# A preliminary study on glucosinolates heterosis in leaf of hybrids in *Brassica napus* L.

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**Abstract**

*Brassica napus* L. is widely grown in Yangzi River valley of China as a major edible oilseed. Because of the unpleasant smell and toxicity from glucosinolates degradation products, genetic improvement has been made to decrease glucosinolates concentration in seed by breeding. However, it was also found that leaf glucosinolates concentration and profiles were closely related to plant diseases resistance and the taste of shoot as vegetable. And anticancer effects from degradation products of indolyl and other glucosinolates were also reported. Recently, new varieties of *Brassica napus* L. used both for vegetable and oilseeds were released and popularized in the middle and lower valley of Yangzi River. Rapeseed with high glucosinolate content in leaves and low content in seeds were considered as ideal for both seed quality and plant diseases resistance. Therefore leaf glucosinolates call for more and more attention. In this paper the variation of leaf glucosinolates content in all growth periods and the heterosis of glucosinolates in leaf were studied with hybrids and their parents as well as the double high material by high performance liquid chromatography (HPLC). The results showed that Leaf glucosinolate content in double-high material Zhongyou 821 decreased from rosette to blooming stage, while in double-low material leaf glucosinolate increased from overwintering to budding period, decreased at stem elongation stage and rose again at early blooming stage. Leaf glucosinolate contents and profiles varied significantly in double-high and double-low rapeseeds although aliphatic glucosinolates were the dominant ones in both leaves. All crosses presented mainly negative heterosis over both parents. The heterosis of leaf glucosinolates of $F_1$ and $F_2$ hybrids were significant and negative in stem elongation and floral period, suggesting that it would be difficult to increase leaf glucosinolates in these periods for high disease resistance and it might be the best time for harvesting shoots as vegetable.

**Key words:** *Brassica napus*, leaves, growth stage, glucosinolates, heterosis

## Introduction

Rapeseed in which *Brassica napus* accounts for more than 90 percent is the most important oilseed cultivated in China as the main edible vegetable oil source and animal feedstuff. The presence of glucosinolates in rapeseed influences the quality of meal obtained after crushing the seeds as break-down products of the glucosinolates are goitrogenic which results in depressed growth of the animals fed with meals containing high level glucosinolates. However, it has also been shown that there are positive effects from glucosinolates degradation products such as the increase of diseases resistance. Indolyl glucosinolates degradation products were reported to increase the mouse immunity. And rapeseed plant organs inoculated with high Indolyl glucosinolates were found to be more resistant to pests. Milford showed that the degradation products of glucosinolates could prevent rapeseed plant from mechanical damage and pests. Seed glucosinolates genetics has been widely studied and the concentration especially aliphatic ones in seed has been significantly reduced by double low breeding program. In this study, glucosinolates profiles, concentration and heterosis in growth stages of leaf in *Brassica napus* were investigated by high performance liquid chromatography.

## Materials and methods

Rapeseed leaves materials from *Brassica napus* 1008,5899,6098,$M_1$,$R_1$,$R_6$, Zhongyou 821 and $F_1$, $F_2$ populations were planted in the Farm of Oil Crops Research Institute of Chinese Academy of Agricultural Sciences. Rapeseed leaves were cut and frozen with liquid nitrogen at resetting, overwintering, budding, stem elongation, early blooming and blooming stages. Leaves samples were stored in freezer at -45°C and dried by vacuum freezing at -45°C.

Leaves samples were grinded with microgrinder and extracted with 70% methanol (70%,v/v solution) by ultrasonic for 18 min after 1min water bath at 75°C for inactivation of myrosinase. Sample was transferred to mini ion-exchange column with sulfatase and kept for 20 hours. After glucosinolate was eluted, samples were analyzed by high performance liquid chromatography. Waters M32 HPLC system with 510 pumps, 470auto-injector, connected to a 2487 ultraviolet detector was used. Glucosinolates were separated with a Novapak $C_{18}$ column at 30°Cand detected at the wavelength of 229 nm.

## Results and discussions

*Leaves glucosinolates profiles at different growth stages*

Concentration and components change of glucosinolates with growth stages of *B.napus* leaves was showed in Fig. 1 and Table 1. Glucosinolates content varied significantly among the materials and growth stages. Glucosinolates content in double high material Zhongyou 821 decreased from rosetting to blooming stage with the maximum content of 33.15 μmol·g$^{-1}$, while in double-low material leaf glucosinolate increased from overwintering to budding period, decreased at stem elongation stage and rose again at early blooming stage. The maximum value of $M_1$, 1008, 6098 × 1008 and 6098 were 9.40, 7.11, 6.35 and 7.51μmol·g$^{-1}$, respectively.

