In vitro Germination and the Effect of Acute Gamma Irradiation on Pollen of Pinus patula Schiede et Deppe

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(Received August / November 1980)

Summary

In-vitro study revealed that the best medium for germinating *P. patula* pollen was 12,5% Sucrose with 0.001% Boron in 2% agar medium. After gamma irradiation the LD-50 based on pollen germination and germination energy index was 1.174 kr and 1.148 kr respectively. Pollen was viable on the 90th day when used after storing at 10° C in desiccator. Gamma doses for haploid breeding and mutation breeding are suggested. ANOVA showed that differences between the different media used for germination and gamma-ray doses were highly significant.

Key words: Pinus patula, in -vitro pollen germination, pollen irradiation, heteroplastic grafting.

Zusammenfassung

In Vitro-Studien zeigten daß das beste Keimmedium für *Pinus patula* Pollen eine 12,5%ige Rohrzuckerlösung mit 0,001% Bor in einem 2%igen Agar-Agar Medium ist. Nach γ-Bestrahlung war die LD-50 bezogen auf Pollenkeimung und GEI 1,174 kr bzw. 1,148 kr. Pollen war noch am 90. Tag lebensfähig, wenn er bei 10° C im Exiccator gelagert wurde. γ-Dosen werden für die Haploidie- und Mutationszüchtung vorgeschlagen. ANOVA zeigte, daß die Unterschiede zwischen den verschiedenen Keimmedien und γ-Dosen statistisch hoch signifikant waren.

Introduction

Information on the effect of ionizing radiations on different organs of tree species is essential if this method is to be used to induce variation for selection. Such information is of fundamental value and essential to the forest geneticist interested in using induced mutations in breeding (STAIRS, 1964). Pollen irradiation is potential use in mutation breeding of forest trees (STAIRS and MERGEN, 1964). Also, the artificial production of haploid individuals is of great importance in forest tree breeding (GUSTAFSSON, 1960). Haploids can be produced if pollen is irradiated at higher levels than LD-50 and used in experiments where genetically inert tube growth would be desirable as a stimulator without taking part in fertilizing the egg cell. RUDOLPH (1965) and EL-LAKANY and SZIKLAI (1971) studied the effect of gamma irradiation on pollen and seed characteristics in White spruce and Douglas fir respectively. However, not much work has been done on these lines in forest tree species (LYNN, 1967). Knowledge of a suitable medium for germinating pollen of forest tree species is also useful. Therefore, studies were begun on pollen germination in-vitro and effect of acute gamma rays on *Pinus patula* to obtain basic information on the radiosensitivity, the medium required for pollen germination in-vitro and later on explore its usefulness in mutation breeding and haploid breeding studies. The results of these studies are reported here.

Materials and Methods

Material: Mature male cones of *P. patula* were collected in the 1st week of April, 1978 from heteroplastic grafts (*P. patula on P. roxburghii* stocks) raised at New Forest Estate (KAPOOR, unpublished). These grafts flowered in the second year from grafting; pollen was collected from 5 grafts and bulked. Male cones were dried in the sun (35° C) and pollen was extracted. Pollen was cleaned by sieving through fine muslin cloth and placed in glass vials plugged

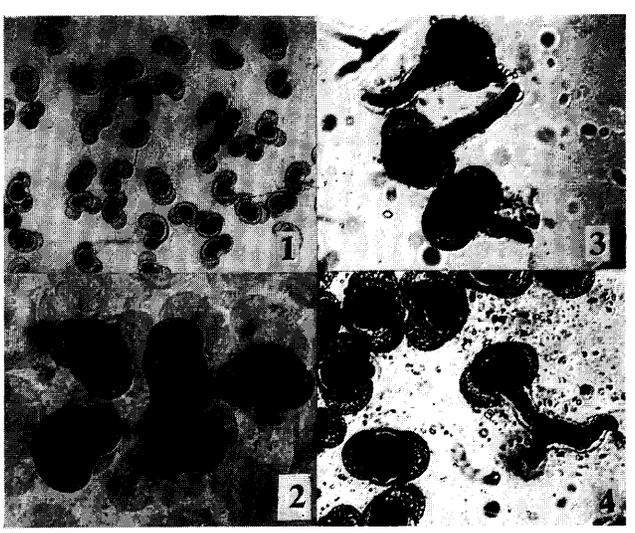

Figure 1. — Pollen grains of *p. patula.* × 100
Figure 2. — Pollen grains of *p. patula.* × 400
Figure 3. — Germinating pollen grain of *P. patula.* × 400
Figure 4. — Branched pollen tube of *P. patula* pollen. × 400

