Plant Cell Physiol. 49(2): 284–289 (2008)
doi:10.1093/pcp/pcm182, available online at www.pcp.oxfordjournals.org

Short Communication

A Novel *orf108* Co-Transcribed with the *atpA* Gene is Associated with Cytoplasmic Male Sterility in *Brassica juncea* Carrying *Moricandia arvensis* Cytoplasm

Ashutosh[1,4], Pankaj Kumar [1,4], V. Dinesh Kumar [2], Prakash C. Sharma [3], Shyam Prakash [1] and Shripad R. Bhat [1,*]

[1] *National Research Centre on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi-110012, India*
[2] *Directorate of Oilseeds Research, Rajendranagar, Hyderabad-500030, India*
[3] *Guru Gobind Singh Indraprastha University, Kashmere Gate, Delhi-110006, India*

Mitochondrial *atpA* transcripts were examined in cytoplasmic male sterile (CMS) and fertility restorer lines of CMS (*Moricandia arvensis*) *Brassica juncea*. Male sterile flowers had longer *atpA* transcripts than male fertiles. The mitochondrial *atpA* region of the CMS line was cloned and sequenced. The 5′ and 3′ ends of the *atpA* transcripts of the CMS and the fertility restorer lines were mapped and full-length transcripts were cloned and sequenced. A novel *orf108* (open reading frame 108) co-transcribed with the *atpA* gene was found in the male sterile flowers. In the fertility restorer line, the transcript was cleaved within *orf108* to yield monocistronic *atpA* transcripts.

Keywords: *atpA* — *Brassica juncea* — Cytoplasmic male sterility — Fertility restoration — *orf108* — Transcript mapping.

Abbreviations: cRT–PCR: circular reverse transcription–PCR; CMS: cytoplasmic male sterility; ORF; open reading frame; TAP; tobacco acid pyrophosphatase.

Mitochondria carry their own genetic information but are heavily dependent on nuclear-encoded proteins for their replication and correct expression of the information. Similarly, cellular mitochondrial activity can influence expression of nuclear genes and is termed retrograde regulation (Liu and Butow 2006). Thus a well-orchestrated expression of mitochondrial and nuclear genes is regarded as essential for normal growth and development of eukaryotic organisms. As mitochondria carry out vital cellular functions, any impairment of their function is likely to be lethal. Hence there are very few phenotypes linked to mitochondrial gene mutations in plants. Maternally inherited male sterility in angiosperms, referred to as cytoplasmic male sterility (CMS), is known to result from mitochondrial dysfunction. This defect, however, can be overcome by the action of appropriate nuclear gene(s) called restorer of fertility (*Rf*). Thus CMS is one of the rare traits that is governed by nuclear–mitochondrial gene interactions and serves as a model to study such interactions (Hanson and Bentolila 2004).

Detailed molecular analyses of a number of CMS systems in various plant species have revealed that male sterility is associated with either the appearance of novel, often chimeric, transcripts or altered expression, usually in a tissue-specific manner, of some essential mitochondrial gene (Schnable and Wise 1998, Hanson and Bentolila 2004). Such changes in mitochondrial gene expression usually result from rare mitochondrial genome mutations, alien mitochondrial substitutions following wide hybridization or mitochondrial recombination in somatic hybrids. However, each CMS system appears to be unique with respect to the mitochondrial transcript associated with male sterility. Further, the action of the restorer gene is also specific and restoration of male fertility is associated with suppression of cognate chimeric transcripts/proteins and the appearance of normal mitochondrial transcripts. For example, in Texas CMS maize, the chimeric *urf13* has been shown to be responsible for male sterility. Similarly, in *Brassica*, a number of novel open reading frames (ORFs) such as *orf224* (polima CMS), *orf222* (nap CMS) and *orf138* (ogura CMS) have been found to be associated with male sterility. Cloning of restorer genes of CMS systems of petunia, radish, rice, etc. in recent years has revealed that they encode proteins belonging to the PPR family (see Horn 2006), which are implicated in organellar transcript processing (Small and Peeters 2000) and thus match their expected function.

