Theor Appl Genet (1994) 89:814–817

H. S. Yu · S. D. Russell

Quantitative differences in sperm cells and organelles of tobacco (*Nicotiana tabacum* L.) grown under differing environmental conditions

Received: 1 February 1994 / Accepted: 10 June 1994

Abstract In flowers grown at warm temperatures in environmental chambers and at cooler temperatures in the greenhouse, eight parameters of the sperm-cell organization of *Nicotiana tabacum* were examined during sperm-cell maturation using serial ultrathin sectioning, transmission electron microscopy and quantitative cytology. Despite employing the same seed source, and similar soil and nutrient conditions, the surface area and volume of the cell, the nucleus and the chondriome were larger in flowers grown in growth chambers under warmer controlled conditions, whereas the number of plastids appeared to be the same, or slightly higher, in flowers grown under cooler greenhouse conditions. These results suggest that environmental conditions may influence the quantity of cytoplasmic organelles, including mitochondria and plastids, thus potentially influencing the likelihood of male cytoplasmic inheritance.

Key words Cytoplasmic inheritance · Environmental conditions · *Nicotiana tabacum* · Sperm cell

Introduction

Although the majority of the angiosperms are believed to express uniparental maternal inheritance of mitochondrial and chloroplast DNA, a significant minority inherit some cytoplasmic DNA from the paternal parent (for review, see Smith 1988). The structural basis of this transmission has been examined in detail using the electron microscope to study sperm cells (see Mogensen 1992) and fertilized embryo sacs (see Russell 1992). DNA-specific fluorochromes have also been used to screen for the presence and condition of male organelles and whether they contain DNA (Corriveau and Coleman 1988). With the advent of three-dimensional reconstruction and quantification, both the number of organelles and the quantity of sperm cytoplasm can be measured accurately. These data provide an indication of the amount of paternal cytoplasm available for fusion with the female reproductive cells during fertilization. Although there is evidence that the phenomenon may be under genetic control (Derepas and Dulieu 1992), the possibility of environmental influences on the organelle content of sperm cells has not been previously examined.

The concept for this analysis arose in studies on the organization and maturation of the male germ cells of tobacco (*Nicotiana tabacum* L.) and is based in part on data from previous studies (Yu et al. 1992; Yu and Russell 1994). In the present report, seed of the same cultivar grown in similar soil conditions under cooler and less-rigidly controlled temperatures in a greenhouse and under warmer and more-controlled conditions in environmentally controlled growth chambers were compared structurally. Sperm cells were serially reconstructed to determine their quantitative cytological organization and that of their organelles.

Materials and methods

Plant material

Plants of *N. tabacum* L. cv Da Qing Ye (Solanaceae) used for this study were cultivated either under greenhouse or environmental-chamber conditions. Plants grown under controlled conditions in growth chambers underwent daily temperature shifts of 19 °C (night-time) to 25 °C (daytime), 80–90% humidity and 15 h daylength. Light intensity was 600 μEinsteins · m^{-2} s^{-1} at 10 cm. Greenhouse-grown plants were raised under ambient conditions in November 1987 at the Department of Biology, Peking University, Beijing, China; temperatures were variable, averaging about 5–7 °C cooler than growth-chamber-grown plants, with a daylength of 9–10 h.

Communicated by R. Hagemann

H. S. Yu[1] · S. D. Russell (✉)
Department of Botany and Microbiology, University of Oklahoma, Norman, OK 73019, USA

Present address:
[1] Department of Pharmaceutics, College of Pharmacy, Rutgers University, P. O. Box 789, Piscataway, NJ 08855, USA

