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Response to radiation and *in vitro* growth of two species of *Luzula* with non-localised centromere

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Abstract. *In vitro* growth preceded by gamma-radiation was studied in two species with non-localised centromere, namely *Luzula elegans* and *Luzula pediformis*. Seed germination was affected by radiation doses. Cytological investigation revealed dose dependent chromosomal aberration and spindle disturbances. Chromosome break and fragments were present in *Luzula elegans* but absent in *Luzula pediformis*. The growth of the callus was affected at higher doses in both the species. The radiosensitivity of the two species of *Luzula* with diffuse centromere has been discussed.

Keywords. *Luzula* spp.; non-localised centromere; gamma-irradiation; *in vitro* growth; cytological investigation; radiosensitivity.

1. Introduction

The species of *Luzula* of Juncaceae are characterised by diffuse centromere in the chromosomes. Increase in chromosome number in this genus has principally been affected by fragmentation instead of duplication (Nordenskiöld 1962). Previous studies on DNA estimation have also indicated similar amount of DNA in both diploid and polyploid species (J Sengupta, S Mukherjee and A K Sharma, unpublished results).

The susceptibility and response to culture of the diploid and polyploid species to *in vitro* treatment are not yet known. Moreover instability in chromosome complement often noted *in vitro* has not been studied for diploid and polyploid forms. This is specially significant as fragments arising from such chromosomes have the possibility of survival. Moreover, the radiosensitivity of diploids and such polyploids is yet uninvestigated.

The present study was undertaken with two species of *Luzula* with $2n = 6$ and 12 chromosomes. The species were subjected to gamma-irradiation and the differential behaviour was studied *in vitro* along with control. The objective was to get an understanding of the *in vitro* behaviour of radiation of diploid and polyploid species of *Luzula* with diffuse centromere.

2. Materials and methods

Seeds of *Luzula elegans* and *L. pediformis* obtained from Department of Genetics, Estacao Agronomica National Oeiras, Portugal, were soaked overnight in water and exposed to two different doses of gamma-ray viz. 10 and 20 Gy from a 900 curie ^{60}Co source. After treatment the seeds were washed with 5% Teepol for 10 min followed by several washings in sterile distilled water. These were then surface sterilized with 0·1% $HgCl_2$ for 10–15 min, washed several times with sterile

distilled water and inoculated on half-strength basal medium of Murashige and Skoog (1962) solidified with 0·5% agar for germination. Cultures were inoculated at 22 ± 1°C with 55–60% relative humidity under cool, white fluorescent lamp providing 2000 lux light at the culture level for 16 h. A parallel set was maintained in complete darkness. Aseptically germinated seedlings, 1–1·5 cm long, were inoculated on basal medium of Murashige and Skoog (1962) supplemented with 3 mg/l of 2,4-dichlorophenoxyacetic acid (2,4-D) and 300 mg/l of casein hydrolysate (Inomata 1982) solidified with 0·5% agar. Cultures were incubated under similar conditions with 16/8 h light/dark period as mentioned above. Callus was maintained by regular subculturing at an interval of 28–30 days for two months in the same medium.

For studies of chromosome number of callus cells, small pieces of calli were fixed, 4–5 days after subculturing in chilled Carnoy's solution overnight. Squash preparations were made following usual aceto-orcein staining schedule (Sharma and Sharma 1980). Chromosome counts were made for two periods of growth namely, 30 and 60 days. At least 50 ± 10 metaphase plates were counted for each preparation for each species.

3. Results and discussion

3.1 *Germination of seeds*

Seed germination was greatly affected by radiation doses (table 1). With increasing dose, there was a decrease in germination in both species. Moreover, in each species there was a delay in the initiation of germination (table 1). Inhibition of germination or production of abnormal seedlings has been reported earlier in other genera with increasing dose (Bajaj *et al* 1970). However, the germinated seedlings were morphologically identical with the control plants (figure 1).

3.2 *Chromosome study*

In callus tissue of both *L. elegans* and *L. pediformis*, cytological investigations exhibited both chromosomal aberrations and spindle disturbances, the frequency of which were generally dose dependent. Similar findings have been reported in other genera by earlier workers (Sengupta *et al* 1984, 1986; Kar and Sen 1985). Chromosomal aberrations in the present experiment included high percentages of

Table 1. Effect of radiation dose on seed germination in *L. elegans* and *L. pediformis*.

