GLUCOSINOLATES ACCUMULATION PATTERN DURING THE DEVELOPMENT OF SINGLE LOW AND DOUBLE LOW OILSEED RAPE (BRASSICA NAPUS L.)

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1-INTRODUCTION

Toxicity and breeding aspects of glucosinolates in rapeseed have been investigated for the last twenty years. As yet, little is known about the role(s) performed by these compounds in the plant.

A comprehensive approach to glucosinolate metabolism in plants would require an intensive study of their distribution between plant organs and changes in these patterns through various developmental stages. Until now, only a few stages have been investigated . Changes in contents of these compounds have been described in seedlings (Mc Gregor 1988) or in seeds during maturation (Sukhija et al. 1985 ; De March et al. 1989). These results are difficult to be compared because of the diversity of plant materials and of the analytical methods used. Our study was performed by using an accurate analytical method for intact glucosinolates (Clossais Besnard et al. 1990). We investigated changes in concentrations of each glucosinolate in various organs, as well as changes in total content per plant of these compounds during a complete plant life cycle from sowing to harvesting.

2-EXPERIMENTAL

Two spring rape cultivars were compared ; Drakkar is a double low cultivar stemming from french breeding programms, whereas Chine 32 is a chinese line, the quality of which has not been genetically improved.

For the first experiment, seeds were germinated on humidified filter paper, in Petri dishes, in the dark, at 25°C for two days. Then they were transferred to 15cm pots containing a sand/soil/supplemented peat mixture (25/25/50, V/V/V). The plants were grown in a growth chamber to day 30. For the second experiment, seeds were sown per 25cm pot filled with blond peat/brown peat supplemented compost (70/30, V/V) and grown in a glasshouse to the harvesting of the next seed generation. All plant material collected was immediatly divided into different parts and frozen in liquid nitrogen. Extraction and analysis of glucosinolates in freeze dried plant material were performed as described elsewhere (Clossais Besnard et al. 1990).

3-RESULTS

3-1-Glucosinolate concentrations and content during germination and the first steps of vegetative life (fig 1)

The total glucosinolate concentration decreased during germination and early stages of development. It stabilized in 15 days old plant. Although the initial concentration in the seed was higher in Chine 32 (57 $\mu mol.g^{-1}$ DM) compared to Drakkar (25 $\mu mol.g^{-1}$ DM), it stabilized for both cultivars around 6 $\mu mol.g^{-1}$ DM at day 15.

In the seed, the major compounds were the 2-hydroxybut-3-enyl, but-3-enyl, and 4-hydroxyindol-3-ylmethylglucosinolates. According to the described changes in glucosinolate concentrations, the first stages of rape development might be divided into three distinct periods. For the first seven days, biomass was kept quite constant whereas seed glucosinolate concentration decreased drastically. At the end of this period, but-3-enyl and 4-hydroxyindol-3-ylmethylglucosinolates were no longer detected. On the other hand, compounds appeared which were not found in seeds, i.e., indol-3-ylmethyl, N-methoxyindol-3-ylmethyl and 2-phenethylglucosinolates. The concentrations of these compounds were found to be quite similar, the total content per plant being always lower in Drakkar because of the lower biomass produced. It has to be pointed out that the 2-phenethylglucosinolate was detected only in roots and hypocotyls ; whereas 2-hydroxy-but-3-enyl, indol-3-ylmethyl and N-methoxyindol-3-ylmethylglucosinolates were present in all organs.

In a second period lasting from day 15 to day 20, young plants were growing quickly and the content per plant of each of the remaining glucosinolates increased significantly. In a third period of time, which occured earlier for Chine 32 cv compared to Drakkar cv, the content of each of the three new compounds identified during the first period were found to decrease rapidly.

3-2-Glucosinolate contents during the end of the vegetative growth period, during flowering and first stages of maturation (fig 2)

During the period from day 35 to day 100, in the two cultivars, the glucosinolate content per plant increased whereas its concentration decreased. However, a break in that trend occurred with onset of flowering. It was more pronounced for Chine 32 cv than for Drakkar cv. The content per plant decreased for a few days (day 66 to day 77) together with a strong decrease of the total concentration. Next, with the beginning of maturation, trends were reversed and glucosinolate accumulation was observed.

