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Isoelectric Zones of Vegetative and Generative Nuclei in Pollen Grains of *Tradescantia*

By

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It is well known that vegetative and generative nuclei in pollen grains differ in their reaction to dyes, e.g. a combination as cyanin (basic dye) and erythrosin (acid dye) staining the vegetative nucleus red and the generative nucleus blue (*cf.* COULTER and CHAMBERLAIN, 1909).

While, from the cytophotometric studies of Feulgen staining in the pollen grains of *Tradescantia*, it has been found that DNA synthesis in the generative nucleus occurs during the first third of interphase while the DNA content of the vegetative nucleus remained unchanged (SWIFT, 1950; WOODARD, 1956).

From the facts mentioned above, it may be assumed that the difference in the reaction to the dyes is due to the different amounts of DNA between the vegetative and generative nuclei in the pollen grains. The purpose of the present study is to see whether difference of isoelectric points between these two nuclei exists or not, and to determine whether the difference of the isoelectric point of these nuclei has an important relation to the amount of DNA in these nuclei or not.

Material and Methods

Young pollen grains of *Tradescantia reflexa*, before the generative nuclei grew up into crescent-shaped ones, were used in the present investigation.

Methods of the study are described in the appropriate places in the descriptive parts of the following pages.

Results

I) *Isoelectric zones determined by the turbidity method.* Isoelectric zones of the vegetative and generative nuclei in the fresh pollen grains were determined by the turbidity method with a dark field microscope (*cf.* SHINKE, 1939).

This method is based on the fact that the maximum flocculation of proteins and other ampholytes takes place at the isoelectric point of the substances.

In this study, the living pollen grains were observed in the media of different pH and the brightness of the nuclei was measured by a densitometric method. The pH of the media was controlled by acetic acid solutions of different concentrations and also by MCILVAINE's buffer solutions.

The results obtained by the above method on the vegetative and generative nuclei in the pollen grains are shown in Plate I and Table 1.

Table 1. Turbidity of nuclei and cytoplasm in pollen grains caused by acetic acid in different concentrations.

Composition of media		pH	Turbidity		
5% sucrose solution	Glacial acetic acid		Generat. nucleus	Vegetat. nucleus	Cytoplasm
20 ml	46.6 ml	1.00	—	—	—
20 ml	3.0 ml	2.35	++	+	—
20 ml	2.0 ml	2.40	++	+	—
20 ml	1.0 ml	2.55	++	+	—
20 ml	0.7 ml	2.62	++	+	±
20 ml	0.4 ml	2.75	+	+	+
20 ml	0.2 ml	2.95	+	+	+
20 ml	0.1 ml	2.98	+	+ or ++	+

In Plate I, the generative nuclei in Figs. B (pH, 2.35), C (pH, 2.40) and D (pH, 2.55) are illuminated brightly, and are easily distinguishable from the vegetative nuclei and the cytoplasm. In Fig. E (pH, 2.95), the generative nuclei decrease somewhat in brightness and are of the same brightness as those of the vegetative ones. In Fig. F (pH, 2.98), the vegetative nuclei and the cytoplasm increase in brightness. These results are summarized in Table 1.

From the Table 1, it is seen that the isoelectric point or zone of the vegetative nucleus is larger than pH 2.98 and that of the generative one is found between pH 2.35 and 2.62.

II) *Isoelectric zones determined by acid and basic dyes.* The isoelectric zones of the nuclei and cytoplasm in the pollen grains were determined by a modified NAYLOR's method (1926). The fixative used in this experiment was 10% neutral formalin. The mixtures of 5 ml of M/10 sodium acetate solution and different amount of M/10 or 1 M acetic acid were used for buffer solutions (*cf.* SHINKE, 1939).

The limits of retention of eosin and toluidine blue by the cytoplasm and both the vegetative and generative nuclei in the pollen grains are shown in Table 2. As shown in this table, the isoelectric zones of the cytoplasm and both the vegetative and generative nuclei are pHi 4.05–4.62, 3.90–4.35 and 3.40–3.90 respectively.

Table 2. Limits of retention of eosin and toluidine blue by nuclei and cytoplasm of pollen grains.

