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QUANTITATIVE ANALYSIS OF DNA AND HISTONES OF NUCLEI IN FERTILE AND STERILE ANTHERS OF *BETA VULGARIS* L.

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ABSTRACT

Quantitative analysis of DNA and histones of nuclei in the anthers of male fertile (MF), cytoplasmic, genic and gamma-ray induced cytoplasmic male sterile (MS) lines of *Beta vulgaris* indicated that in MS anthers, the quantity of DNA and histones corresponded to the MF lines upto microspore tetrad stage but in subsequent stages there was a progressive retrogression and the nuclei of vacuolate pollen grains and tapetal cells completely lost their stainability. It is concluded that this is due to an impaired synthesis of nuclear DNA and histones associated with their progressive degradation.

Key Words : DNA, histones, male sterility, sugarbeet

Cytoplasmic male sterility (CMS) has been found in 140 species of 40 genera from 20 families including sugarbeet species [1]. Several investigators are of the opinion that there must be a biochemical dialogue between the tapetal cells and the developing pollen grains and when normal communication between these two tissues is disturbed in any way, abnormalities in development occur leading to male sterility [2]. Present investigation has been undertaken to estimate the quantity of DNA and histones in meiocytes and tapetal cells in the anthers of male fertile, cytoplasmic, genic and gamma-ray induced male sterile lines of *Beta vulgaris* L.

MATERIALS AND METHODS

Present investigation was carried out on male sterile lines of various origins in *Beta vulgaris* (Table 1). The seeds of male sterile lines alongwith their fertile counterparts were grown at Plant Breeding Institute, Faculty of Agriculture, Hokkaido University. Sapporo, Japan. The seedlings at 5-7 leaf stage were kept in growth chambers for two months at 4-8°C in constant illumination for vernalization and photoperiodic induction of flowering. The floral buds of various strains were fixed in 1:3 acetic-alcohol and formalin-acetic alcohol respectively for quantitative estimation

Table 1. Nuclear DNA levels in arbitrary units in the sporogenous (S) and tapetal (T) tissues of male fertile and male-sterile lines

Strain	Pheno-type	Tissue	Developmental stage				
			a	b	c	d	e
TK 76-O	MF	S	1.96(0.08)	4.12(0.19)	1.82(0.10)	1.90(0.06)	2.16(0.05)
		T	2.10(0.03)	4.32(0.07)*	2.42(0.04)	1.98(0.07)	0.34(0.03)
TK 76-S	CMS	S	1.93(0.05)	4.04(0.06)*	1.32(0.03)	0.79(0.03)	-
		T	1.87(0.04)	4.02(0.05)	1.83(0.09)	0.88(0.84)	0.68(0.02)*
H-2002	MF	S	1.98(0.03)	4.02(0.04)	1.81(0.04)	2.01(0.04)	2.23(0.07)
		T	1.97(0.06)	4.12(0.06)	2.50(0.05)	2.14(0.02)	0.52(0.02)
r-60 γ-ray induced	CMS	S	1.99(0.07)	4.30(0.07)	1.22(0.06)	0.81(0.03)	-
		T	1.88(0.08)	4.09(0.08)*	1.91(0.07)	0.83(0.06)*	0.43(0.02)
H-19	MF	S	1.87(0.09)	4.20(0.04)	1.81(0.08)	2.02(0.07)	2.32(0.06)
		T	1.88(0.08)	4.09(0.08)*	1.91(0.07)	0.83(0.06)*	0.43(0.02)
BM-2	Genic	S	1.92(0.04)	4.11(0.04)*	1.34(0.08)	0.76(0.07)	-
	MS	T	1.71(0.06)	4.01(0.06)	1.92(0.07)	0.86(0.08)	0.34(0.02)*

Standard errors in brackets; *Phenotype difference significant at 5% level.
Stage a : Sporogenous tissue, b : PMC, c: microspore tetrad, d : vacuolate microspore and e : vacuolate pollen.

of DNA and histones. Localization of DNA in the anthers was done by the Feulgen procedure and for histones alkaline fast green test was followed and two wave length method was used for the quantitative determination of DNA contents in the interphase nuclei by MPM microscope photometer as described in [3]. The wave-lengths used were 550 and 508 nm. Triplicate readings per nucleus were made on a set of 20 nuclei per development stage, yielding mean relative amounts and standard deviation. The t-test was applied in estimating the significance of difference between male fertile and sterile phenotypes. Similarly, histones were quantitatively measured in the interphase nuclei at 630 and 622 wave lengths [3].