2-Hydroxybut-3-enylglucosinolate was the major glucosinolate in both cultivars during the whole period. Most of it was located in the leaves. But-3-enyl glucosinolate was present only in inflorescences where it accumulated in great amount during maturation. 4-hydroxyindol-3-ylmethylglucosinolate was not detected in the roots. It was abundant in the leaves and in smaller amount in inflorescences. Indol-3-ylmethyl glucosinolate was found in all the different plant organs studied. Its maximal content per plant was reached at early flowering for both cultivars. At first, the major part of it was located in the shoot. However, at the late stages of development, it was mainly in the roots. A very small part of indol-3-ylmethylglucosinolate was present in the inflorescences. Changes in N-methoxyindol-3-ylmethylglucosinolate contents gave a quite similar profile to that of indol-3-ylmethylglucosinolate, mainly in the shoot till the beginning of flowering, and then exclusively in the roots. 2-Phenethyl glucosinolate was found only in the roots where it accounted for 40 to 75% of total glucosinolates in Drakkar cv and 48 to 86 % in Chine 32 cv.

4-DISCUSSION

The characterization of the distribution of glucosinolates throughout the whole life cycle in two varieties of *B. napus* is considered as a prerequisite for understanding the metabolic traits associated with synthesis, catabolism, fluxes between plants organs and interconversion. In our study, during the first days following imbibition, a rapid decrease in the concentration was observed for all the glucosinolates initially present in the seed (2-hydroxybut-3-enyl, but-3-enyl, 4-hydroxyindol-3-ylmethyl and pent-4-enylglucosinolates). At the same time, compounds initially not present in seeds appeared (i.e. indol-3-ylmethyl, N-methoxyindol-3-ylmethyl and 2-phenethylglucosinolates). However, as their concentrations were very low, their synthesis does not seem to be directly related to the catabolism of aliphatic glucosinolates. In addition, we have shown that the trends are similar for double low cultivar as well. The data suggest a rapid metabolization of glucosinolates for sustaining plant growth. They apparently constitute a storage form for nitrogen, carbon and especially sulfur. When the young seedlings have the capacity to perform photosynthesis, their biomass increases rapidly. At the same time, glucosinolates are accumulated mainly in the shoots where large amounts of 2-hydroxy-but-3-enyl glucosinolate and significant quantities of the three indolic glucosinolates were found. Accumulation of glucosinolate occurred also in roots, the total content per plant being smaller but the concentration higher than in leaves. This accumulation phase was followed by a general decrease corresponding to the flowering period. Thus, it seems again that glucosinolates, accumulated during the vegetative growth period, are catabolized during the reproductive growth period and especially during flowering. Later on, during maturation, glucosinolate accumulation occurred in the silique and also at a lower extent in the roots.

Intense activity periods of glucosinolates metabolism and catabolism succeeded one another. This rises the question about the regulation of enzymes involved, and about the localization of these reactions. A few investigations indicate a fast turnover of glucosinolates. However, it is not known whether the reaction catalysed by myrosinase is a preferential pathway *in vivo* for glucosinolates catabolism or whether other catabolic routes exist.

Despite recent characterization of some enzymes of the glucosinolates biosynthesis (Jain et al. 1989 ; Ludwig-Müller et al. 1990), the localization of synthesis and transport systems are also unknown. In particular, it is difficult to decide whether glucosinolates synthesized and accumulated in vegetative parts are partly deposited in the seeds. However, our results showed that their absolute quantity in the shoots is not sufficient to explain the final content in the seeds. In addition, the presence in seeds of specific compounds such as but-3-enyl and pent-4-enylglucosinolates suggests an *in situ* synthesis. This could occur either in pod shells or in the seed. Graffting studies (Lein 1972) suggested that biosynthesis of glucosinolates for seed filling took place partly in the silique. The remaining organs of the plant might supply the other fraction or their precursors. Recently, De March et al (1989) reported that an increase of glucosinolates in the seeds was associated with a decrease in pod

shells. However, the relationship was not quantitative and transport from other parts or synthesis in the seed seemed possible. Because of the existence of seed- specific glucosinolates, it is suggested that vegetative parts provide mainly precursors and that the final steps for glucosinolate synthesis occur in the seed.