Parts	Dyes	pH values							
		3.10	3.40	3.70	3.90	4.05	4.35	4.62	5.00
Generative nucleus	Eosin	++	++	+	+ or ±	± or ---			
	Toluidine blue		±	+	+	+	+ or ++	++	++
Vegetative nucleus	Eosin	++	++	+	+	+	±		
	Toluidine blue				±	+ or ±	+	++	++
Cytoplasm	Eosin	++	++	+	+	+	+	±	
	Toluidine blue					± or +	+	++	++

III) *Effect of DNAase treatment.* The pollen grains were fixed with 10% neutral formalin, washed with dist. water and were treated with the enzyme solution such as 0.5 mg of crystaline DNAase[1] per 1 ml of dist. water containing 0.00125 M $MgSO_4$ for 20 hours at 35°C (PEARSE, 1960).

In the pollen grains treated with the DNAase solution, the isoelectric zones of both the vegetative and generative nuclei determined by the staining method are shifted to alkaline side by the treatment and the zone is pHi 4.05–4.62, which is the same zone as that of the cytoplasm. These results are shown in Table 3.

Table 3. Limits of retention of the dyes by nuclei and cytoplasm of pollen grains treated with DNAase.

Parts	Dyes	pH values							
		3.10	3.40	3.70	3.90	4.05	4.35	4.62	5.00
Generative nucleus	Eosin	++	++	+	+	+	+	±	
	Toluidine blue					± or +	+	++	++
Vegetative nucleus	Eosin	++	++	+	+	+	+	±	
	Toluidine blue					± or +	+	++	++
Cytoplasm	Eosin	++	++	+	+	+	+	±	
	Toluidine blue					± or +	+	++	++

IV) *Effect of proteinase treatment.* The pollen grains fixed with 10% neutral formalin, after being washed with dist. water, were treated with a solution containing 1 mg of proteinase[2] per 1 ml of dist. water for 20 hours at 35°C.

The isoelectric zones of the cytoplasm and both the vegetative and generative nuclei determined by the staining method are pHi 4.05–4.62, 3.90–

1) The DNAase used was the products of Sigma Chemical Co.

2) The proteinase used was Pronase A S, the products of Kaken Chemical Co.

4.35 and 3.40–3.90 respectively, and these zones do not show any changes by the enzyme treatment as compared with those of the untreated pollen grains.

Discussion and Conclusion

The isoelectric zone of the generative nucleus in the fresh pollen grains determined by the turbidity method is found to be smaller value than pHi 2.62, while that of the vegetative one is larger than pHi 2.98. On the other hand, the isoelectric zones of the cytoplasm and both the vegetative and generative nuclei in the fixed pollen grains as determined by the dye staining are pHi 4.05–4.62, 3.90–4.35 and 3.40–3.90 respectively. In both cases, the iso-electric zones of the generative nuclei are in more acid side than those of the vegetative ones, and those of the vegetative nuclei are in more acid side than those of the cytoplasm. These results are harmonious with the reports of PISCHINGER (1926), YAMAHA (1932) and others that the isoelectric point of the nucleus is in more acid side than that of the cytoplasm. These results are also consistent with the report that the isoelectric point of nucleoprotein is near pHi 4.0 (HALL, 1941).

In the pollen grains treated with the DNAase, the isoelectric zones of both of the vegetative and generative nuclei are shifted to more alkaline side by the treatment, and the difference in isoelectric zones among the cytoplasm and the vegetative and generative nuclei does not exist. Whereas, in the pollen grains treated with the proteinase, the isoelectric zones of the cytoplasm and the vegetative and generative nuclei do not change by the treatment, and the zones are the same as those of untreated pollen grains.

From these facts mentioned above, it may be concluded that the difference in the reactions to the dyes between the vegetative and generative nuclei is based upon the difference of the DNA content in these nuclei. This is in agreement with the results of SWIFT (1950) and WOODARD (1956).

Summary

1) Isoelectric zones of the vegetative and generative nuclei in the fresh pollen grains of *Tradescantia reflexa* were determined by the turbidity method. These zones of the generative nucleus are between pHi 2.35 and 2.62, and those of the vegetative one are of larger value than pHi 2.98.

2) Isoelectric zones of the vegetative and generative nuclei in the fixed pollen grains of *Tradescantia* were also determined by acid (eosin) and basic (toluidine blue) dyes. The isoelectric zones of the generative and vegetative nuclei and the cytoplasm were pHi 3.40–3.90, 3.90–4.35 and 4.05–4.62 respectively.