	Minimum days required for seed germination			Percentage of seed germination		
Plant species	Control	10 Gy	20 Gy	Control	10 Gy	20 Gy
L. elegans	21	30	35	16	8	9
L. pediformis	18	30	30	56	45	25

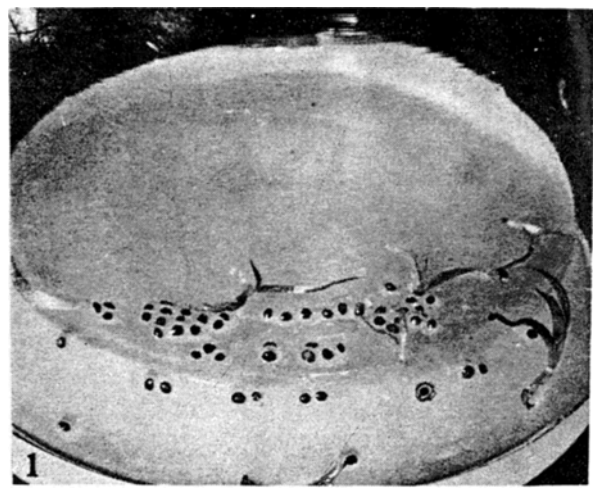


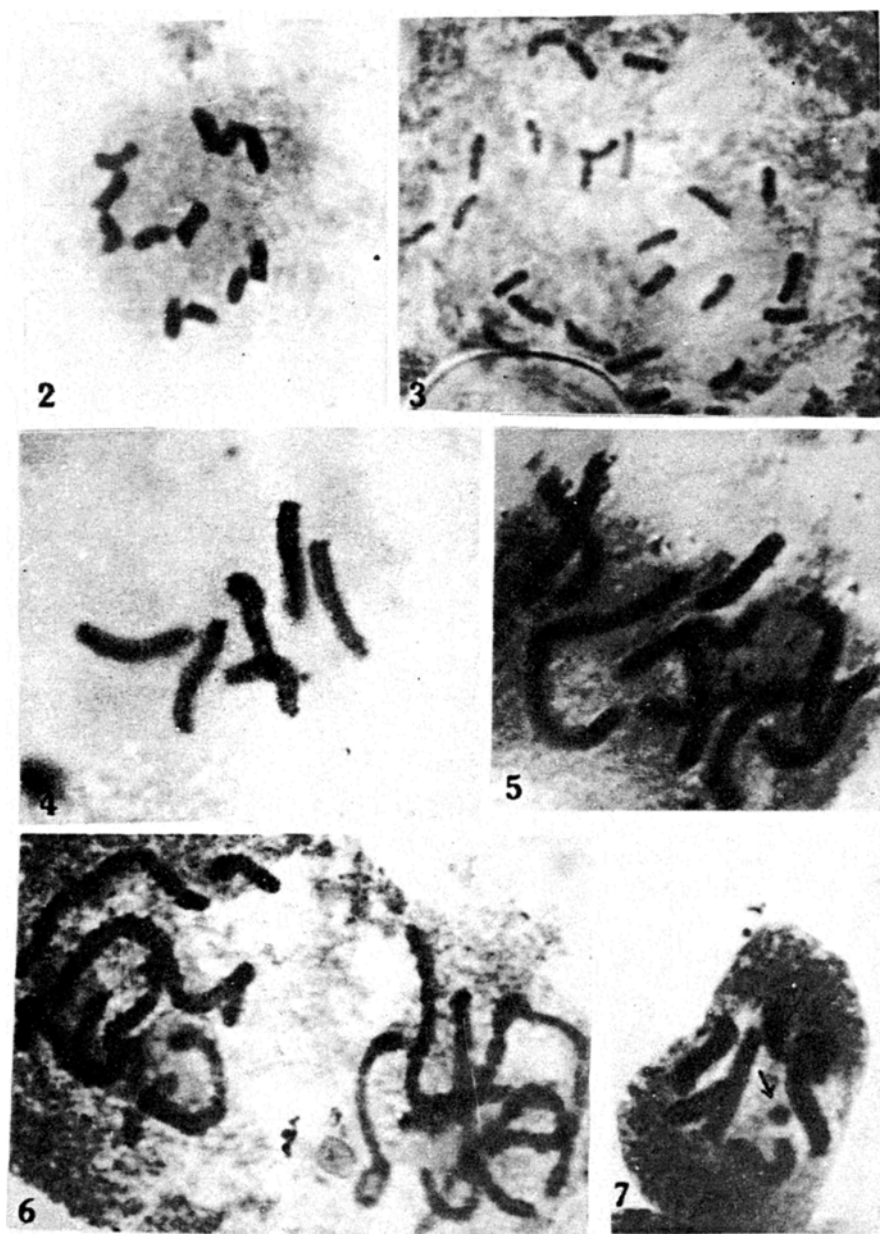
Figure 1. *In vitro* germinated seedlings of *L. elegans*.

metaphase and anaphase stages with fragmentation, clumping, lagging, fusion of chromosome ends, sticky bridge formation as well as disorientation of spindle (figures 2–7).