RESULTS AND DISCUSSION

Quantitative analysis of DNA and histones of nuclei of fertile and sterile anthers

Tables 1 and 2 show the trends of nuclear DNA and histone levels during five successive stages of anther development of sporogenous and tapetal tissues in TK

Table 2. Nuclear histone levels in arbitray units in the sporogenous (S) and tapetal (T) tissues of male of fertile and male-sterile lines

Strain	Pheno-type	Tissue	Developmental stage				
			a	b	c	d	e
TK 76-0	MF	S	1.54(0.07)	3.21(0.08)	1.15(0.04)	1.36(0.05)	1.62(0.10)
		T	1.41(0.06)	2.78(0.04)	1.87(0.06)	1.16(0.03)	1.12(0.08)
TK 76-S	CMS	S	1.61(0.06)	3.80(0.09)	1.31(0.05)	1.02(0.04)*	-
		T	1.64(0.08)	2.92(0.06)*	1.21(0.07)*	1.05(0.06)	0.76(0.02)*
H-2002	MF	S	1.81(0.06)	3.49(0.09)	1.21(0.05)	1.42(0.06)	1.71(0.09)
		T	1.62(0.07)	3.23(0.08)	1.96(0.04)	1.21(0.06)	1.31(0.06)
r-60 γ-ray		S	1.71(0.08)	3.31(0.08)	1.42(0.06)*	0.91(0.07)	-
		T	1.50(0.02)	3.11(0.07)	1.34(0.04)	1.21(0.06)	0.33(0.07)*
H-19	MF	S	1.52(0.09)	3.67(0.03)	1.36(0.08)	1.47(0.08)	1.92(0.09)
		T	1.53(0.07)	3.40(0.07)	2.03(0.07)	1.37(0.07)	1.12(0.06)
BM-2	MS	S	1.81(0.06)	3.58(0.05)*	1.52(0.09)	0.99(0.09)	-
		T	1.67(0.04)	3.42(0.06)	1.41(0.02)	1.24(0.07)*	1.42(0.07)

Standard errors in brackets; *Phenotype difference significant at 5% level.
Stage a : Sporogenous tissue, b : PMC, c : microspore tetrad, d : vacuolate microspore and e : vacuolate pollen.

76-0 (MF), TK 76-S (CMS), H-2002, gamma-60 (gamma-ray induced cytoplasmic male sterile line) and genic male sterile line BM-2 and its fertile counter part H- 19. The stages of development are : stage a : sporogenous tissue, stage b : microspore mother cells before meiotic division, stage c : microspore tetrad, stage d : vacuolate microspore and stage e : vacuolate pollen grain.

It is evident from Tables 1 and 2 that at stage a, the quantity of DNA and histones in sporogenous and tapetal cells in both fertile and sterile lines was more or less equal. Although the histone values were comparatively lower than the DNA values, the terminal DNA/histone ratios were, however, above one and mostly in a range of 1:1 to 1:6. However, between the stages a and b, the quantity of both DNA and histones in different lines increased more or less double to that recorded in the previous stage. In male fertile line (TK 76-0), DNA increased from 1.96 to 4.12 in the nuclei of sporogenous cells and from 2.10 to 4.32 in the nuclei of tapetal cells indicating complete duplication of the genome. Similarly, in sterile strain

(TK 76- S), the quantity of DNA increased from 1.93 at stage a to 4.04 at stage b (prior to meiosis). The other two male fertile (H-2002 and H-19) and male sterile (r-60 and BM-2) lines also exhibited similar trend.

