EFFECT OF COTYLEDON WOUNDING ON THE MYROSINASE ENZYME SYSTEM IN *SINAPIS ALBA* SEEDLINGS.

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ABSTRACT

An investigation about the effects of mechanical wounding on the expression and activity of the myrosinase enzyme system were undertaken on the *Sinapis alba* seedlings. Plants were grown *in vitro* on MS medium for 15 days thereafter one cotyledon was mechanically wounded while the sister cotyledon was left without any damage. The expression of myrosinase polypeptides and the activity of myrosinase were studied on the wounded, unwounded cotyledons and stem/root at five different time intervals after wounding and normal plant was taken as control. The expression and activity studies showed no remarkable response of wounding between unwounded and wounded cotyledons.

INTRODUCTION

Myrosinase glucosinolate system have attracted attention, because of their physiological roles in the plants as well as their effects on human and animals. This system is believed to play important roles in the metabolism of the plants and defence against pathogens and pests (Chew 1988, Schnug 1990). Myrosinase has been shown to occur in multiple forms and it is associated within a group of cells known as myrosin cells. The presence of myrosinase within these cells has been confirmed with the use of immunochemical and *insitu* hybridization techniques (Thangstad *et al.* 1991, Lenman *et al.* 1993). A subcellular compartmentalisation of glucosinolates and myrosinase has been suggested (Luthy & Matile 1984). Upon cellular disruption, the glucosinolates immediately undergo hydrolysis by myrosinase and the hydrolysis products are responsible for the biological activity. Many different compounds are induced in plants during wounding, some of which are associated with the wound healing (Bowels 1990) while others enhance resistance against micro-organisms. It has been reported that in some Brassicaceae plants infestation with pathogens (Doughty *et al.* 1991), insects (Koritsas *et al.* 1989, Birch *et al.* 1990) and mechanical wounding (Koritsas *et al.* 1991) can affect the concentration of individual glucosinolates.

The objective of this study was to determine whether artificial (mechanical) cotyledon damage produce responses with respect to components in the myrosinase glucosinolate enzyme system and to ascertain whether such changes depend on the time elapsed after wounding.

EXPERIMENTAL

Seeds of *Sinapis alba* cv. Trico were kindly provided by Svalöf, Sweden. Seeds were sterilised and sown on solidified MS medium (Murashige and Skoog 1962). After 15 days one cotyledon of the plant was pricked twice with 3 toothed edge forceps and the sister cotyledon was kept without damaging. Samples were collected 0 hr, 6 hr, 24 hr, 3 days, and 7 days after wounding and normal plant was also harvested as control. Extracts were prepared and immunoblots were performed as described by Bones *et al.* (1994) after running SDS-PAGE on Mini-Protean II dual slab gels (Bio-Rad). Myrosinase activity was measured as liberated glucose with the hexokinase assay (Sigma). Activation of ascorbic acid was measured with the hexokinase assay in the presence of 0.4 mM ascorbic acid. Protein was measured by the Bio-Rad protein reagent using bovine serum albumin as standard.

Effect of mechanical wounding in myrosinase activity

The total activity of myrosinase measured as liberation of glucose is generally higher in all organs in the presence of 0.4 mM ascorbic acid than in the absence of ascorbic acid. There was not much variation in the activity between normal and wounded organs of the plants (Fig.1). It was observed that in the wounded cotyledon total activity went down immediately after wounding compare to sister cotyledon and normal cotyledon then it is increasing with time up to 7 days in the absence of ascorbic acid (Fig.1A).

