Protoplasma (1993) 174: 74–78

PROTOPLASMA

Pollen dimorphism in soybean

Rapid communication

Eliane Kaltchuk-Santos[1], Maria Helena Bodanese Zanettini[1,*], and Elsa Mundstock[2]

[1] Departamento de Genética, and [2] Departamento de Estatística, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul

Received January 27, 1993
Accepted March 23, 1993

Summary. Microspores of soybean plants (*Glycine max* (L.) Meer.) of four cultivars were cytologically analysed. The pollen grains showed a clear dimorphism when stained with propionic-carmine from binucleate stage onwards. The majority of the grains are large, deeply stained and with asymmetric division ("normal type") while the remainder grains are smaller, lightly stained, uninucleate or with two similar nuclei ("P-pollen"). The different frequencies of "P-pollen" on the four cultivars suggest a genotype effect of microspore dimorphism.

Keywords: Androgenesis; *Glycine max*; Pollen dimorphism; "P-pollen"; Symmetric division.

Introduction

An interesting finding which has come to light in recent years is the phenomenon of pollen dimorphism. Interspersed among the regular starch-filled pollen grains (normal grains) is a second class of microspores, much smaller (ca. three-quarters the normal size), lacking in starch and staining lightly. They may be uninucleate, or binucleate, with two equal or two unequal nuclei. Altogether the atypical class accounts for 15 to 20% of the total populations and they are clearly retarded in relation to the microspore mitosis of the normal pollens (Sunderland 1974). Such atypical grains have been named "E pollen" (Sunderland and Wicks 1969), "S pollen" (Horner and Street 1978), or "P pollen" (Heberle-Borns and Reinert 1979). The microspore dimorphism can be seen in anther maturing in vivo or even in culture and has been reported in many species, such as *Tradescantia bracteata* (La Cour 1949), *Paeonia hybrida* (Sunderland 1974), *Hordeum vulgare* (Dale 1975), *Nicotiana tabacum* (Horner and Street 1978), *Triticum aestivum* (de Buyser and Picard 1975).

In nature, the "P pollen" is a non-functional gametophyte and can be considered a kind of male sterility (Heberle-Bors 1982 a) but given appropriate culture conditions it may follow the embryogenic pathway instead of gametogenesis (Heberle-Bors and Reinert 1980). Then, these atypical pollen grains are a possible way in the formation of haploid embryos.

Since the successful inductions of haploids from anthers cultured in vitro in 1964, a great deal of attention has been given to this problem by those interested in obtaining pure lines and mutants for crop improvement and biochemical genetics (Maheshwari et al. 1980).

Based on the few initial divisions in the microspores, four routes of in vitro androgenesis have been identified (Sunderland 1974, Maheshwari et al. 1982). In one of these routes the microspore division is symmetric, resulting in two equal cells with diffuse vegetative-type nuclei. These pollen grains have been considered as one of the recognized pathways of pollen embryogenesis as well as to be one of the principal routes in the formation of haploid embryos (Nitsch and Norreel 1973, Rashid and Street 1974, Wilson et al. 1978).

In the last years the anther culture technique has been refined and extended to many agronomically important crop species. However, in soybean the successful development of haploid plantlets is not reported yet (Yuyu et al. 1986).

Studies about the modes of early androgenic segmen-

* Correspondence and reprints: Departamento de Genética, Universidade Federal do Rio Grande do Sul, Caixa Postal 15053, 91501-970 Porto Alegre, RS, Brasil.

tation and occurrence of dimorphism in this species are not available.

The present study was undertaken to verify if pollen dimorphism exists in *Glycine max*, and if it does, to describe the different microspore types.

Material and methods

Four *Glycine max* (L.) Merril cultivars (Década, IAS 5, BR 4 and Ipagro 21) served as a source of material and 15 plants each were investigated. Young floral buds were collected from field grown plants and fixed in ethanol-acetic acid (3 : 1). For microscopic analysis, the pollen grains released from the anthers were stained with propionic-carmine and left for 2 h before scoring. The pollen grains were analysed at binucleate stage (mid or late).

The microspore dimorphism was studied by counting the number of normal looking pollen grains as well as other possible types (with different size or staining properties) in a sample of 500 grains per plant. A total of 7500 microspores were analysed from each cultivar. The pollen diameter and the distance between two pores were measured (Fig. 1) with measurement eye pieces (Zeiss Axioplan Universal microscope).