We have earlier reported synthesis of a CMS system in *Brassica juncea* carrying the mitochondrial genome of *Moricandia arvensis* (Prakash et al. 1998). Floral-specific alteration in mitochondrial *atpA* expression was found to be associated with male sterility in this system

[4]These authors contributed equally to this work.
*Corresponding author: E-mail, srbhat22@rediffmail.com; Fax, +91-11-25843984.

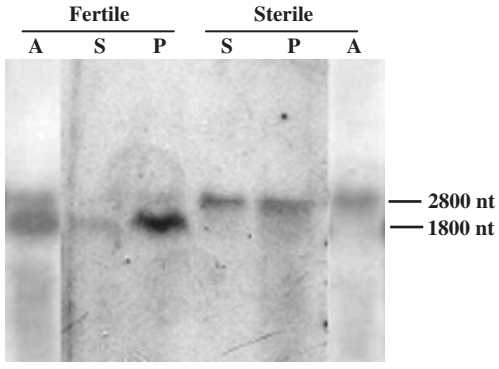

Fig. 1 Expression of the mitochondrial *atpA* gene in sepals (S), petals (P) and anthers (A) of the male sterile (rr) (Sterile) and the fertility-restored (Rr) (Fertile) plants. Northern blot was prepared using about 10 µg of total RNA and probed with the *atpA* coding sequence.

(Gaikwad et al. 2006). Also, we have tagged the *Rf* gene, which acts gametophytically to restore male fertility (Ashutosh et al. 2006). The present study was undertaken to characterize this CMS system further with respect to the *atpA* gene. We report here the identification of a novel *orf108*, which is co-transcribed with the *atpA*. In fertility-restored plants, the bicistronic *orf108-atpA* transcripts are specifically cleaved within the *orf108* to yield monocistronic *atpA* transcripts.

A previous study showed that *atpA* transcripts in flower bud tissues of the CMS plants are longer as compared with normal *B. juncea*, *M. arvensis* and the fertility-restored plants (Gaikwad et al. 2006). However, genetic studies revealed that the *Rf* gene acts gametophytically to confer male fertility to the CMS plants. To verify whether the *Rf* gene was active in all floral tissues or was restricted to the anthers, we examined the transcript pattern of *atpA* in sepals, petals and anthers of the CMS and the fertility-restored (i.e. F_1) plants. Northern analysis revealed a 2,800 nt long *atpA* transcript in all the three whorls of the CMS flower, whereas in the fertility-restored plants, a shorter (1,800 nt) *atpA* transcript was found in sepals and petals (Fig. 1). Anthers of the fertility-restored plants expressed both 2,800 and 1,800 nt transcripts, but the intensity of the longer transcript was weak. Thus the restorer gene action was detected in both sporophytic and gametophytic tissues of the flower, and was inferred to be dominant. When restoration is gametophytic, it is difficult to determine the dominant/recessive nature of gene action through genetic studies. In maize, Kamps et al. (1996) generated tetraploid restorer lines and showed that the diploid gametes of R/r constitution are viable, thereby confirming the dominance of the restorer. The present study demonstrated that Northern analysis of individual whorls of flowers could be helpful in determining the dominance relationship of the gametophytic restorers.