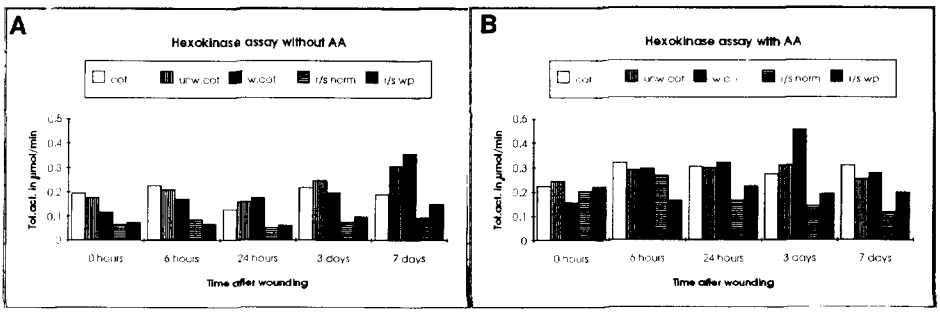

Fig.1. Total myrosinase activity of the extracts from normal and wounded *Sinapis alba* plants grown *in vitro* on MS medium for 15 days before wounding. **A.** Activity in the absence of ascorbic acid. **B.** In the presence of 0.4 mM ascorbic acid. Plant organs: Cot - Cotyledon; Unw. cot - Unwounded cotyledon; W.cot. -wounded cotyledon; r/s norm - root and stem of normal plant; r/s wp - root and stem of wounded plant. Total activity is expressed as µmol/min. Values represent average of duplicates.

Wounding effect on myrosinase expression

Mechanical wounding of cotyledons of fifteen day old plants did not produce any significant change in the expression pattern of myrosinase polypeptides (Fig.2). The two main classes of myrosinase polypeptides were detected after immunoblots with all samples examined. Three polypeptide bands could be observed in the 68 kD class and two in the 77 kD class of polypeptides. It has been suggested that the 77 kD (MI) class of myrosinase polypeptide could be involved in the defence system of the plants (James and Rossiter 1991). Robert (1992) reported that the concentration of aliphatic and aromatic glucosinolates in *Brassica juncea* and *Sinapis alba* were unchanged in the wounded cotyledons. Birch *et al.* (1990) on the other hand reported that both the concentration and relative proportions of individual glucosinolates were changed considerably in response to turnip root fly larvae attacks. In a more recent report Griffiths *et al.* (1994) found that artificial damage of roots of oil seed and forage rape (*Brassica napus*) reduced the total glucosinolate content of the roots. In similar plants attacked by turnip root fly larvae an increase in the proportion of indole glucosinolates was observed.

In conclusion our results indicate only minor responses on the myrosinase enzyme system after mechanical wounding of cotyledons of 15 day old *Sinapis alba* plant. However, these results do not exclude the possibility that artificial damage affects the system. Responses may be induced after repetitive wounding or by wounding of other plant parts or of plants at other developmental stages. Several possibilities exists if our results are valid for all plant parts and developmental stages. The myrosinase enzyme system can represent a premade system where the components are ready to be used at any time. No induction of synthesis is necessary, only some signals to activate the compounds that are already present. Some results could also indicate that the nutritional status of the plants can affect the expression of the components in the enzyme system. These possibilities will be further examined.

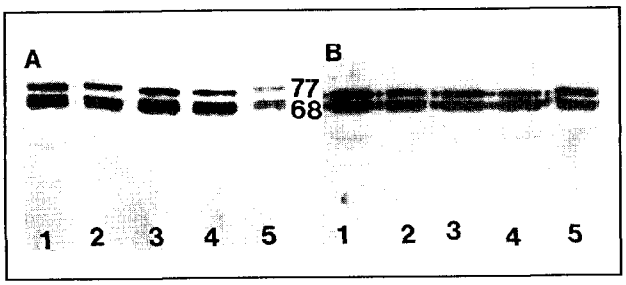

Fig. 2. Immunoblots after SDS-PAGE with extracts from **A:** 6 hrs after wounding **B:** 24 hrs after wounding of 15 day old *Sinapis alba* plants cultured *in vitro* on MS medium. Plant organs: **Lane 1.** cotyledon of normal plant; **Lane 2.** root and stem of normal plant; **Lane 3.** wounded cotyledon; **Lane 4.** unwounded cotyledon; **Lane 5.** root and stem of wounded plant.

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