Pollination and semi-vivo culture of pollen tubes

Emasculated flowers were hand pollinated using mixed pollen from several plants. Styles with attached stigmas were harvested just ahead of the tip of the pollen tubes at 16.5 h after pollination for greenhouse-grown flowers and at 24.5 h for environmental-chamber-grown flowers. Pollen tubes were then allowed to grow an additional 1.5 h at 28–30°C on a 10% sucrose/0.01% boric acid medium in semi-vivo culture (Yu et al. 1992; Yu and Russell 1993, 1994). At 18 h and 26 h after pollination, pollen tubes emerged from the cut end of the style and short stylar segments were fixed using glutaraldehyde followed by osmium tetroxide (Yu et al. 1989). The styles were then dehydrated, infiltrated, embedded and serially sectioned at 100 nm using a diamond knife (Yu and Russell 1993). Each micrograph in a series was printed at the same magnification and numbered according to its order. All observed sperm cells were at least 200 μm away from the cut surface of the style to minimize direct exposure to the culture medium.

Quantitative cytology

Electron micrographs of serial sections were traced into an IBM AT or an IBM compatible 386-33 computer to obtain three-dimensional datasets using methods described previously (Yu and Russell 1992; Yu et al. 1992). Volumes and surface areas of sperm cells, nuclei and mitochondria were obtained from datasets using two utility programs (MEASURE obtained from Dr. S. Young, author of the reconstruction program, and VOLUME written by S. D. R.). The numbers of organelles were determined by comparing organellar profiles throughout the series, counting each organelle once. Sperm cells were statistically compared using an anova test.

Results and discussion

The two sperm cells of tobacco differ in organization: one, S_{vn}, is physically associated with the vegetative nucleus throughout development, whereas the other, S_{ua}, is unassociated with the vegetative nucleus (Fig. 1). This association of the two sperm cells and the vegetative nucleus, forming a male germ unit, has been described in detail in tobacco (Yu et al. 1989, 1992, 1994; Yu and Russell 1993, 1994) and in other flowering plants (see Mogensen 1992). There were no evident changes in this association under the different growth regimes, but quantitative differences in some parameters occurred. There were no ultrastructural differences in sperm cells grown under the two described conditions, but differences as a result of age were evident (Fig. 1) with respect to the increased length of the protuberance of the S_{vn} and increased thickness of the sperm cell wall.

The volume of the sperm cells increased under warmer growth-chamber conditions (Table 1), exceeding the greenhouse-grown sperm cells by 90.9 μm^3 for the S_{vn} (an increase of 71.5%) and 87.7 μm^3 for the S_{ua} (an increase of 62.8%). The nuclear volume of the sperm cells increased by only 10.5 μm^3 in the S_{vn} (an increase of 19.4%) and 9.4 μm^3 in the S_{ua} (an increase of 16.5%). The volume of the cytoplasm, however, increased by 80.5 μm^3 in the S_{vn} (an increase of 110.4%) and 78.6 μm^3 in the S_{ua} (an increase of 95.2%). Increases in cytoplasmic volume accounted for over 85% of the change in the volume of the S_{vn} and over 89% of the change in the S_{ua}. Thus, changes in sperm size may be principally attributed to cytoplasmic rather than nuclear enlargement.