Following meiosis in the meiocytes and karyokinesis in the tapetal nuclei, the quantity of both DNA and histones in the fertile anthers fell back to the original pre-synthetic levels. The completion of meiosis was marked by a second period of DNA and histone synthesis in microspore nuclei which was gradual and nearly linear. On the other hand, nuclei of sporogenous cells with the advance in development, quantity of the DNA and histone linearly decreased in tapetal nuclei from microspore tetrad stage and by the vacuolate microspore stage and with the degeneration of tapetum (stage e), the quantity was at its lowest.

In the sporogenous tissue of male sterile anthers, the relative DNA and histone values corresponded to the normal levels upto microspore tetrad stage. However, subsequently, a progressive quantitative retrogression in DNA and histone quantity occurred and by the vacuolate pollen stage, the nuclei completely lost their stainability. Similarly, a departure from the normal metabolic activity of both DNA and histones was recorded in male- sterile tapetal cells.

The role of tapetum as a possible source of nucleic acid precursor for the meiocytes has been debatable for long. The reduction in the quantity of DNA and histones in the tapetal nuclei may be either due to localized breakdown or its transport to the sporogenous cells and latter seems more plausible. It is well known that tapetum is active in the synthesis of all the major classes of compounds such as protein, lipids, carbohydrates, pollen wall material-sporopollenin and pollenkitt [4-6].

The observations of the present author on male fertile lines of *Beta vulgaris* are more or less similar to those in genic MS lines of barley [7]. According to them, the progressive loss of DNA, histones and RNA in genic sterile anthers of barley may be due to one of the three possible modes of action, viz. (i) The biosynthesis of each of these three crucial macromolecules is impaired, or (ii) the biosynthesis proceeds at the potential rate but there is a high degree of degradation or (iii) there is an impaired synthesis associated with the progressive degradation. The quantum values recorded in the late stages of development actually fall below the basic levels tend to indicate that the third alternative is actually operative. The sharp decline in the quantity of DNA and histones in the sterile anthers of sugarbeets seems to be a result of blockage or impairment in the synthesis associated with a regular breakdown. As suggested earlier, the inhibition of vascular differentiation in sterile

anthers of sugarbeets may be responsible for the blockage and deficiency of these important macromolecules which act as limiting factor for normal pollen development [8].

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REFERENCES

1. M. L. H. Kaul. 1988. Male sterility in higher plants. Springer-Verlag, Berlin.
2. J. P. Mascarenhas. 1975. The biochemistry of angiosperm pollen development. Bot. Rev., **41**: 259-314.
3. G. P. Berlyn and J. P. Miksche. 1976. Botanical microtechnique and cytochemistry. Iowa State Univ.press. Ames, Iowa.
4. J. P. Mascarenhas. 1990. Gene activity during pollen development. Ann. Rev. Plant Physiol. Plant Mol. Biol., **41**: 317-338.
5. D. Staiger, S. Kappler, M. Muller and K. Apel. 1994. The protein encoded by two tapetum-specific transcripts. *Sa* tap 35 and *Sa* tap 44, from *Sinapis alba* L. are localized in the exine cell wall layer of development microspores. Planta., **192**: 221-231.
6. T. Tscuchiya, K. Toriyama, M. Yoshikawa, S. Ejiri and K. Hinata. 1995. Tapetum-specific expression of the gene for and Eudo-B-1, 3-glucanase causes male sterility in transgenic tobacco. Plant Cell Physiol., **36**: 487-494.
7. H. R. Mian, J. Kuspira, G.W.R. Walker and N. Mutjeweriff. 1981. Macromolecular changes and submicroscopic structure in the differentiation of *msg* 16 male-sterile barley anthers. Proc. IV. Int. Barley Genetics Symposium. Edinburg. pp. 804-813.
8. S. V. S. Chauhan and T. Kinoshita. 1979. Histochemical localization of histones, DNA and proteins in the anthers of male-sterile and male-fertile plants. Japan. J. Breed., **29**: 287-293.