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Table 1. — Medium used for pollen germination of P. patula

S. No.	Medium	Percentage of Sucrose	Percentage of Boron
1.	Tap water	Nil	Nil
2.	Distilled water	Nil	Nil
3.	Agar Agar 2%	Nil	Nil
4.	Agar Agar 2%	5, 7.5, 10, 12.5, 15	Nil
5.	Agar Agar 2%	5, 7.5, 10, 12.5, 15	0.01 & 0.001

air-tight with cotton wrapped in muslin cloth, and then stored in a refrigerator at 10° C in a desiccator containing silica gel.

Media and germination procedure: Bulked mature pollen of *P. patula* was placed for germination in the following media (*Table 1*).

Bacteriological grade agar agar powder was used to prepare 2 % agar agar medium. Different quantities of sucrose and boron were added to the medium to get the required concentrations. Medium was autoclaved at 122° C under 15 Lb/sq. inch pressure for 30 minutes. This medium was allowed to solidify, out of which one cm. square blocks were prepared. A fine layer of pollen was dusted on these blocks with the help of sterile cotton and kept in petridishes. These petridishes were placed in germinator at 70 percent humidity and 33 ± 1° C temperature for germination. Cavity slides were used for liquid media. Each treatment was replicated thrice, single block representing a replicate.

Gamma irradiation: Pollen was irradiated with acute gamma rays from a Cobalt — 60 source (Dose rate 1kr/min.) with 1kr, 2kr and 4kr doses. Un-irradiated pollen was used as control. Irradiated and control pollen was kept for germination in the last week of May, 1978 at 33 ± 1° C under three replications on 2 % agar agar, in combination with 12.5 % sucrose & 0.001 % boron in petridishes as this had proved to be the best medium for germination.

Preparation of slides: Samples were taken at random daily on 2nd to 5th day from the start of the experiment: 5 slides were prepared from each replicate of the treatment. Stains used were 2 % Acetocarmine having 10 % Glycerol and Cotton blue separately. A drop of stain was placed on the slide, and material was put on it with the help of a needle and the coverslip was applied. The slides, thus prepared, were sealed with molten wax.

Observations: Daily observations were made upto 5th day on germination, length & width of pollen tube and pollen size. From each slide 10 observations were recorded. Pollen fertility was also recorded based on the acetocarmine staining. Photomicrographs were taken from the preparation showing morphology of the pollen, germination of pollen grains and pollen tube showing branching.

Statistical methods: All data recorded were computed and subjected to analysis of variance (ANOVA) to test the significance of differences observed between the different treatments. Germination percentage was transformed to angular values before ANOVA. From germination records taken daily upto 5th day, Germination Energy Index (GEI) was calculated, according to GROSE and ZIMMER (1958). Standard error was also calculated for pollen measurements. LD-50 was calculated based on germination of pollen using FINNEY (1952) probit analysis method.

Results and Discussion

1. *General:* The pollen grains of *P. patula* are winged and full of starch *(Fig. 1 and 2)*. The mean length of pollen grains along the vertical line to the wings was 43.75 ± 0.564 and the width along horizontal line with and without wings was 76.72 ± 0.689 and 57.69 ± 1.324 microns respectively. Pollen was found viable on the 90th day when it was used for germination after storing at 10° C in the desiccator. Mean fertility percentage of pollen based on acetocarmine staining was 97.60 and percentage of unstained and under-developed pollen was 1.62 and 0.78 respectively. Branching of the pollen tube was also observed *(Fig. 4)* and this has also been reported by MAHESHWARI (1950) in *Amentiferae*. Cotton blue gave good staining and contrast.

2. *Effect of medium:* Pollen germinated only on 2 % agar agar having 10 %, 12.5 % & 15 % sucrose and 0.001 % or 0.01 % Boron in each case. Based on germination *(Fig. 3)*,

Table 2. — Mean values of germination, GEI and Pollen tube measurements of *P. patula* pollen.