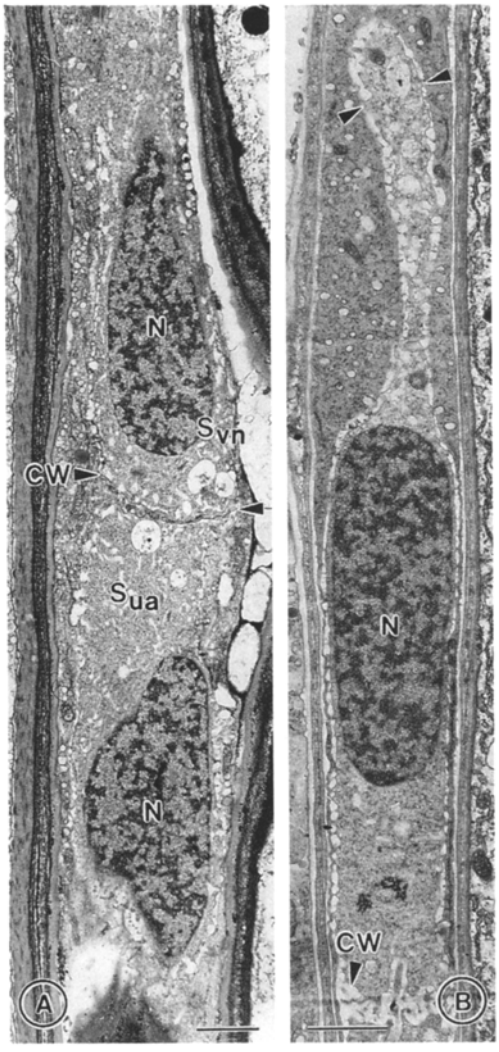

Fig. 1 A, B Transmission electronmicrographs of sperm cells of tobacco grown under differing growth conditions. **A** The sperm cell associated with the vegetative nucleus (S_{vn}) and the sperm cell unassociated with the vegetative nucleus (S_{ua}) travel in tandem after 9 h of growth in an environmental chamber. A slender cell wall (*CW*) separates the cells soon after inception; ×5 250. **B** The sperm cell associated with the vegetative nucleus after 18 h growth under greenhouse conditions. This older sperm cell has a prominent projection (*arrowheads*) at its leading edge and a thicker cell wall. Although the ultrastructure of sperm cells may differ as a result of relative age, no significant cytological differences are evident as the result of different growth conditions; ×13 200. *N* nucleus. Bars 2 μm

It might be expected that differences in surface area would not vary as dramatically as volume, but the surface area of the S_{vn} increased by 65.7% – almost as much as the increase in volume (Table 1). Initially, the surface area of the S_{vn} was 44.6% larger than that of a sphere of equivalent volume; its surface area increased to 67.3% larger than that of a sphere of equivalent volume at 26 h. This presumably reflects the elongation of the S_{vn} during development. At the time of sperm formation only a small protu-

Table 1 Comparison of selected cellular parameters in cooler greenhouse-grown and warmer environmental-chamber-grown sperm cells of *N. tabacum* determined from three-dimensional reconstructions

Parameters	Sperm cells – cooler greenhouse-grown grown flowers (n=8) (Yu et al. 1992)		Sperm cells – warmer environmental chamber-grown flowers (n=14)		Difference	
	S_{vn} [a]	S_{ua} [b]	S_{vn}	S_{ua}	S_{vn}	S_{ua}
Cell volume (μm^3)						
Range	64.1–189.4	68.0–234.8	178.7–276.2	170.8–276.2		
Mean±SEM	127.1±13.8	139.7±18.7	218.0±11.7	227.4±12.4	+71.5%	+62.8%
Cell surface (μm^2)						
Range	117.5–214.7	103.4–239.5	212.7–389.0	170.5–337.5		
Mean±SEM	176.8±10.8	187.8±16.7	293.0±9.7	254.8±15.5	+65.7%	+35.7%
Cytoplasmic volume (μm^3)						
Range	36.7–115.4	40.2–159.8	113.1–202.4	107.3–217.8		
Mean±SEM	72.9±9.1	82.5±13.3	153.4±11.1	161.1±11.1	+110.4%	+95.2%
Nuclear volume (μm^3)						
Range	27.4–74.0	27.8–74.9	53.9–78.2	52.4–75.8		
Mean±SEM	54.2±5.5	56.9±5.8	64.7±2.8	66.3±2.6	+19.4%	+16.5%
Nuclear surface (μm^2)						
Range	42.9–76.6	38.2–95.25	3.8–107.1	55.0–84.2		
Mean±SEM	64.2±4.6	67.6±6.7	70.1±4.7	71.3±3.0	+9.2%	+5.5%
Mitochondrial volume (μm^3)						
Range	0.76–2.77	0.98–3.32	2.60–4.93	2.67–5.55		
Mean±SEM	1.69±0.23	2.19±0.32	3.35±0.26	3.69±0.35	+98.2%	+68.5%
Mitochondrial surface (μm^2)						
Range	10.5–29.3	13.5–30.5	22.2–41.4	24.2–44.6		
Mean±SEM	18.3± 2.0	22.6±3.4	29.6±2.5	31.8±2.8	+61.7%	+40.7%
Mitochondrial number						
Range	12–33	15–31	26–49	32–72		
Mean±SEM	22.6±2.12	23.0±2.85	39.6±4.6	42.9±3.7	+75.2%	+86.5%
Plastid number						
Range	0–2	0–2	0	0–1		
Mean (± SEM)	0.38±0.26	0.25±0.25	0	0.12±0.12	–100%	–52%

