EFFECTS OF AN ANTISENSE CRUCIFERIN GENE ON SEED STORAGE PROTEIN IN TRANSGENIC *BRASSICA NAPUS* SEEDS.

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ABSTRACT

To manipulate the quality and quantity of seed storage proteins of *Brassica napus* we introduced an antisense gene of cruciferin by *Agrobacterium*-mediated transformation. Three trasngenic plants were regenerated and all plants produced self-pollinated seeds (T1 seeds). In T1 seeds of all transgenic plants there was a reduction in the levels of $\alpha 1\beta 1$ and $\alpha 2/3\beta 2/3$ subunits of cruciferin, whereas the level of the $\alpha 4\beta 4$ subunit was unchanged. The total protein and lipid contents of transgenic seeds did not differ significantly from that of normal seeds. Seeds with reduced amounts of cruciferin accumulated higher amounts of napin than non-transformed seeds, but the level of oleosin was unaffected. Amino acid analysis of the seed storage protein revealed that the T1 seeds with reduced amounts of cruciferin contained higher relative levels of three essential amino acids, namely, lysine, methionine and cysteine, with increases of 10%, 8% and 32% over the respective levels in non-transgenic seeds (*B. napus* cv. Westar). These results revealed that the antisense method generate palnts that produced seeds with a modified amino acid compositions without any of the disadventages often associated with mutant lines.

INTRODUCTION

A great deal of effort has been devoted to attempts at improving the quality and quantity of seed storage proteins by genetic engineering. For example, Altenbach et al. introduced the gene for the sulfur-rich 2S albumin of Brazil nut into *B. napus* , and the seed protein of trnasgenic plants contained up to 33% more methionine than the controls (Altenbach et al. 1992).

Recently, antisense genes for various proteins have been introduced into plants to investigate the biological functions of the products of the corresponding sense genes or to improve economically important characteristics (Krol et al. 1988; Mol et al. 1990). As compared to classical mutagenetic methods, one of the advantages of the antisense method is that expression of the target gene can be inhibited without any effect on other genes. When the antisense gene for napin, 2S storage protein of *B. napus* was transformed into *B. napus* cv. Westar, total storage protein and total storage lipid contents did not affected in the transgenic seeds with reduced amount of napin. However, cruciferin, 12S seed storage protien of *B. napus*, increased in the seeds with decreased levels of napin and fatty acid compositions differed form those of the non-transformant, *B. napus* cv. Westar (Kohno-Murase et al. 1994).

Cruciferin contains lower levels of methionine and cysteine than the second most prominent seed storage protein, napin (Simon et al. 1985) Here, we report the effects of an antisense gene for cruciferin on the accumulation of

cruciferin and other storage proteins. The cruciferin content of seeds was reduced by the introduction of the antisense gene and the reduction was balanced by an increase in napin content. This change resulted in an increase in relative levels of essential amino acids in the seed storage proteins.

RESULTS

Transformation of *B. napus* and the accumulation of seed storage protein in transgenic plants.

Cruciferin is composed of six subunits and each subunit contains two chains, the α and β chain, encoded by one gene (Schwenke et al. 1981). Four different subunits exist, namely α1β1, α2β2, α3β3 and α4β4 subunit. The α2 and α3 chains do not differ from each other in terms of molecular weight, and α2β2 and α3β3 subunits are the most abundant in the cruciferin hexamer (Rödin and Rask 1990).

The antisense construct contained a sequence that was coding complementary to part of *cruA* gene that corresponded to the α2/3 and β2/3 subunits (Rödin and Rask 1990; Ryan et al. 1989). The promoter region of the gene for napin, used for this experiment, controlled expression of a gene for β-glucuronidase (GUS) in a seed-specific manner in the seeds of *B. napus* (Kohno-Murase et al. 1994).

The antisense construct was introduced into *B. napus* by *Agrobacterium*-mediated transformation. Three transgenic plants were obtained and all produced self-pollinated seeds (T1 seeds). The analysis of storage protein in T1 seed by SDS-PAGE revealed that seeds of all the transgenic plants examined had reduced amounts of cruciferin, and one transgenic plant (CA#3) showed a clear reduction in cruciferin content. T1 seeds of the CA#3 plant were further analyzed. The analysis by SDS-PAGE also revealed that all the T1 seeds contained reduced levels of α1, α2/3, β1, β2 and β3 chains. However, intensities of bands that corresponded to α4 and β4 chains were not reduced (Fig. 1).

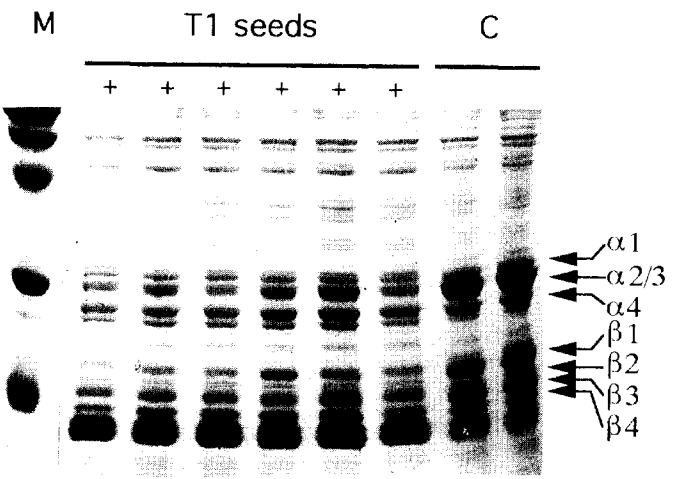

Fig. 1 Analysis by SDS-PAGE of seed proteins from the transgenic plants. Cruciferin was examined on a gel with 12% polyacrilamide. Plus signs above lanes indicate that the presence of the antisense gene was confirmed by PCR. M, Molecular size markers; T1 seeds, transgenic plants CA#3; C, non-transformant B. napus cv. Westar. α1 through 4 and β1 through 4 indicate α and β chains of cruciferin, respectively.

The total protein and the total lipid content per seed of the CA#3 plant did not differ significantly from those of seeds of the non-transformant, *B. napus* cv. Westar. For the analysis of napin content, aliquots of extracts were fractionated by electrophoresis. SDS-PAGE revealed that all the seeds of the CA#3 plant had increased levels of napin. These results were the same as the results of analysis of the transgeinc seeds with decreased amount of napin. However, fatty acid compositions in T1 seeds of CA#3 were not different form those of non-transgenic plants.

Amino acid composition of the transgenic T1 seeds.

Amino acids in the T1 seeds of the CA#3 plant were analyzed. The results showed that the seeds contained increased amounts of lysine, methionine and cysteine. The transgenic seeds contained 32% more cysteine than non -transgenic seeds, and lysine and methionine contents were 10% and 8% greater, respectively, than those in control seeds. By contrast, levels of tyrosine and asparagine plus aspartic acid were significantly reduced in the transgenic seeds (Table 2).

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