In *L. elegans*, the percentage of abnormal cells indicated an increase with increasing doses as well as period of culture. This was correlated with a decrease in the frequency of diploid cells under similar condition (table 2).

In *L. pediformis* the same trend was observed in relation to abnormal cells. In this species, however, both control as well as treated cells revealed principally diploid number in different doses as well as after different periods of culture. While chromosome break, fragments and dot chromosomes were most dominant in *L. elegans*, these were absent in *L. pediformis*. Similar fragments and dot chromosomes were observed in radiation induced cells of *L. elegans* by previous workers (Nordenskiöld 1962; Kusanagi 1967). The complete absence of fragments despite diffuse centromeric constitution in any of the sets in *L. pediformis* is of special interest. In species of *Luzula*, increase in chromosome number from $n = 3$, is principally through fragmentation of chromosomes (Nordenskiöld 1962; Camara 1951) as fragments are not devoid of centromere. Such a mode of increase in number has also been confirmed through DNA estimation of diploid and polyploid cells. Origin of $2n = 12$ in *L. pediformis* is thus through chromosome fragmentation. Once fragmented, the distribution of gene loci in 12 chromosomes has become selectively adapted in the genome. It is not unlikely that any further breakage may destroy the positive balance of the genes and thus lead to non-viability of the cells. Thus the absence of fragments can be accounted for.

Following 20 Gy treatment, the cultures obtained from seeds turned brown after 40 days and could not be studied further. The growth of the callus of two species is thus strongly affected at higher doses of radiation. Similar inhibitory effect of radiation on callus growth has been reported earlier in other plant species (Bajaj *et al* 1970).



Figures 2–7. **2.** Diploid metaphase cell of *L. pediformis* (2n = 12) showing fusion of chromosome ends. **3.** Tetraploid metaphase plate of *L. pediformis* with 24 chromosomes. **4.** Diploid metaphase plate of *L. elegans* (2n = 6) from control set. **5.** Tetraploid metaphase plate of *L. elegans* showing 12 chromosomes. **6.** Diploid anaphase cell of *L. elegans* showing disorientation of spindle. **7.** Diploid metaphase cell of *L. elegans* showing six normal chromosomes and a dot-like fragment (→).

Table 2. Frequency of normal and abnormal cells in cultures of *L. elegans* and *L. pediformis* following gamma treatment.

		L. elegans (2n = 6)*			*L. pediformis* (2n = 12)*		
Days in culture	Treatment	No. of diploid cells (%)	No. of >2n cells (%)	No. of abnormal cells (%)	No. of diploid cells (%)	No. of >2n cells (%)	No. of abnormal cells (%)
	Control	46·8 ± 2·3 (93·6)	3·2 ± 1·0 (6·4)	2·0 ± 0·98 (4·0)	50·00 ± 0·0 (100)	—	—
30 days	10 Gy	44·0 ± 1·7 (88·0)	6·0 ± 1·2 (12·0)	5·0 ± 1·89 (10·0)	49·45 ± 0·11 (98·9)	0·55 ± 0·23 (1·1)	13·5 ± 1·11 (27)
	20 Gy	37·5 ± 2·8 (75·0)	12·5 ± 1·9 (25·0)	15·6 ± 0·5 (31·2)	48·00 ± 1·1 (96·0)	2·00 ± 0·56 (4·0)	20·45 ± 2·95 (40·9)
	Control	47·0 ± 3·75 (94·0)	3·0 ± 1·1 (6·0)	2·5 ± 1·25 (5·0)	50·00 ± 0·0 (100)	—	—
60 days	10 Gy	30·65 ± 2·5 (61·3)	19·35 ± 1·75 (38·7)	11·25 ± 0·75 (22·5)	41·25 ± 2·05 (82·5)	8·75 ± 1·96 (17·5)	10·0 ± 1·78 (20·0)
	20 Gy	—	—	—	—	—	—

*No. of metaphase cells studied in each species in each set = 50.

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