

# Sequence Analysis and Expression of *orf224* Gene Associated with Two Types of Cytoplasmic Male Sterility in *Brassica napus* L.

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Polima and Shaan 2A are the two most widely used forms of cytoplasmic male sterility (CMS) in the utilization of heterosis of rapeseed (*Brassica napus*) in China. A previous study indicated that the mitochondrial gene, *orf224*, was the only gene with a differential expression pattern among the normal, sterile and fertility-restored lines in rapeseed. DNA sequences of *orf224*, including coding sequences from Shaan 2A and Polima CMS, were then amplified and analyzed. DNA sequence alignment indicated both the coding sequences were 675 bp in length and had 99.9 and 99% homology in nucleotides and amino acids, respectively, and shared certain similarity to homologues from other *Brassica* spp. and *Arabidopsis thaliana*. The probable promoter regions of *orf224* were conserved between *B. napus* and *A. thaliana*, but the upstream regions of probable promoter regions were completely divergent from each other. Additionally, analysis of the primary and secondary structure of the proteins encoded by *orf224* from the two lines predicted that the proteins contain a  $\alpha$ -helix, extended strand, and random coil. After cloning a *in vitro* experiment showed that these two proteins could be expressed in *Escherichia coli* BL21.

**Key words:** Shaan 2A CMS, Polima CMS, *orf224* Gene