We had found distinct restriction fragment length polymorphism (RFLP) patterns between the CMS and normal *B. juncea* lines with the *atpA* probe (Gaikwad et al. 2006). To know the exact sequence differences in the *atpA* region, we cloned and sequenced the *atpA*-containing 6.0 and 5.8 kb *Hin*dIII fragments from the CMS and euplasmic lines, respectively (male sterile line genomic clone, GenBank accession No. EF483940). The *atpA* coding sequences (1,524 bp) in both clones were identical. Sequences downstream of *atpA* also did not show a significant difference between the two clones. On the other hand, no significant homology was found between the CMS and *B. juncea* beyond 52 bp upstream of the *atpA* initiation site. Northern hybridization with the sequences from the upstream region of *atpA* (1,728–2,173 of the 6.0 kb clone) had revealed a single transcript of 2,800 nt only in the CMS line, indicating that the *atpA* transcript of the CMS line differs from normal *B. juncea* in the 5′ region (unpublished results). Hence, we first tried to determine the 5′ end of the transcripts. Based on genomic DNA sequence information, we designed PCR primers that could amplify various upstream regions of the *atpA* gene (Table 1, Fig. 2A). To identify the location of the 5′ ends of the *atpA* transcripts, we performed reverse transcription–PCR (RT–PCR) using different sets of primers. RNA from the male steriles gave amplicons of the expected size when primers located up to 1,019 nt upstream of the *atpA* start codon were used in RT–PCR (i.e. up to the F8 primer), but no amplicon was obtained with the primers R6 + F9 (Fig. 2B). This indicated that the 5′ end of the transcript is located between the F9 and F8 region (i.e. −1,411 and −1,019 of the *atpA* start codon). In the male fertiles, primers R3 + F3 gave a 170 bp RT–PCR product, while no amplicon was obtained with R4 + F5 primers. Hence, the 5′ end of the *atpA* transcript of the male fertile line was inferred to lie between −503 and −260 nt upstream of the *atpA* start codon (Fig. 2C).

The circular RT–PCR (cRT–PCR) method (Khun and Binder 2002) was employed to identify the exact 5′ and 3′ ends of the *atpA* transcripts. To distinguish between the primary and processed transcripts, two sets of cRT–PCRs were set up. In one, RNA was treated with tobacco acid pyrophosphatase (TAP) prior to self-ligation whereas the other was subjected to self-ligation without TAP treatment. A linear RT–PCR was also included to serve as negative control. The absence of amplicons in linear RT–PCR ruled out DNA contamination and also confirmed that only circularized RNA molecules served as templates for DNA amplification. In the male sterile line, a 1,050 bp, intense cRT–PCR amplicon was obtained only in the treatment where RNA was treated with TAP (Fig. 3). Identical cRT–PCR products were observed in both TAP(+) and TAP(−) treatments in the male fertile line (Fig. 3). Since transcripts with 5′ triphosphate cannot undergo self-ligation,

Table 1 Details of primers used in various experiments

Primer	Sequence (5′–3′)	Position[a]
R1	ATATGGTATCGATAGCAATAG	3,259–3,238
R2	CTAATTCGACTTTCGAATAGATTCG	2,759–2,735
R3	CCGCCCGTTAAATTGATAATATTGAA	2,615–2,590
R4	CAAGGTCATATCATCTCCAAG	2,520–2,500
R5	CGGGTTTCACGATTAGATTA	2,281–2,262
R6	GCTAACTCCTGTAAGCAGCT	1,919–1,900
F1	GGAATTATGGAATTATCTCCCAGAG	2,698–2,722
F2	ACCTTGGGAAATGTTGCTTC	2,515–2,534
F3	ACCATAATGTTCTTTTGCCCG	2,444–2,464
F4	CCCGAAAATCAACTTCTACTTATGAATAC	2,278–2,306
F5	CTTCACTACTCCTAGAGGCT	2,201–2,220
F6	GATCTCTATGCCCCCTGTTCTTGG	2,126–2,149
F7	GCTCCCTCCAAGTGTTGG	1,801–1,818
F8	CTTGCAGACCTACTCGGAAC	1,685–1,704
F9	CTCAGCCAGATGGAAGGTCA	1,293–1,312
F10	CCACTAGACAGAATCTCTCAAT	4,081–4,102

[a]With reference to GenBank sequence No. EF483940.