[a] Leading sperm cell
[b] Trailing sperm cell
[c] Standard error of the mean

berance may be evident on the S_{vn}, but this later extends to form a projection that may be over 20 μm long. The surface area of the S_{ua} stays essentially the same, but decreases slightly, from 44.2% to 41.4% larger than the surface area of the equivalent sphere. The surface area of the S_{ua} increased by 35.7%. In contrast, the surface area of the nuclei increased by only 9.2% in the S_{vn} and 5.5% in the S_{ua} (Table 1); sperm nuclei remained essentially spheroidal throughout their development.

There are significant increases of mitochondrial quantity from 22.6 to 39.6 mitochondria in the S_{vn} (an increase of 75.2%) and from 23.0 to 42.9 mitochondria in the S_{ua} (an increase of 86.5%) (Table 1). The total volume of mitochondria in each cell increased significantly, by 98.2% in the S_{vn} and 68.5% in the S_{ua}, and the total surface area of mitochondria increased by +61.7% in the S_{vn} and +40.7% in the S_{ua}. Individual mitochondria increased only slightly in volume in the S_{vn} (+13.1%) and decreased slightly in volume in the S_{ua} (–9.7%); the surface area of individual mitochondria decreased by 7.7% in the S_{vn} and 24.6% in the S_{ua}.

The quantity of plastids displayed a slight, but statistically insignificant, decrease in sperm-cells grown in environmental chambers (P=0.10 anova, P=0.20 anova for unequal variances). Of 16 sperm cells grown in cooler temperatures under greenhouse conditions, three plastids were seen in S_{vn} cells and two plastids were seen in S_{ua} cells, whereas in the 28 sperm cells grown under warmer environmental chamber conditions, only two plastids were seen and these both in the S_{ua} (Table 1).

Although environmental conditions appear to be the most critical factors, several additional components may contribute to the observed differences, including: (1) asynchronous maturation of the sperm cells during pollen incubation and (2) the effects of semi-vivo culture on sperm cells. Pollen tubes were collected at different times in the greenhouse-grown flowers (collected at 18 h after pollination) and the growth-chamber-grown flowers (collected 9 and 26 h after pollination). A prior study comparing developmental changes during sperm maturation found no significant alterations in the volume of the sperm cells, cytoplasm, nuclei and mitochondria, or in the surface area

of the nucleus and mitochondria during the period between 9 h and 26 h after pollination. Only one measured parameter differed significantly: the surface area of the sperm cells increased from an average of 228 μm^2 to 318.8 μm^2 (an increase of 39.8%). Despite this difference, even newly formed sperm cells of pollen tubes grown in the warmer growth-chamber-grown plants have a greater surface area than the cooler greenhouse-grown sperm cells by 25.1%. Therefore, none of the differences in cellular parameters could be attributed principally to differences in age. The sperm cells used in the present study were incubated briefly (1.5 h) using semi-vivo culture conditions. That such a brief exposure would have had an appreciable affect on either pollen tube growth rates or the quality of the ultrastructural preservation seems unlikely. Also, a study by Read et al. (1993) on the culture of tobacco pollen tubes reports that their growth under semi-vivo conditions closely resembles that of in vivo grown tubes and is preferable to in vitro culture.

Since the seed source and pollen culture conditions were essentially the same for both studies, it appears that differences in the environment of the plant, including but not limited to temperature and daylength, can influence sperm-cell parameters. Growth under different environmental conditions could potentially change the ratio of male to female cytoplasm within the zygote and so affect cytoplasmic inheritance. This is particularly true if the embryo sac does not display similar changes. The potential influence of the environment on the cells of the embryo sac, including the egg and central cells, has not been examined, but the frequency of organelles and other cytoplasmic parameters may vary in these tissues also.