The physiological role of glucosinolates might be different during the two periods of utilization. The concentration (in µmol.g-1 DM) in dry seeds is about 5-10 times higher than in vegetative parts. Further, during germination and initial plant development, the concentrations in the seedlings decreased drastically. The behaviour of the seed glucosinolates suggests them to be storage compounds. The requirement for nutrients, especially sulfur, in young seedlings migh be higher in single low than in double low cultivars. After seedling development, such variations are usually much smaller. In addition, the structure of glucosinolates concerned are different. The aromatic structure of glucosinolates in vegetative parts suggests some kind of interaction with the control of flowering process. An implication in phytohormonal regulation has to be considered.

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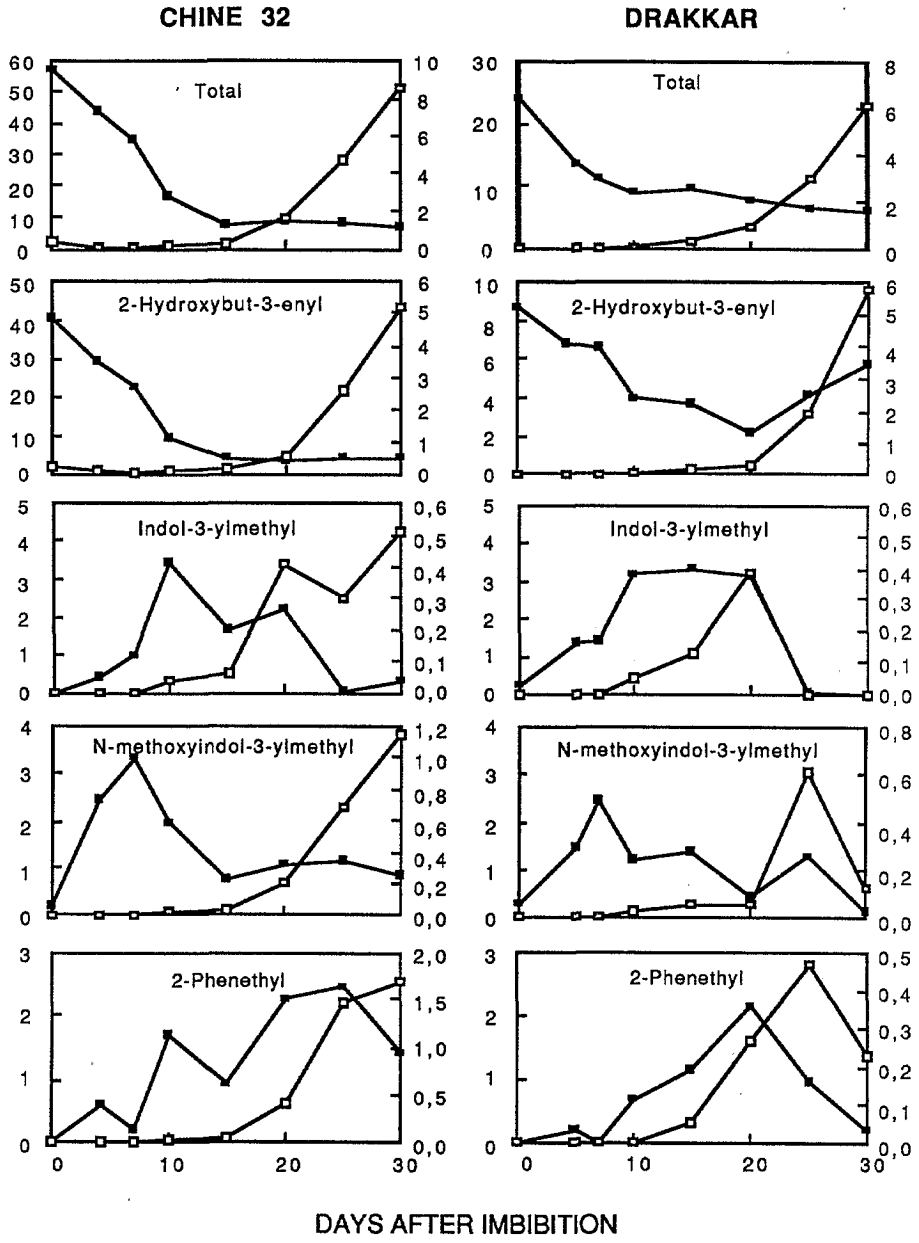

Fig 1 : Changes in glucosinolates concentrations (—■—, µmol.g -1 dry weight) and contents (—□—, µmol per plant) during germination and early vegetative development.

