Transcriptional control of mitochondrial genes in different tissues of *NCa* CMS system in *Brassica napus* L. and their relationship with sterility

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Abstract

Northern blot analysis was conducted with mitochondrial RNA from seedling leaves, floral buds, and developing seeds of *NCa* CMS, maintainer line and fertile F_1 using ten mitochondrial genes as probes. Eight genes, such as *orf*139, *orf*222, a*tp*1, *cox*1, *cox*2, *cob, rrn*5S, and *rrn*26S, showed their expression was not regulated by nuclear genes and was not tissue-specific. And the transcription of *atp*9 was neither controlled by nuclear gene nor tissue-specific while the expression of *atp*6 might be tissue-specific under regulation of nuclear gene. Moreover, three transcripts of *orf*222 were detected in the floral buds of *NCa* cms and fertile F_1, but no transcript was detected in floral buds of the maintainer line. The transcription of *orf*139 was similar to that of *orf*222 but only two transcripts of 0.8 kb and 0.6 kb were produced. The *atp*9 probe detected a single transcript of 0.6 kb in *NCa* cms and in maintainer line and an additional transcript of 1.2 kb in fertile F_1. The relationship of expression of *orf*222, *orf*139, and *atp*9 with *NCa* sterility was discussed**.**

Key words: *Brassica napus* L.; cytoplasmic male sterility (CMS); mitochondrial gene expression; restorer gene; transcriptional regulation

Most of the investigations of CMS focused on the transcriptional regulation of sterility-related mitochondrial gene by restorer gene [1-7]. In higher plants, mitochondria are not only pivotal as an organelle supplying energy and carbon framework, but also play an important role in regulation of pollen development [8]. The differential expression of mitochondrial genes in different tissues was detected in several crops [9-12]. And most of the mitochondrial proteins, especially the constituents of transcription apparatus for mitochondrial genes in almost all eukaryotes, are encoded by nuclear genes [13]. It is of great importance to understand the interaction between mitochondrial genes (sterility-related gene included) and nuclear genes (restorer gene included) in different plant tissues and developmental stages.

Pol CMS and *nap* CMS in rapeseed were thoroughly investigated. Sterility-related mitochondrial DNA region of *pol* CMS was *orf*224/*atp*6 [1, 2, 14], and that of *nap* CMS was spanned *orf*222/*nad*5c/*orf*139 [2, 15]. Menassa *et al.* [16] revealed that *pol* CMS was post-transcriptionally regulated by restorer gene and fertility was restored with decrease in the number of the transcripts of sterility-related gene region.

Cytoplasm monotony is emerging in rapeseed and of great risk for hybrid production because hybrid with *pol* cytoplasm is widely used in China and even in other parts of the world, and so it is of high priority to introduce and utilize novel sterile cytoplasm in rapeseed breeding program.

NCa CMS system was showed diverse performance with respect to isozyme profile [17], maintainer–restorer relationship [18], abortion stage and characters of pollen [19], and mitochondrial DNA polymorphism of several sterile cytoplasm in CMS systems of rapeseed [20], and was confirmed to be a novel CMS. So *NCa* CMS system would be important for improving cytoplasm monotony for rapeseed hybrid utilization and for facilitating breeding and production of rapeseed.

In this article, transcriptional regulation of mitochondrial genes in different tissues and developmental stages was studied by analyzing the expression of mitochondrial genes in seedling leaves, floral buds, and developing seeds (20 days after pollination) of *NCa* cms, maintainer line, and fertile F_1.

1 Materials and Methods

1.1 Plant materials

NCa cms, maintainer line, restorer line, and fertile F_1, which was obtained by crossing *NCa* cms with its restorer line, were investigated in this study.

1.2 Methods

Seedling leaves of *NCa* cms, maintainer line, and fertile F_1, and their floral buds lesser than 3 mm in diameter, and the developing seeds 20 d after pollination, were harvested. The mitochondria were extracted as described by Zhang *et al.* [6] and the mitochondrial RNA was extracted using Trizol® kit (Invitrogen™, Beijing, China) according to the manufacturer's description. Northern blot analysis was performed according to Sambrook *et al.* [21].

2 Results

The Northern blot results are summarized in Table 1 and shown in Fig. 1. The transcripts of *atp*1, *cox*1, *cox*2, *cob*, *rrn*5S, *rrn*26S, *orf*139 and *orf*222, suggested that the expression of all these eight mitochondrial genes did not involve interaction with nuclear genes and the expression not tissue-specific.

Only one transcript of 0.6 kb with the same abundance in the three tissues of *NCa* cms, maintainer line and two transcripts of 0.6 kb and 0.8 kb with the same abundance in all three tissues of fertile F_1 were detected by *atp*9. This indicated that the expression of *atp*9 might involve interaction with nuclear gene, but the expression not tissue-specific. However, *atp*6 detected a band of 1.1 kb in length with different abundance in the three tissues of *NCa* cms, maintainer line, and fertile F_1, indicating that the expression of *atp*6 might be influenced by nuclear genes and the expression be tissue-specific.

The transcripts of *orf*222 and *orf*139 were detected with the same size and abundance in floral buds of *NCa* cms and fertile F_1, whereas no transcript was detected in the maintainer line. And a*tp*9 gene corresponded to a band of 0.6 kb in the floral buds of *NCa* cms and maintainer line and two bands of 1.2 kb and 0.6 kb in the fertile F_1. These suggested the transcription of mitochondrial genes of *orf*222, *orf*139, and *atp*9 might be related with sterility of *NCa* CMS.