That environmental conditions may influence plant growth and development has been reported for somatic and floral development, but has not been reported specifically for either male or female gametes of any flowering plant. In tobacco, except for plastid frequency, all parameters measured appeared to increase under warmer environmental-chamber growth conditions, whereas plastids were the same or slightly more abundant under the cooler greenhouse conditions.

When these experiments were conducted, we did not expect to find any differences in the condition of the male cytoplasm based on environmental conditions. Therefore, we did not attempt to experimentally alter the variables and other methods should be developed to address this problem given the labor-intensive nature of sampling in the current study. Undoubtedly, the experiments would have been designed differently if we had anticipated this type of variation.

Angiosperm growth, in general, is known to be affected by temperature, lighting, and a variety of other conditions. According to Yu et al. (1994), most if not all of the cytoplasm of the sperm cells of *Nicotiana* is transmitted during fertilization and enters the egg and central cells during gametic fusion. Combined with the evidence of plastid inheritance provided by Medgyesy et al. (1986), the expression of male cytoplasmically transmitted genetic material is possible, although the input in numbers of paternal plastids during gametic fusion is highly restricted (Yu et al. 1992, 1994; current study). Future research will be required to investigate whether this sort of environmental influence occurs in other flowering plants and whether growth conditions may be altered in breeding regimes in order to mdify some of the characteristics of cytoplasmic inheritance.

Acknowledgements We thank Mr. Gregory W. Strout for technical assistance and gratefully acknowledge use of the Samuel Roberts Noble Electron Microscopy Laboratory of the University of Oklahoma. This research was supported in part by U.S. Department of Agriculture grant 91-37304-6471.

References

Corriveau JL, Coleman AW (1988) Rapid screening method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperm species. Am J Bot 75:1443–1458

Derepas A, Dulieu H (1992) Inheritance of the capacity to transfer plastids by the pollen parent in *Petunia hybrida* Hort. J Hered 83:6–10

Medgyesy P, Páy A, Márton L (1986) Transmission of paternal chloroplasts in *Nicotiana*. Mol Gen Genet 204:195–198

Mogensen HL (1992) The male germ unit: concept, composition and significance. Int Rev Cytol 140:129–147

Read SM, Clarke AE, Bacic A (1993) Requirements for division of the generative nucleus in cultured pollen tubes of *Nicotiana*. Protoplasma 174:101–115

Russell SD (1992) Double fertilization. Int Rev Cytol 140:357–388

Smith SE (1988) Biparental inheritance of organelles and its implications in crop improvement. Plant Breed Rev 6:361–393

Yu H-S, Russell SD (1992) Male cytoplasmic diminution and male germ unit in young and mature pollen of *Cymbidium goeringii*: a 3-dimensional and quantitative study. Sex Plant Reprod 5: 169–181

Yu H-S, Russell SD (1993) Three-dimensional ultrastructure of generative cell mitosis in pollen tubes of *Nicotiana*. Eur J Cell Biol 61:338–348

Yu H-S, Russell SD (1994) Populations of plastids and mitochondria during male reproductive cell maturation in *Nicotiana tabacum* L.: a cytological basis for occasional biparental inheritance. Planta 193:115–122

Yu H-S, Hu S-Y, Zhu C (1989) Ultrastructure of sperm cells and the male germ unit of *Nicotiana tabacum*. Protoplasma 152:29–36

Yu H-S, Hu S-Y, Russell SD (1992) Sperm cells in pollen tubes of *Nicotiana tabacum* L.: Three-dimensional reconstruction, cytoplasmic diminution and quantitative cytology. Protoplasma 168:172–183

Yu H-S, Huang B-Q, Russell SD (1994) Transmission of male cytoplasm during fertilization in *Nicotiana tabacum*. Sex Plant Reprod 7:312–323