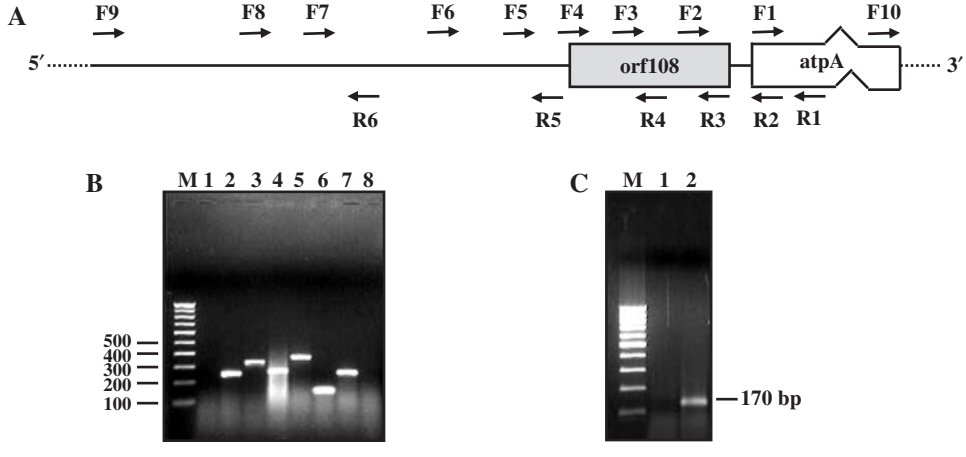

Fig. 2 Identification of the *atpA* transcript initiation region in the male sterile and the male fertile lines. (A) Schematic representation of the mitochondrial *atpA* region of the *Brassica juncea* CMS line carrying *Moricandia arvensis* mitochondria. The position and direction of various primers used in the study are indicated with arrows (figure not to scale). (B) Gel photograph of RT–PCR with various primer combinations using RNA from the male-sterile flowers. M, 100 bp DNA ladder, Bangalore Genei, India; 1, negative control (PCR with R2 + F2 primers without a reverse transcription step); 2, R2 + F2 (58); 3, R2 + F3 (60); 4, R4 + F4 (58); 5, R4 + F6 (58); 6, R6 + F7 (56); 7, R6 + F8 (58); 8, R6 + F9 (58). (C) RT–PCR results obtained using RNA from the male fertile (RR) flowers with primers (1) R4 + F5 (56) and (2) R3 + F3 (60). M, 100 bp DNA ladder (Bangalore Genei, India). The primer annealing temperature (°C) is given in parentheses.

the appearance of identical cRT–PCR products in both TAP(+) and TAP(−) treatments indicated that *atpA* transcripts are processed in the male fertile line. These results show that the male steriles contain mostly the primary *atpA* transcript while male fertiles predominantly carry the processed transcript. Additional weak amplicons observed in both TAP(+) and TAP(−) treatments may have arisen from processed or partially degraded RNA. Cloning and sequencing of the longest and intense amplicons from the male sterile and the male fertile lines helped to identify the 5′ and 3′ ends of *atpA* transcripts. In the male steriles, the 5′ end of the *atpA* transcript was determined to be the T at 1,200 nt upstream of the *atpA* start codon while in the male fertiles it was the T nucleotide situated 390 nt upstream of the *atpA* ATG codon (Fig. 4A). In both the CMS and the fertility restorer lines, the *atpA* transcripts terminated at the same position, which was located 160 nt downstream of the *atpA* stop codon. The complete *atpA* transcripts of both the lines were amplified by RT–PCR and sequenced (GenBank accession Nos. EF483941 and EF483942).

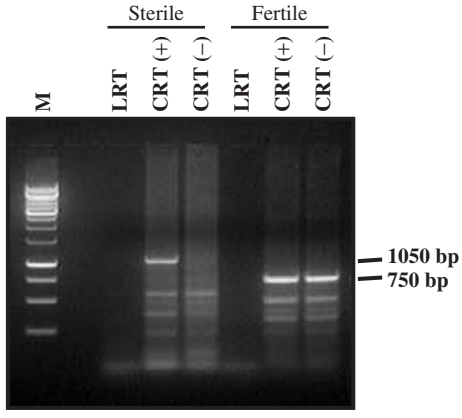

Fig. 3 cRT–PCR amplification of *atpA* from the male sterile and the fertility restorer lines for the identification of transcript extremities. M, 1 kb DNA ladder (Fermentas); LRT, linear RT–PCR; CRT(+), circular RT–PCR with TAP treatment; CRT(−), cicular RT–PCR without TAP treatment.

The *atpA* transcript in the CMS was 2,884 nt long, whereas in the fertility restorer line it was 2,074 nt long (Fig. 4A). These results confirmed our earlier size estimates of *atpA* in the two lines. Further, the 3′ ends of the *atpA* transcripts of the CMS and the fertility restorer lines were not polyadenylated and thus conformed to the norm for mitochondrial transcripts.