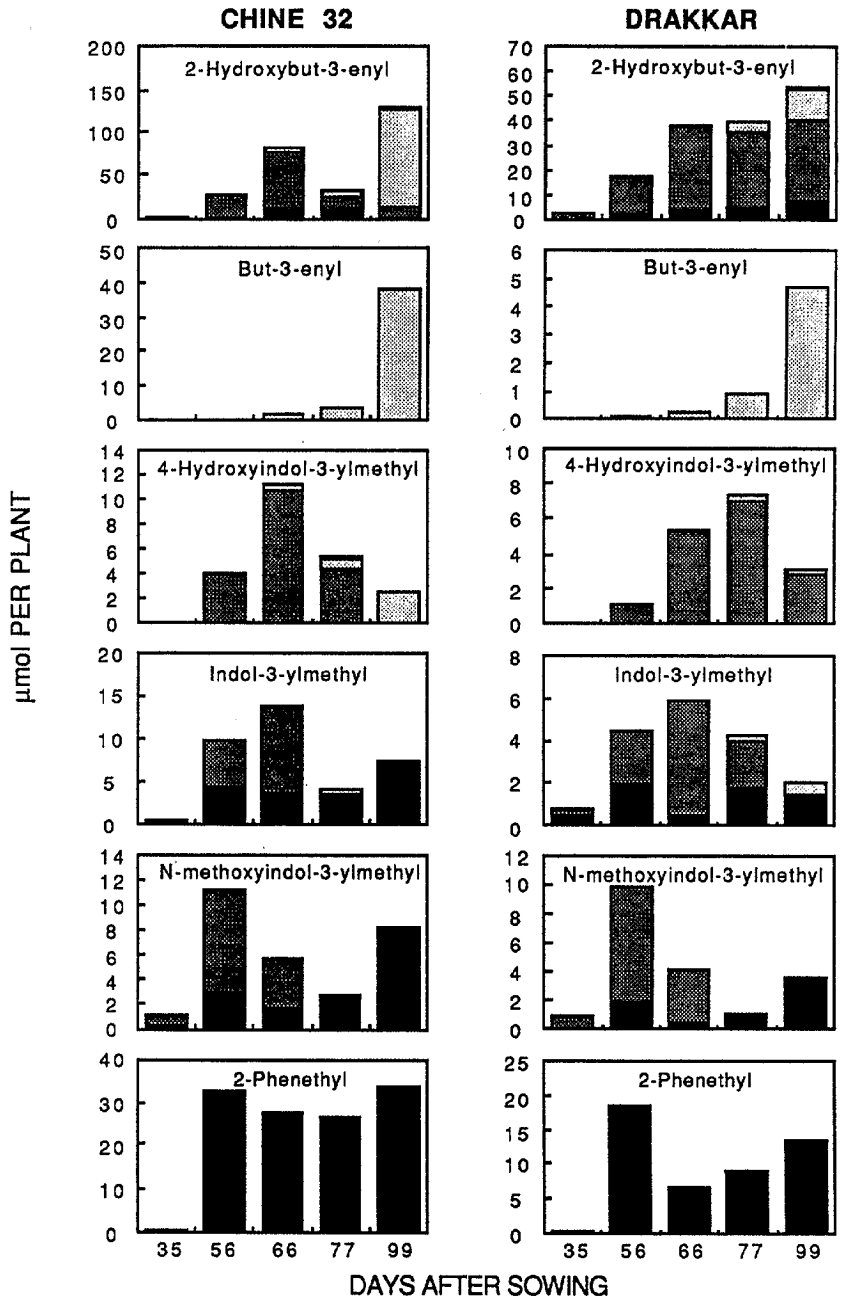

Fig 2 : Glucosinolate contents in different part of the plant : roots and hypocotyl (■), stem and leaves (▩), inflorescence (□).