Character	Boron 0.01% Sucrose %			Boron 0.001% Sucrose %		
	10	12.5	15	10	* 12.5	15
Germination	40.75	60.12	55.84	40.61	70.58	29.45
GEI	29.93	29.07	29.42	18.02	54.05	16.66
P. T. Length (microns)	38.67	50.63	44.31	63.61	76.48	38.43
P. T. Width (microns)	28.71	31.44	27.77	30.71	31.61	27.77

* used as control in irradiation studies.

Table 3. — ANOVA for different parameters

Source of variation	Germination			Germination		Pollen tube Length			Pollen tube Width	
	d. f.	MSS	F	MSS	F	d. f.	MSS	F	MSS	F
Between replication	2	150.95	2.50 NS	6.04	2.50 NS	2	29.48	0.59 NS	1.58	0.28 NS
Between treatment	7	10907.93	*** 180.80	436.19	*** 180.60	5	694.24	*** 14.01	9.69	1.72 NS
Error	14	60.33		2.41		10	49.53		5.63	

NS = Statistically Non-significant

*** = Statistically significant at 0.01% probability level.

Table 4. — Mean values of germination, GEI and pollen tube Measurements of *P. patula* pollen after gamma irradiation.

Characters	Doses			LD-50
	Control 0 kr	1 kr	2 kr	
Germination %	70.58	36.50	29.00	1.174 kr
GEI	54.05	28.46	17.80	1.148 kr
P. T. Length (mircrons)	76.48	52.31	48.36	N.A.
P. T. Width (mircrons)	31.61	29.09	28.37	N.A.

N.A. = not achieved as kr is low dose.

germination energy and pollen tube length and width *(Table 2)* best results were achieved with 12.5% Sucrose and 0.001% Boron. ANOVA *(Table 3)* showed that the differences between treatments were highly significant at 0.1% probability level based on all the above parameters except for pollen tube width which was non significant. Boron appears to be an essential requirement for pollen germination and pollen tube growth in combination with 10—15% Sucrose concentrations in agar agar medium because pollen germination was not observed in any other medium. The pH of 2 % agar agar medium having sucrose and boron varied from 7.0 to 7.1 in the case of 0.01 % Boron series and 7.3 to 7.5 for 0.001 % Boron series. To understand the physiology of pollen (Johri and Vasil, 1961) bio-physical and bio-chemical studies are required.

3. *Effect of gamma irradiation: P. patula* pollen tolerated gamma dose up to 2kr *(Table 4)*. At 4kr dose no germination was observed showing this dose to be lethal. In a few pollen grains small protuberances were observed but the pollen tube failed to grow further. For haploid breeding, doses between 2kr and 4kr could be tried for pollen irradiation which may be able to cause physiological activity but no participation in fertilization. Based on pollen germination, calculated LD-50 was 1.174 kr and based on GEI, LD-50 was 1.148 kr which could be tried for mutation studies. Fifty percent reduction in pollen tube length and width was not achieved, therefore LD-50 could not be calculated for these characters. Delay in pollen germination was observed after gamma irradiation *(Table 4)* as germination energy was reduced. The gamma rays followed a linear trend in relation to dose and observed effect on the Characters germination, germination energy index (GEI) and pollen tube length, ANOVA showed that the differences between doses and control were highly significant *(Table 5)* at 0.1% probability level.

Acknowledgements

We are grateful to Sh. R. C. Ghosh, Director, Forestry Research of this Institute for taking interest in this work and providing facilities.

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Table 5. — ANOVA for different parameters after gamma irradiation.

Source of variation	Germination			Germination Energy Index		Pollen tube Length		Pollen tube Width	
	d. f.	MSS	F	MSS	F	MSS	F	MSS	F
Between replication	2	8.87	4.48 NS	4.67	0.67 NS	4.35	1.72 NS	8.93	5.73 NS
Between treatment	2	513.08	*** 259.51	1041.27	*** 150.42	695.54	*** 274.52	8.70	5.59 NS
Error	4	1.98		6.92		2.53		1.56	

NS = Statistically Non-significant.
*** = Statistically significant at 0.01% probability level.