Comparative analysis of the *atpA* transcripts of the CMS and the fertility restorer lines revealed a novel *orf108* upstream of the *atpA* region in the male sterile line (Fig. 4A). In the fertility restorer line, the *orf108* was abolished as the transcript was cleaved at 12 nt downstream of the *orf108* initiation codon. Thus the *atpA* transcript of the male sterile line was bicistronic, whereas the transcript of the male fertile line was monocistronic, capable of coding for only *atpA* protein. When primers F4 and R3, capable of yielding a product only from the male sterile transcript, were used in RT–PCR, a 300 bp amplicon was obtained

A

ttgttctggacgagagagtaggaataggtacctgcttcaaccatagaaagtcaacagaggggtgatcctgcttaggtttgaaaac
ctaactcggaatgacgaggttaagaagaggaagatagaagggaaggggtagctgattaaccaactgtaatacaaaaggtacga
tgatcctcctaggcttgcagacctactcggaactgtggaaagaaagcaaagatatttaaaaacggggcttgcgcccctgctttag
ctgcacttgcagaggtagctacagtcgcgagcaggtccttgctcgctccctccaagtgttggttagctacgatagaacacggata
ataaacagataattgcagtgaaggaagaatgacagagaaatacgtatatgataagaaagctgcttacaggagttagccagctca
cttccctcttcgaatggggagatagataaaagaaacgaaactctataaaatatcattaagagaagaagattctcattccagcttaaa
taagtaagacttgactctttgttcactttgttttctcgctccttgctggcgaagaagcggcatatcaaaaataaagaaagaagcaaaa
tctcattcaatttgaaatataagagatctctatgccccctgttcttggttttctcccatgctttgttggtcaacaaccaaccacaactttc
tatagttcttcactactcctagaggcttgacggagtgaagctgtctggagggaattgttgaaatcaattaatctaatcgtgaaacccg
aaaatcaacttctacttA**TGAATACTATCAAA**▼**TCACTTTCCCAAGACATCGAAGAGTTAAAAAA**
AAATGGAAATCTCTTCGATGTCTCCGATCAGTTGTATAACAACGCGCTTGTTAGCGTAAC
TGAAGCACTGACCGGGACGGTCCAACGCCTTTTACAGCACCATAATGTTCTTTTGCCCG
AAAATTGGACCCACGCACAAATAGTACAAGATATACTTGGAGATGATATGACCTTGGGA
AATGTTGCTTCTGTATTGAGCAACATCACTCAGTTGGGACTCGCAAGTGACGAGTTTCAT
CAAATCTTCAATATTATCAATTTAACGGGCGGGGGGGTTTAGttaaggtactatatccgaaggtatac
ttttttttctaaagcggtattcctccttgcctctgaattcaaaaatggaatt***atggaattatctcctagagctgcggaactaacgaatc***
tattcgaaagtcgaattaggaacttttacgcgaattttcaagtggatgagatcggtcgagtggtctcagttggagatgggattg
cacaagtttatggattgaacgagattcaagctggggaaatggttcttttgccaacggtgtgaaaggaatggccttgaatcttg
agaatgagaatgtcgggattgttgtctttggtggtgataccgctataaaagaaggagatcttgtcaagcgcactggatctattg
tggatgttcccgcgggaaaggctatgctagggcatgtggtcgacgcgatgggagtacctattgatggaagaggggctctaa
gcgatcacgagcaaagacgtgtcgaagtgaaagccctgggattcttgaacgtaaatcagtgcacgagcctatgcaaaca
gggttaaaagcggtagatagcctggttcctataggccgtggtcaacgagaacttctaatcggggaccgacaaactggaaa
aacgactattgctatcgataccatattaaaccaaaagcaaataaactcaaggggccacctctgagagtgagacaatgtattgt
gtctatgtagcgattggacagaaacgctcgactgtgggacaattaattcaaactcttgaagaagcgaatgctttggaatattc
cattcttgtagcagccaccgcttcggatcctgctcctctgcaatttttggccccatattccgggtgtgccatgggggaatatttcc
gcgataatggaatgcacgcattaataatctatgatgatcttagtaaacaggcggtggcatatcgacaaatgtcattattgttac
gccgaccaccaggccgtgaggctttcccaggtgatgttttctatttacattcccgtctcttagaaagagcggctaaacgatcgg
accagacaggtgcaggtagcttgaccgccttacccgtcattgaaacacaagctggagacgtatcggcctatattcccacca
atgtgatctccattactgatggacaaatctgtttggaaacagagctcttttatcgcggaattagacctgctattaacgtcggctta
tctgtcagtcgcgtcgggtctgccgctcagttgaaagctatgaaacaagtatgcggtagttcaaaactggaattggcacaata
tcgcgaagtggccgcctttgctcaatttggctcagaccttgatgctgcgactcaggcattactcaatagaggtgcaaggctga
cagaagtaccgaaacaaccacaatatgcaccacttccaattgaaaaacaaatactagtcatttatgcagctgtcaatggatt
ctgtgatcgaatgccactagacagaatctctcaatatgagaaagccattctaaatagtgtcaaacctgaattactacaagccc
ttaaaggtggattaactaacgaaagaaaaatggaactagatgctttcttaaaagaaagagctttagctttaatttagctttcatc
acattaggaatcctcaatcagtaagaaagaaaagttcttaagcaaagactggagaggcccccggtcgagatgtagtaagtaggt
ctccaatactgggagactgaggagaatgggagtttgtgggttgagggtggcatgctccccagggccct

