate. Seeds with less than 11% water content or more than 20%, especially the latter, showed decreased germination percentage. Similar results were obtained on the seedling height at 30 days after γ-rays irradiation. Seeds with less than 8% water content or more than 20% decreased in seedling height, and those with more than 23% died soon after germination (Table 6).

Table 5. Relation between water content of mint seeds and seedling height after exposure to various doses of γ-rays (*M. arvensis* L.)

Water content of seed (%)	Exposure dose (KR)			
	5	10	20	40
5	33.68%	13.24%	5.89%	1.58%
8	36.14	19.83	6.96	9.13
11	60.52	38.91	10.09	9.53
14	64.93	44.12	21.49	9.52
17	66.21	45.13	20.41	8.03
20	65.72	44.89	19.09	7.99
23	21.09	29.81	10.21	1.01
26	19.91	20.39	6.96	0.91

Table 6. Relation between water content of mint seeds and seedling height after exposure to various doses of γ-ray (*M. arvensis* L. var. *piperascens*)

Water content of seed (%)	Exposure dose (KR)			
	5	10	20	40
5	1.51%	3.96%	1.82%	1.13%
8	4.59	4.31	5.73	1.52
11	69.91	53.09	36.24	30.13
14	74.13	56.21	41.53	34.14
17	75.14	57.19	43.12	34.01
20	74.85	55.21	41.93	33.24
23	15.99	7.58	9.50	6.58
26	10.18	9.11	8.16	5.59

For the dosage of 5KR to 40KR of γ-rays (^{60}Co) showed but little difference in germination percentage of seeds between 8 and 23% water content. The seedling hight following irradiation decreased with the increase in dosage of γ-rays, which was especially shown when the water content of the dormant seeds was less than 11% or more than 20%.

Discussion

This experiment was intended to obtain as much data on the response of dormant seeds with various water contents to γ-rays. The results are summarized in Fig. 1. From a comparison of all the data presented, it seems fair to conclude that the sensitivity of most dormant seeds to radiation is at a minimum when they contain about 11 to 20% water (8 to 17% for *M. arvensis* L. 40KR) and they approach the maximum

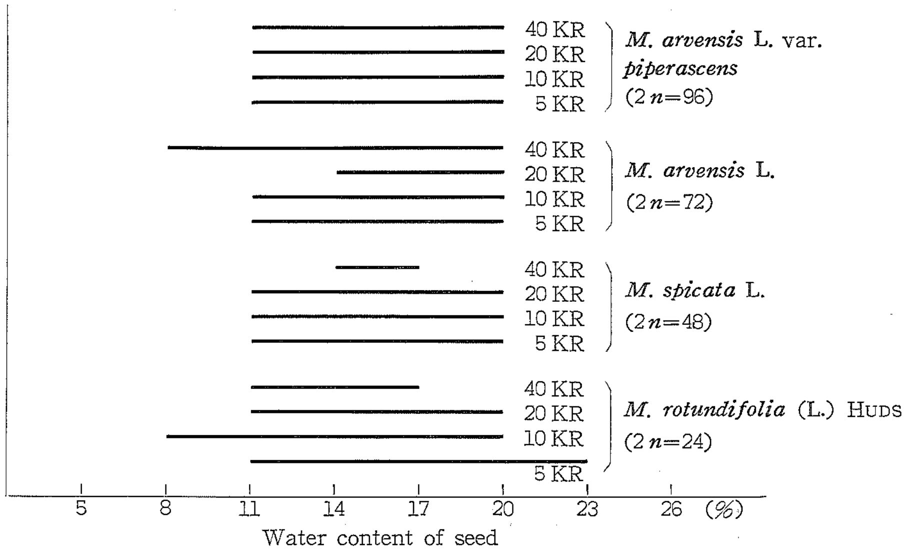

Fig. 1 Relation between the water content of dormant seeds and their sensitivity to different doses of γ-rays, showing the range of low sensitivity in species

sensitivity when they contain less than 11% (8% for *M. arvensis*) or more than 20% (17% for *M. arvensis*) water.

For many years, it has been recognized that seeds with a high water content are more sensitive to γ-rays irradiation than those with a low water content, and it is considered by many workers that the increased radiosensitivity of the seeds with a high water content results largely from the nature and frequency of the radical species formed along the tracks of ionizing electrons.

Recently, in barley, relationship between dormant seeds with different water contents and radiation sensitivity has been studies in detail (CALDECOTT 1954, 1955a, 1955b, and EHRENBERG and NYBOM 1954). EHRENBERG and NYBOM (1954) found that seeds with an 8.5% water content showed lowest sensitivity. In barley seeds EHRENBERG (1955a, 1955b) reported that the effectiveness of sparsely ionizing radiation decreased as the water content of seeds increased from 7 to 20%. CALDECOTT (1954, 1955a, 1955b) also decribed that barley seeds with an 8 to 16% water content were most resistant to X-ray inhibition. In *Agrostis stolonifera*, EHRENBERG (1959) observed that the some amount of free radicals was primarily produced in the seeds independently of their water content and that the radicals remain unchanged in the driest samples but an increasing proportion of them decayed as the water content increased.

EHRENBERG and NYBOM (1954) suggested that hydrated dormant seeds produced a protective substance to radiation. From the fact that there was not a 1-to-1 relationship between the water content of seeds and their sensitivity to γ-rays, CALDECOTT (1954) suggested that reduced seedling height resulting from γ-irradiation was not simply related to the production of active radicals in the presence of water. In general, production and decay of radicals were influenced by the environmental conditions of irradiation, various biological compounds (amino acid, proteins etc.) and storage. Free radicals induced by irradiation were quite stable and lasted much longer in drier seeds than in those with a high water content where they decayed very rapidly to a semi-constant level. Moreover, in a case where the seeds contain still more water, it was supposed that the sensitivity may be increased by a new type of chemical reaction following the metabolic activity of the organism at the higher water content.

Summary

1. Relationship between the water content of dormant seeds of genus *Mentha* and their sensitivity to γ-rays (^{60}Co) was studied.
2. Sensitivity of dormant seeds to radiations was at a minimum when they contained from about 11 to 20% water, except for *M. arvensis* L. (40KR) seeds.
3. Resistance of dormant *M. arvensis* (40KR) seeds to γ-rays was at a maximum when they contained from 8 to 20% water.
4. In general, sensitivity of dormant seeds of genus *Mentha* to radiation is increased by either a low or a high water content.

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

(8報) ハッカの種子含水量と放射線感受性に関する研究

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要　　約

ハッカ属植物の染色体数の異なる ($2n=24$, 48, 72 および 96) 種を材料として，種々の含水量の休眠種子に γ 線 ($^{60}C_0$) を照射し，種子含水量と放射線感受性の関係を調べた．その結果，本実験の範囲では，種子含水量11~20% ($2n=72$ の40KRでは 8 ~17%) の範囲において放射線感受性が低く，含水量がこれより少なくても多くても感受性は高いことが認められた．