FREEZE STRESS INDUCED CHANGES IN GENE EXPRESSION, HORMONE AND CHLOROPHYLL LEVELS IN RELATION TO ACCELERATED DESICCATION OF *BRASSICA NAPUS* SEED

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ABSTRACT

When exposed to mild freezing stress, *Brassica napus* seeds fail to lose all their chlorophyll (Chl) at maturity, probably because of rapid moisture loss. We have found transient increases in cytokinins (CKs) especially zeatin (Z), and abscisic acid (ABA) within the first two days after the freeze stress. In some experiments, this was accompanied by a transient increase in Chl. This is the first report of a freeze stress-induced increase in cytokinin levels in seeds.

INTRODUCTION

During normal development, *B. napus* seeds lose all their Chl well before maturation. However, an early frost during seed maturation can induce retention of some Chl in the mature seed, thus causing the "green seed" problem (Johnson-Flanagan *et al.*, 1990). This appears to be related to an accelerated developmental programme which includes more rapid desiccation of seed (Johnson-Flanagan *et al.*, 1992). The green pigments in the seed are undesirable and must be removed from the oil during processing procedures, which reduces the oil yield and the value of the crop.

Plant hormones, especially cytokinins (CKs) are known to be involved in the processes of Chl synthesis, cab gene expression and chloroplast development (Parthier, 1979). We therefore investigated the effect of freezing on CK, ABA and Chl levels in developing *B. napus* seeds in relation to their desiccation as a result of exposure to a mild freeze stress.

EXPERIMENTAL

Brassica napus cv. Westar plants were grown in styroblocks in controlled environment chambers with 16h light (24°C) and 8h dark (18°C). Flowers were tagged daily and hand pollinated. The control plants remained in the growth chamber while the experimental plants were subjected to freeze stress. Plants were placed in a programmable freezer at +2°C for 1h,, the temperature was lowered by 5°C/h to -5°C; kept at -5°C for 3h , warmed to +2°C at a rate of 5°C/h. Plants were then kept at 4°C for 12h and returned to growth chamber.

Seed samples were taken from both control and frozen siliques of same age groups e.g., 30 days post anthesis (DPA). Samples were taken at 1,2,3 and 7 days post freezing to study the kinetics of Chl, water loss, CK and ABA levels. The data represent an average of values from two independent experiments. Moisture content of seeds was calculated from the difference in their fresh and dry weights. Chl content was determined by the method of Arnon (1949). CK and ABA were purified by high performance liquid chromatography and quantified by their respective enzyme-linked immunosorbent assays (Singh and Sawhney, 1992).

RESULTS AND DISCUSSION

In 30 days post anthesis (DPA) seeds of *B. napus*, the moisture levels declined very little over the next 7 days of development. However, freezing caused a significantly greater decline in moisture content (Figure 1). Similarly, Johnson-Flanagan *et al.* (1992) reported an accelerated developmental programme including rapid drying down of the seed under freeze stress conditions. Figure 1 shows that freezing induced a transient increase in ABA levels, one day after the treatment, after which the levels of ABA dropped below the control levels. Smaller increases in ABA levels were found with seeds of 27 and 34 DPA.

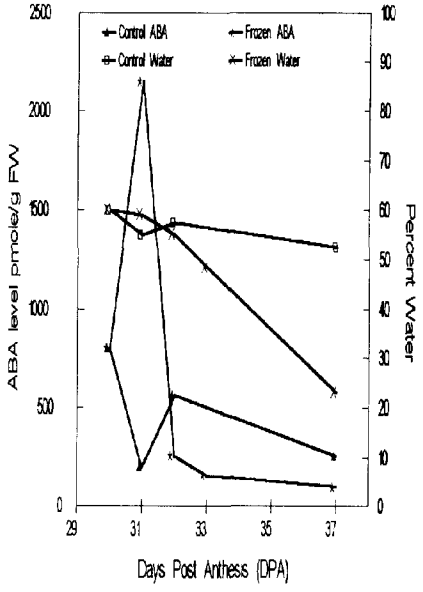

FIGURE 1. Effect of freezing on ABA level and percent water content in 30 DPA seeds of *Brassica napus*.

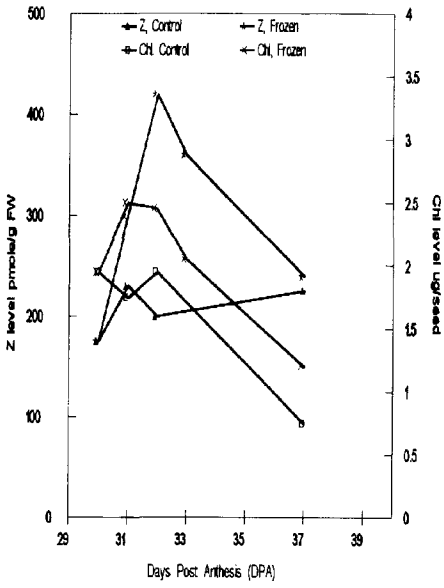

FIGURE 2. Effect of freezing on Chl and Z levels in 30 DPA seeds of *Brassica napus*.

Freezing caused a transient increase in zeatin (Z) levels in 30 DPA seed (Figure 2). We also found freeze-induced transient increases in dihydrozeatin and isopentenyladenine levels (data not shown). Similar increases in CK levels were observed in seeds of 27 and 34 DPA. In this experiment, Chl levels showed a small transient increase during the first couple of days after freezing (Figure 2). However, this transient increase in chl was not significant in all experiments. Nevertheless, freezing at mid-maturation stage caused retention of Chl in fully mature *B. napus* seed. The cab mRNA levels declined during development of both normal and frozen seed (results not shown). Hence, although freezing caused a transient increase in CK levels, which is known to promote cab gene expression and Chl synthesis, it is more likely that freeze-induced rapid desiccation of seed is a more important factor in the green seed problem in *B. napus*. Whether or not the freeze-induced increase in CK levels has any role in the formation of green seed is not known. However, this study is the first report of a freeze stress-induced increase in CK levels.

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