3 Discussion

Nuclear–cytoplasmic interaction could be cell-specific and functioned throughout the developmental stages of plants. However, it was not investigated in detail with regard to the expression of mitochondrial genes in different tissues of CMS system. Menassa *et al.* [16] investigated the expression of *orf*224/*atp*6 region, in sepal, carpel, petal, and stamen of *pol* cms, maintainer line, and fertile F_1 and the result suggested that the fertility of the fertile F_1 was restored by restorer gene by decreasing the abundance of the 2.2 kb and 1.9 kb transcripts and increasing that of the 1.3 kb and 1.4 kb transcripts.

In this article, 9 mitochondrial genes, except for *atp*6, were constitutively expressed mitochondrial genes, which was similar to the results of Menassa *et al.* [16]. Among the nine constitutively expressed expressed without the influence of nuclear genes and with no tissue-specific effect. The transcripts of mitochondrial genes, *atp*1, *cox*1, *cox*2, *cob*, *rrn*5S, *rrn*26S, *orf*222, and *orf*139 were the genes *orf*222 and *orf*139 were with the same size and abundance, respectively, in the three tissues of *NCa* cms and fertile F_1, but no transcript was detected in the maintainer line (Fig. 1). It was hard to explain why *orf*222 (or *orf*139) generated same transcripts in *NCa* cms and fertile F_1 even though *NCa* cms and fertile F_1 had the same cytoplasm and different nuclear context but different transcripts in *NCa* cms and maintainer line that had different cytoplasm and the same nuclear context. Further investigations on the regulation of *orf*222 or *orf*139 in different developmental stages are in progress.

Table1 Transcripts of 10 mitochondrial genes in seedling leaves, floral buds, and developing seeds of *NCa* cms, maintainer line, and fertile F_1

mt gene	transcripts (kb)	cms line			maintainer line			fertile F_1		
		leaf	flower	seed	leaf	flower	seed	leaf	flower	seed
orf 222	1.1	+	+	+	—	—	—	+	+	+
	0.9	+	+	+	—	—	—	+	+	+
	0.6	+	+	+	—	—	—	+	+	+
orf 139	0.8	+	+	+	—	—	—	+	+	+
	0.6	+	+	+	—	—	—	+	+	+
atp 9	1.2	—	—	—	—	—	—	+	+	+
	0.6	+	+	+	+	+	+	+	+	+
atp 6	1.1	+	+	+	+	+	+	+	+	+
atp 1	1.9	+	+	+	+	+	+	+	+	+
cox 2	2.4	+	+	+	+	+	+	+	+	+
	1.6	+	+	+	+	+	+	+	+	+
cox 1	1.8	+	+	+	+	+	+	+	+	+
cob	2.0	+	+	+	+	+	+	+	+	+
	1.7	+	+	+	+	+	+	+	+	+
rrn 5S	1.9	+	+	+	+	+	+	+	+	+
rrn 26S	2.6	+	+	+	+	+	+	+	+	+

+: with visible band; —: without visible band.

The sterility-related mitochondrial gene region generally detected transcriptional diversity between cms and maintainer lines or between cms and fertile F_1, which was verified in CMS investigation of rice, maize, sunflower, and rapeseed [1, 14, 22-26]. In this article, *orf*222, *orf*139, and *atp*9 showed transcriptional diversity between *NCa* cms and maintainer line or between *NCa* cms and fertile F_1, which indicates that these three genes might be associated with *NCa* sterility. But how restorer gene regulated the expression of the sterility-associated mitochondrial gene and then caused fertility restoration in progenies of CMS×restorer line (i.e., fertile F_1) was not clear and a detailed research is underway.

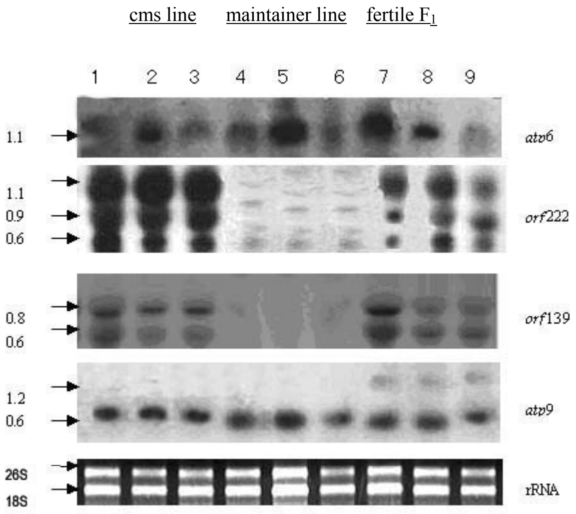

Fig. 1 Transcription analysis of *atp*6, *orf*222, *orf*139, and *atp*9 in seedling leaves, floral buds, and developing seeds of *NCa* cms (lanes 1-3), maintainer line (lanes 4-6), and fertile F_1 (lanes 7–9)

Mitochondrial genome of plants is more diverse in size and structure compared with other higher eukaryotes. Sequences of mitochondrial genome of *Arabidopsis thaliana* [27], rice [28], *Brassica napus* [29], and tobacco (*Nicotiana tabacum*) [30] have been accomplished and would be helpful to investigate the expression characters of mitochondrial genes, especially their tissue-specific manner and regulation mechanism of nuclear–cytoplasmic interaction and to introduce novel CMS to meet the demands of rapeseed breeding and production.

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