POLLEN VIABILITY IN *POL* CMS LINES OF *BRASSICA NAPUS*

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ABSTRACT

In a population of *pol* cytoplasmic male sterile plants, anthers can be found with varying degrees of male sterility. A study was initiated to determine the level of self-pollination that can occur due to the presence of pollen in blistered anthers. The viability of pollen of plants from A- and B-lines was determined with acetocarmine and fluorescein diacetate assays. The acetocarmine assay consistently gave higher viability estimates than did the fluorescein diacetate assay. In A-line plants, anthers that had no blisters did not contain pollen grains. Blistered anthers contained pollen grains but their viability was lower than that of pollen from B-line anthers. Pollen from shedding anthers of A-line plants had a lower viability than did pollen from non-shedding anthers.

INTRODUCTION

High levels of heterosis for seed yield in *Brassica napus* have stimulated interest in commercial production of F_1 hybrids. An effective pollination control system is required in order to produce hybrid seed on a commercial scale. All of the currently registered *B. napus* hybrids in Canada are produced using the 'Polima' cytoplasmic male sterility system (*pol* cms). There is a limited number of restorer genes for this system; most cultivars are maintainers or partial maintainers of sterility (Fan *et al.*, 1986; Fang and McVetty, 1989).

Canadian regulations for pedigreed hybrid canola seed production stipulate that there must be at least 75% hybrid seeds in the seedlot. In order to ensure that this level of hybridity is reached with the *pol* cms hybrids, a number of factors must be controlled. One of the factors is the breakdown of male sterility resulting in self-pollination of the A-line plants (male sterile lines). This breakdown can be minimized by selecting B-lines (maintainer lines) with improved ability to maintain sterility.

Three types of anthers have been identified in the *pol* cms system: small, arrow-shaped white anthers that are completely male sterile; normal anthers that shed pollen; and an intermediate form in which the anther has a yellow blister, or protrusion, containing pollen grains (Tai and McVetty, 1988). When breeding for maintaining ability in the *pol* cms system, attempts are usually made to select plants with flowers containing six completely male sterile anthers. In this system, however, most plants have blistered anthers; few plants are completely male sterile. A study was initiated at the AAFC Research Centre at Saskatoon to determine the level of selfing that could occur in A-lines as a result of the presence of blistered anthers. This paper reports on the first part of the study, i.e. the viability of the pollen grains found in blistered anthers.

EXPERIMENTAL

Flowers were harvested from plants of A- and B-lines of the Swedish spring oilseed rape cv. Karat and the spring oilseed rape breeding line KM7921 developed at the AAFC Research Centre, Saskatoon. Plants were grown in soilless mix under controlled environmental conditions and in a greenhouse. Flowers that were harvested were those

that had just opened, with the exception of several flowers from the B-lines that were to open the following day (non-shedding anthers). Anthers were removed from the flowers and measured for length, and in A-lines, the length of the blisters was also measured. Whether blisters were shedding pollen or not was noted. Single anthers were then crushed in a drop of an aqueous solution of fluorescein diacetate (0.025 mg/ml) on a microscope slide. Pollen grains were examined with a Zeiss Photomicroscope III equipped with an epi-fluorescence condenser III RS and a blue excitation filter set. The number of fluorescing (viable) and non-fluorescing (non-viable) pollen grains was determined. A drop of acetocarmine stain was then drawn by capillary action across the slide to colour the pollen grains. Pollen grains whose contents stained red were counted as viable, whereas those that remained clear were considered to be non-viable.

In A-line plants, pollen grains were found in all anthers with blisters. Anthers that showed no signs of blistering did not contain pollen grains. Tai and McVetty (1988) reported that, in *pol* cms, pollen mother cells degenerated without developing into microspores. Theis and Röbbelen (1990) found premeiotic inhibition of microspore development in Canadian spring oilseed rape cv. Andor with *pol* cytoplasm. Blistered anthers from A-line plants contained fewer pollen grains than did anthers from B-line plants.

Pollen viability in anthers from A-line plants was consistently higher when estimated with the acetocarmine assay than with the fluorescein diacetate assay (Figure 1). Acetocarmine stains cytoplasmic contents which may still be present after cell death, perhaps leading to an overestimate of viability. The fluorescein diacetate assay is based on the production of a fluorescing compound, fluorescein, following hydrolysis by esterase (Heslop-Harrison and Heslop-Harrison, 1970). The fluorescein is retained only in cells with an intact plasmalemma, which are presumed to be viable.

As the size of the blisters on the anthers from A-line plants increased, the proportion of viable pollen grains first increased, then decreased (Figure 1). Based on the fluorescein diacetate assay, pollen viability peaked in blisters measuring about 60-70% of anther length; based on the acetocarmine assay, viability was the highest in blisters measuring about 40-60% of anther length (Figure 1). The proportion of viable pollen did not exceed 63% in non-shedding anthers from A-line plants (Figure 2). Viability of pollen from shedding anthers of A-line plants did not exceed 50% and was generally lower than that of pollen from non-shedding anthers. In contrast, pollen viability of B-line plants was approximately 70% for both shedding and non-shedding anthers. These results suggest that the pollen of A-line plants may be more sensitive to the environment than is the pollen of B-line plants.

The difference in proportion of viable pollen between shedding and non-shedding anthers from A-line plants was less pronounced when the acetocarmine assay was used. A possible explanation may be that pollen from shedding anthers dies soon after release and, although still containing cytoplasm, cannot undergo the enzymatic reaction required for fluorescence.

The next step in determining the importance of blistered anthers as a source of pollen for the A-lines is to conduct *in vivo* studies of pollen germination and tube elongation, and ovule fertilization.

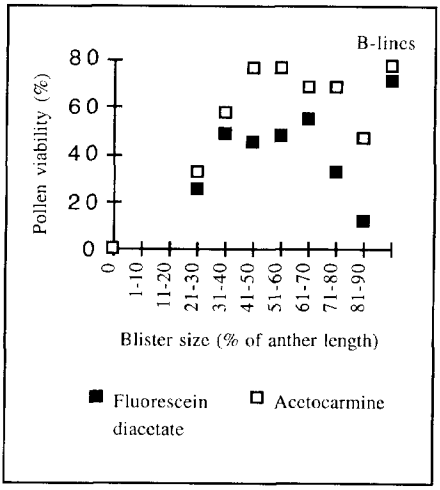

FIGURE 1. Average pollen viability of shedding and non-shedding anthers of *B. napus pol* CMS A- and B-line plants as determined by acetocarmine and fluorescein diacetate assays

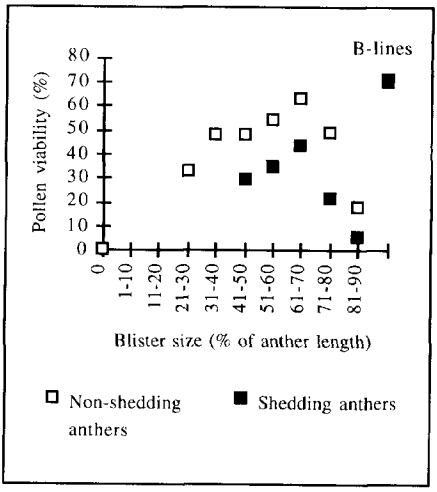

FIGURE 2. Pollen viability of shedding and non-shedding anthers of *B. napus pol* CMS A- and B-line plants as determined by a fluorescein diacetate assay

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