Statistical analysis was performed using analysis of variance on the arcsen $\sqrt{x}$ transformation for percentage of pollen (normal, uninucleate, and binucleate). Duncan's multiple range test was performed when F resulted significantly. In relation of pollen size, a t-paired comparison was made.

Results and discussion

In this study were examined 3000 microspores of 60 *Glycine max* plants. The cytological analysis detected the existence of pollen dimorphism in four cultivars of soybean. Three types of microspores were observed.

(a) Normal grain: Most of the microspores had undergone an asymmetric division always resulting two unequal cells on size (vegetative and generative). These typical pollen grains had large size and stained densely with propionic-carmine (Fig. 2 A and B). About 98% of the microspores were of this type.

(b) Atypical uninucleate grain: This pollen type occurred in lower frequency than the normal pollen (1.5%; Table 1) and was smaller. It had a big central nucleus and stained lightly with propionic-carmine. These microspores were cleary retarded in the sense that they underwent mitosis at a much later stage than the principal pollen populations (Fig. 2 A and B).

(c) Atypical binucleate grain: In relation to size and staining properties, this pollen was equal to the preceding type, but occurred in lower frequency, just 0.54%. This kind of microspore was two-celled with no differentiation into vegetative and generative cells, they are identical (Fig. 2 C and D). Probably this is a result of symmetric mitosis.

It is possible that the atypical binucleate pollen is a result of the first symmetric mitosis underwent by the atypical uninucleate grain. Thus, the two atypical types would be different stages of the same pathway. For this reason the atypical uninucleate and binucleate microspores are both grouped in the same category as "P pollen" for statistical analysis.

The frequency of "P pollen" varied between cultivars (Table 1) and the statistical analysis showed that the differences in averages are significant. The averages of IAS 5, BR 4, and Ipagro 21 did not differ between one another. The Década cultivar had the highest frequency of "P pollen". These results indicate a genotype effect over the "P pollen" occurrence. This effect has been observed in several species (Maheshwari et al. 1980). Heberle-Bors (1984) pointed to the genotype as a major limiting factor for pollen plant production from anther cultures. However, nowadays strategies are being developed to overcome recalcitrance of genotypes.

In average, the normal microspores measured 26.23 μm in diameter and 23.09 μm in distance between two pores, while "P pollens" had a diameter of 23.87 μm and a distance of 18.49 μm between the pores (Table 2). The difference of size between normal and atypical microspores was statistically significant in all 4 cultivars. These data as well as the staining properties indicate that these grains can be considered "P pollens".

According to literature, these atypical uninucleate microspores are defined as "P pollen". The theory of "P pollen" (pre-mitotic pollen) was developed by Heberle-Bors and Reinert (1979). They verified the existence of a clear dimorphism in tobacco pollen grains.

Heberle-Bors (1982 b) proposed that the androgenetic capacity of a microspore is determined at meiosis. The author detected thad during the meiotic process some microspores ("P pollen") do not eliminate completely the maternal cytoplasmic information (mRNA and polysomes) as occurs in normal grains. The cytoplasmic particles could reactivate the sporophytic program of the pollens, that will follow the embryogenic pathway.

In relation to the atypical binucleate pollen, there are some articles reporting this kind of dimorphism (Sax

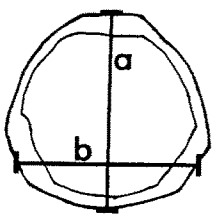

Fig. 1. Drawing showing the two measurements used to analyse the pollen size. *a* Pollen diameter; *b* distance between pores