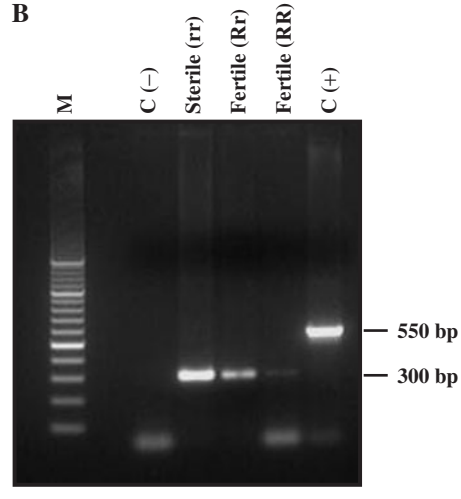

Fig. 4 (A) Complete cDNA sequence of the *orf108*/*atpA* transcript of the male sterile line. Coding sequences of *orf108* (upper case) and *atpA* (bold italics) are highlighted. The triangle indicates the 5′ site of the *atpA* transcript in the male-fertile line. (B) RT–PCR amplification of *orf108* using RNA from flower buds of the male-sterile (rr), fertility-restored (Rr) and fertility restorer (RR) lines. M, 100 bp ladder (Fermentas); C(−), negative control; C(+), positive control for intact RNA in the fertility restorer (RR) with primers F1 + R1 (56°C).

in the CMS and the fertility-restored (i.e. F_1 with R/r genetic constitution) lines (Fig. 4B). The intensity of the band in the fertility-restored plants was nearly half of that observed in the CMS line. In the fertility restorer line (i.e. R/R), a faint band was detectable only when an excess of RT–PCR product was loaded on the gel. These results are in accordance with the gametophytic nature of fertility restoration. In most of the CMS systems studied so far, novel transcripts coding for new ORFs have been found to be associated with male sterility. Such novel ORFs may appear as independent transcripts or co-transcribed with one of the essential mitochondrial genes. For example, in CMS *B. napus* with '*nap*' or '*pol*' cytoplasm, the novel *atp6* transcripts contain the additional *orf222* and *orf224*, respectively (L'Homme et al. 1997, Brown 1999). The fertility restorer genes have been found to cleave these bicistronic transcripts to release monocistronic *atp6* transcripts. The *mori* CMS system appears to follow a similar pattern. Examination of the 5′ sequences of the male sterile *atpA* clone revealed the presence of a consensus sequence of 19 nucleotides (AAAATATCATTAAGAGAAG), which has been shown previously to be the promoter element of the mitochondrial genes (Dombrowski et al. 1999). However, this sequence, being a part of the *atpA* transcript, may not serve as a promoter in this case. We studied the secondary structure of *atpA* transcripts of the CMS and the fertility restorer lines using the software DNASTAR version 4.05 program. A perfect hairpin loop structure was detected at the 5′ end of the *atpA* transcript of the CMS line, which was absent in the *atpA* transcript of the fertility restorer line (Fig. 5). Such a hairpin loop structure was suggested to provide stability to the male sterility-associated *orf355-orf77* transcripts in CMS(S) maize (Xiao et al. 2006).