3) Isoelectric zones of the generative and vegetative nuclei and the cytoplasm in the pollen grains treated with DNAase or proteinase were also determined by the dyes. After the treatment of the DNAase, the isoelectric zones

of both the generative and vegetative nuclei were shifted to more alkaline side by the treatment and the difference in isoelectric zones among the generative and vegetative nuclei and the cytoplasm did not exist. The treatment of pollen grains with proteinase did not give any changes in the isoelectric zones.

4) The difference in the reactions to the dyes between the generative and vegetative nuclei in the pollen grains may be based upon the different amounts of DNA contained in these nuclei.

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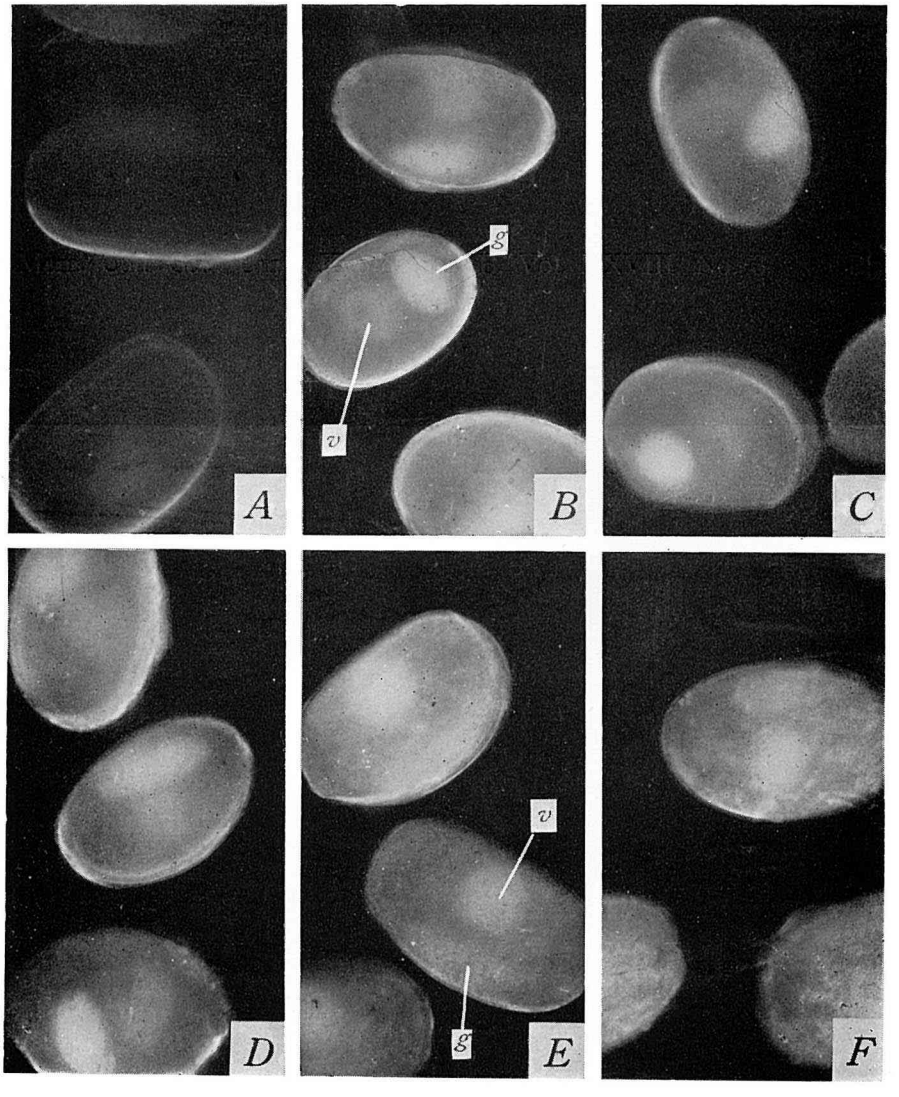
Explanation of Plate I

Figs. A–F. All these figures are photomicrographs taken by a dark field illumination showing turbidity of nuclei and cytoplasm in pollen grains of *Tradescantia reflexa*.

Fig. A shows the pollen grains observed in the medium at pH 1.00; Fig. B at pH 2.35; Fig. C at pH 2.40; Fig. D at pH 2.55; Fig. E at pH 2.95; Fig. F at pH 2.98.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