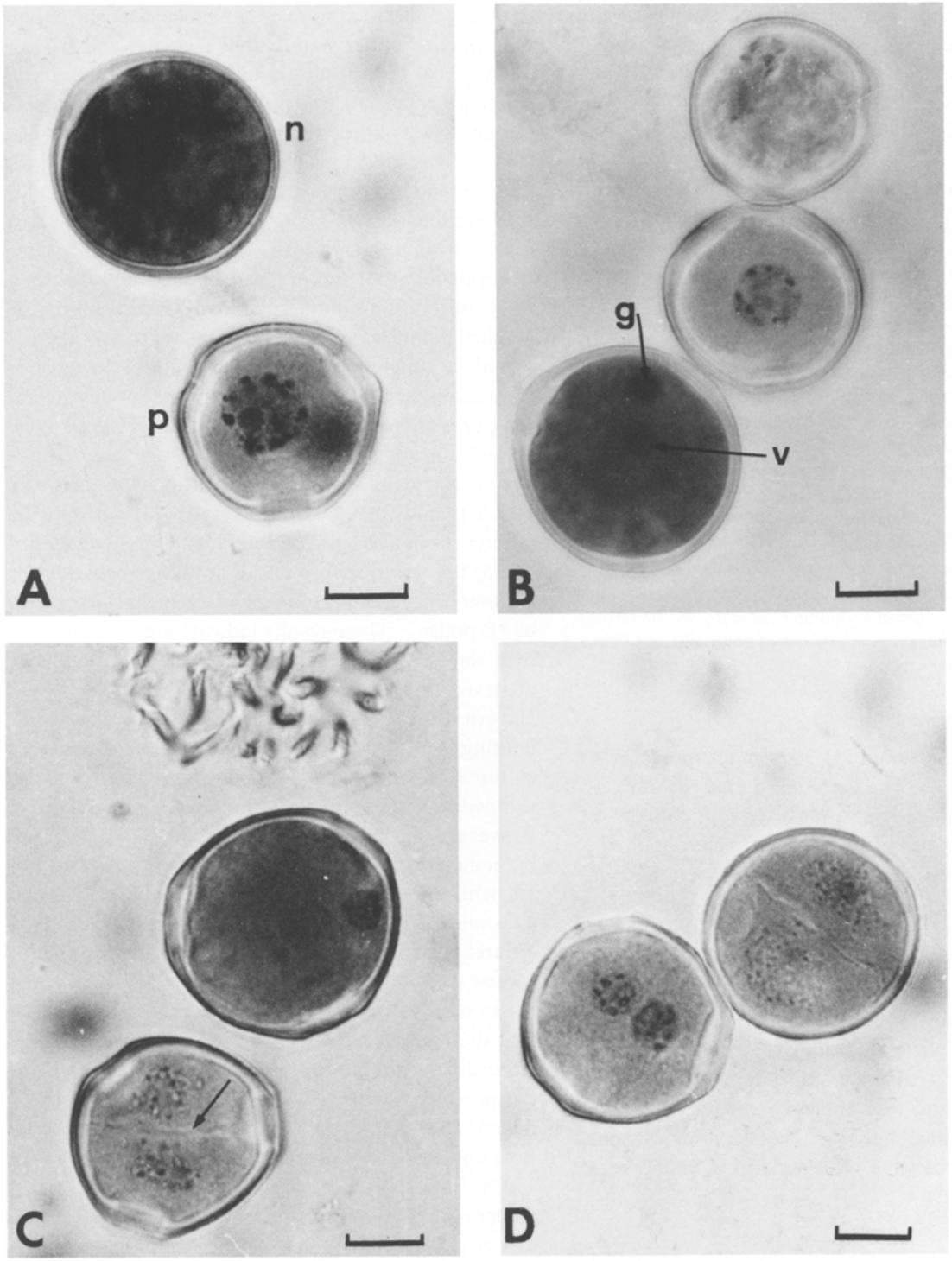

Fig. 2. **A** Normal binucleate pollen (*n*) containing starch grains and cytoplasm which stains so deeply that the nuclei are almost totally obscured; *p* uninucleate "P pollen" clearly retarded in relation to the normal type and showing a weaker staining reaction; note difference in pollen size. **B** Two "P pollen" grains and a normal young bicellular grain. *g* Generative nucleus; *v* vegetative nucleus. **C** A normal binucleate pollen and a binucleate "P pollen" with two equal nuclei; note the wall separating the nuclei (arrow). **D** "P pollen" showing identical nuclei with different contraction levels. Bars: 10 μm

Table 1. Frequencies of "P pollen" in four cultivars of *G. max*

Cultivar	No. of plants examined	No. of pollen grains examined	"P pollen"			
			Uninucleate		Binucleate	
			%	Duncan test[a]	%	Duncan test[a]
Década	15	7500	3.69 (± 3.55)	A	1.86 (± 1.84)	A
IAS5	15	7500	0.25 (± 0.24)	B	0.04 (± 0.11)	B
BR4	15	7500	0.94 (± 1.19)	B	0.09 (± 0.18)	B
Ipagro 21	15	7500	1.32 (± 4.13)	B	0.17 (± 0.47)	B
Total	60	30000	1.55 (± 3.07)		0.54 (± 1.22)	

[a] Means followed by the same character are not significantly different ($P < 0.005$)

Table 2. Measurements related with pollen size

Cultivar	Pollen diameter		Prob. > T	Distance between pores		Prob. > T
	normal	"P pollen"		normal	"P pollen"	
Década	25.23	22.28	0.0001*	22.75	19.15	0.0001*
IAS5	26.33	23.12	0.0020*	23.12	19.33	0.0023*
BR4	26.16	22.53	0.0001*	23.04	18.19	0.0001*
Ipagro 21	26.85	23.85	0.0001*	23.45	17.31	0.0001*
Total	26.39	22.87	0.0001*	23.09	18.49	0.0001*

1935, Nitsch and Norreel 1973, Sunderland 1974, Horner and Street 1978). Microspores with two identical cells can be formed by two ways: non-formation of the vacuole or change of the division axis (Sax 1935, La Cour 1949, Sunderland and Wicks 1971).