A BLAST search (www.ncbi.nlm.nih.gov/BLAST) detected no sequences matching the *orf108* in the database. Similarly, the deduced amino acid sequence did not show significant similarity to any protein sequences in the database. In silico analysis using a homology-based modeling program (www.expasy.org) suggested that the product of *orf108* does not contain any transmembrane domain and might be a soluble protein. The novel ORF of BO-CMS rice was found to be toxic to cells (Wang et al. 2006). It remains to be verified whether *orf108* is toxic to cells or if its presence upstream affects efficient translation of *atpA* and leads to male sterility.

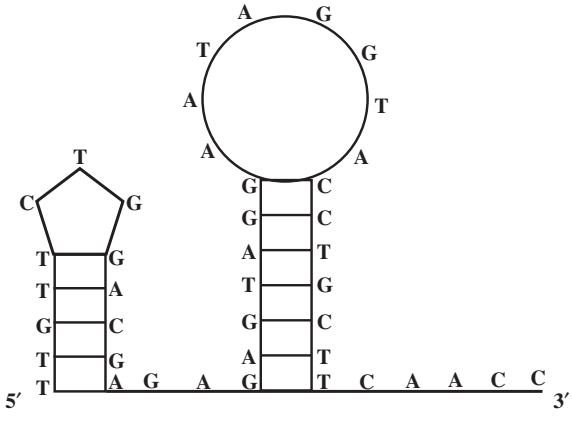

Fig. 5 Structure of the 5′ stem–loop in the bicistronic *orf108/atpA* transcript of the male sterile line.

Materials and methods

The CMS and the fertility restorer lines used in the present study have been described earlier (Prakash et al. 1998). Mitochondrial RNA isolation and Northern analysis were done as per Pathania et al. (2003).

Mitochondrial atpA genomic clones isolated from the CMS and normal *B. juncea* lines (Gaikwad et al. 2006) were sequenced using an automated DNA sequencer (ABI-prism 310) and data were analyzed using the DNASTAR software version 4.05 program.

RT–PCR was performed using about 2 μg of total flower bud RNA with 10 pmol of specific primers, 4 μl of 5× RT buffer, 2.4 μl of 25 mM $MgCl_2$, 1 μl of 10 mM dNTP mix and 1 μl of Improm-II Reverse Transcriptase (Promega, Madison, WI, USA) in a 20 μl reaction. For the first-strand cDNA synthesis, the reaction was incubated at 46°C for 60 min. PCR amplification was carried out for 35 cycles using 10 μl of this RT reaction as template. Depending on the primer combination, appropriate annealing temperatures were applied.

Mitochondrial RNA from flower buds was divided into two 10–15 μg aliquots; one aliquot was treated with with 25 U of TAP (Epicentre Technologies, Madison, WI, USA) at 37°C for 60 min. After TAP treatment, the RNA was extracted with phenol-chloroform, precipitated with ethanol and dissolved in water. Subsequently, both lots of RNA were subjected to self-ligation with 40 U of T4 RNA ligase (New England Biolabs, Ipswich, MA, USA) in a 20 μl reaction and incubated at 16°C for 18 h. After ligation, RNA was again purified through phenol–chloroform extraction and dissolved in water. The mitochondrial RNA from the male sterile and the fertile flower buds was reverse transcribed with the primers R5 and R2, respectively, using reverse transcriptase (Promega). PCR amplification was achieved using primers R5 and F10 in the male sterile lines and R2 and F10 in the male fertile lines.

Funding

The Indian Council of Agricultural Research, New Delhi.