Sangwan and Sangwan-Norreel (1987) reported that pollens with symmetrical division, resulting from non-existence of vacuole, could not eliminate molecules carrying sporophytic information and follow the androgenesis, like the "P pollen".

A clear microspore dimorphism was observed in soybean cultivars. In future experiments, it will be investigated whether the two types of pollen show differential response in culture and if these "P" grains are really embryogenic. In this sense, we intend to test the androgenic capacity of the "P pollen" by culturing them in special culture conditions.

Acknowledgements

The authors are thankful to Dr. Costa for the plant care. Seeds were kindly provided by Fundacep-Fecotrigo. This study was supported in part by Funação Banco do Brasil, Fapergs and Finep.

References

Dale PJ (1975) Pollen dimorphism and anther culture in barley. Planta 127: 213–220

De Buyser J, Picard E (1975) Observation de divisions supplémentaires dans les grains de pollen de plants homozigotas de blé tendre (*Triticum aestivum* L.) obtenues par androgènese in vitro. C R Acad Sci 281: 1153–1156

Heberle-Bors E (1982 a) In vitro pollen embryogenesis in *Nicotiana tabacum* L. and its relation to pollen sterility, sex balance, and floral induction of pollen donor plants. Planta 156: 396–401

– (1982 b) On the time of embryogenic pollen grain induction during sexual development of *Nicotiana tabacum* L. plants. Planta 156: 402–406

Heberle-Bors E (1984) Genotypic control of pollen plant formation in *Nicotiana tabacum* L. Theor Appl Genet 68: 475–479

– Reinert J (1979) Androgenesis in isolated pollen cultures of *Nicotiana tabacum*: dependence upon pollen development. Protoplasma 99: 237–245

– – (1980) Isolated pollen cultures and pollen dimorphism. Naturwissenschaften 67: 311–312

Horner M, Street HE (1978) Pollen dimorphism – origin and a significance in pollen plant formation by anther culture. Ann Bot 42: 763–777

La Cour LF (1949) Nuclear differentiation in the pollen grain. Heredity 3: 319–337

Maheshwari SC, Rashid A, Tyagi AK (1982) Haploid from pollen grains – retrospect and prospect. Amer J Bot 69: 865–879

– Tyagi AK, Malhotra K (1980) Induction of haploidy from pollen grains in angiosperms – current status. Theor Appl Genet 58: 193–206

Nitsch C, Norreel B (1973) Effect dum choc thermique sur le pouvoir embryogènese du pollen de *Datura innoxia* cultivé dans l'anthére ou isolé de l'anthére. C R Acad Sci 276 D: 303–306

Rashid A, Street HE (1974) Segmentations in microspores of *Nicotiana tabacum* which lead to embryoid formation in anther cultures. Protoplasma 80: 323–324

Sangwan RS, Sangwan-Norreel BS (1987) Biochemical cytology of pollen embryogenesis. Int Rev Cytol 107: 221–272

Sax K (1935) The effect of temperature on nuclear differentiation in microspore development. J Arnold Arbor 19: 301–310

Sunderland N (1974) Anther culture as a means of haploid induction. In: Kasha KJ (ed) Haploid in higher plants: advance and potential. University of Guelph Press, Guelph

– Wicks FM (1969) Cultivation of haploid plants from tobacco pollen. Nature 224: 1227–1229

– – (1971) Embryoid formation in pollen grains of *Nicotiana tabacum*. J Exp Bot 22: 213–226

Yuyu J, Liu D, Luo X, Zhao G (1986) Studies on induction of pollen plants in *Glycine max* (L.) Meer. Chiang-su-Nung-Yeh-Hseuh-Pao J Agri Sci (Nanjing) 2 (Suppl): 26–30

Wilson HM, Mix G, Foroughi-Wher B (1978) Early microspore divisions and subsequent formation of microspore callus at high frequency in anthers of *Hordeum vulgare*. J Exp Bot 29: 227–238