Acknowledgments

P.K. is grateful to the Council for Scientific and Industrial Research for providing a senior research fellowship.

References

Ashutosh, Sharma, P.C., Prakash, S. and Bhat, S.R. (2006) Identification of AFLP markers linked to the male fertility restorer gene of CMS (*Moricandia-arvensis*) *Brassica juncea* and conversion to SCAR marker. *Theor. Appl. Genet*. 114: 385–392.

Brown, G.G. (1999) Unique aspects of cytoplasmic male sterility and fertility restoration in *Brassica napus*. *J. Hered*. 90: 351–356.

Dombrowski, S., Hoffmann, M., Guha, C. and Binder, S. (1999) Continuous primary sequence requirements in the 18-nucleotide promoter of dicot plant mitochondria. *J. Biol. Chem*. 274: 10094–10099.

Gaikwad, K., Baldev, A., Kirti, P.B., Mohapatra, T., Bhat, S.R., Chopra, V.L. and Prakash, S. (2006) Organization and expression of the mitochondrial genome in CMS (*Moricandia*) *Brassica juncea*: nuclear–mitochondrial incompatibility results in differential expression of the mitochondrial *atp*α gene. *Plant Breed*. 125: 623–628.

Hanson, M.R. and Bentolila, S. (2004) Interactions of mitochondrial and nuclear genes that affect male gametophyte development. *Plant Cell* 16: S154–S169.

Horn, R. (2006) Cytoplasmic male sterility and fertility restoration in higher plants. *Prog. Bot*. 67: 31–52.

Kamps, T.L., McCarty, D.R. and Chase, C.D. (1996) Gametophyte genetics in *Zea mays* L.: dominance of a restoration-of-fertility allele (*Rf3*) in diploid pollen. *Genetics* 42: 1001–1007.

Kuhn, J. and Binder, S. (2002) RT–PCR analysis of 5′ to 3′-end-ligated mRNAs identifies the extremities of cox2 transcripts in pea mitochondria. *Nucleic Acids Res*. 30: 439–346.

Liu, Z. and Butow, R.A. (2006) Mitochondrial retrograde signaling. *Annu. Rev. Genet*. 40: 159–185.

L'Homme, Y., Stahl, R.J., Li, X-Q, Hameed, A. and Brown, G.G. (1997) Brassica *nap* cytoplasmic male sterility is associated with expression of a mt DNA region containing a chimeric gene similar to the *pol* CMS associated *orf 224* gene. *Curr. Genet*. 31: 325–335.

Pathania, A., Bhat, S.R., Dinesh Kumar, V., Ashutosh, Kirti, P.B., Prakash, S. and Chopra, V.L. (2003) Cytoplasmic male sterility in alloplasmic *Brassica juncea* carrying *Diplotaxis catholica* cytoplasm: molecular characterization and genetics of fertility restoration. *Theor. Appl. Genet*. 107: 455–461.

Prakash, S., Kirti, P.B., Bhat, S.R., Gaikwad, K., Kumar, V.D. and Chopra, V.L. (1998) A *Moricandia arvensis*-based cytoplasmic male sterility and fertility restoration system in *Brassica juncea*. *Theor. Appl. Genet*. 97: 488–492.

Schnable, P.S. and Wise, R.P. (1998) The molecular basis of cytoplasmic male sterility and fertility restoration. *Trends Plant Sci*. 3: 175–180.

Small, I.D. and Peeters, N. (2000) The PPR motif—a TPR-related motif prevalent in plant organellar proteins. *Trends Biochem. Sci*. 25: 46–47.

Wang, Z., Zou, Y., Li, X., Zhang, Q., Chen, L., et al. (2006) Cytoplasmic male sterility of rice with Boro II cytoplasm is caused by a cytotoxic peptide and is restored by two related PPR motif genes via distinct modes of mRNA silencing. *Plant Cell* 18: 676–687.

Xiao, H., Zhang, F. and Zheng, Y. (2006) The 5′ stem–loop and its role in mRNA stability in maize S cytoplasmic male sterility. *Plant J*. 47: 864–872.

(Received October 8, 2007; Accepted December 22, 2007)