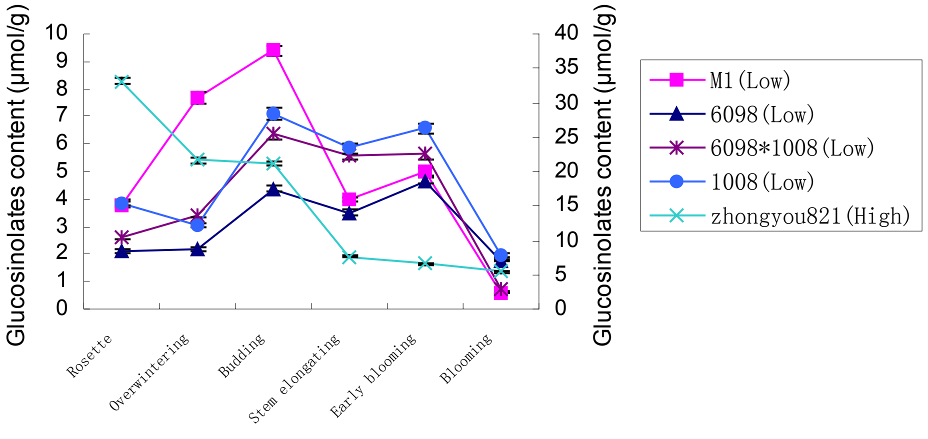


**Table 1 Concentration of glucosinolates at different grow stages of *B.napus* leaves (μmol·g$^{-1}$)**

| Material | Total | A | B | C | D | E | F | G | H | I | J | K |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1008 | 5.45 | 1.97 | 0.10 | 0.23 | 0.19 | 0.52 | 0.27 | 0.81 | 0.67 | 0.59 | 0.13 | 0.09 |
| 5899 | 4.97 | 0.63 | 0.97 | 0.03 | 0.09 | 0.98 | 0.17 | 0.17 | 0.39 | 2.07 | 0.10 | 0.12 |
| 6098 | 4.71 | 0.77 | 0.26 | 0.44 | 0.48 | 1.35 | 0.26 | 0.44 | 0.31 | 0.79 | 0.13 | 0.13 |
| $M_1$ | 5.20 | 0.46 | 0.28 | 0.12 | 0.12 | 0.26 | 0.02 | - | 0.09 | 3.64 | 0.30 | 0.08 |
| 6098 × 1008 | 4.52 | 1.09 | 0.15 | 0.33 | 0.33 | 0.82 | 0.32 | 0.38 | 0.44 | 0.73 | 0.16 | 0.14 |
| 6098 × $M_1$ | 3.02 | 0.71 | 0.16 | 0.24 | 0.12 | 0.53 | 0.08 | 0.17 | 0.15 | 0.84 | 0.12 | 0.07 |
| 5899 × 1008 | 3.10 | 0.73 | - | - | 0.20 | 0.43 | 0.23 | 0.20 | 0.46 | 0.65 | 0.09 | 0.16 |
| $R_1$ | 0.85 | 0.15 | 0.23 | 0.03 | 0.06 | 0.05 | 0.02 | - | 0.07 | 0.50 | 0.04 | 0.08 |
| $R_6$ | 4.18 | 1.64 | - | 0.10 | 0.10 | 0.71 | 0.38 | - | 0.61 | 0.50 | 0.07 | 0.12 |
| 821 | 17.53 | 8.94 | 0.49 | 0.07 | 0.75 | 4.17 | 1.17 | 1.06 | 2.17 | 1.80 | 0.23 | 0.32 |

A.Desulfoprogoitrin; B.Desulfoglucoraphanin; C. Desulfogluconapoleiferin; D. Desulfoglucoalyssin; E. Desulfogluconapin; F. Desulfo-4-hydroxyglucobrassicin; G. Desulfoglucobrassicanapin; H. Desulfoglucobrassicin; I. Desulfogluconasturtin; J. Desulfo-4-methoxyglucobrassicin; K. Desulfo-1-methoxyglucobrassicin

Aliphatic, aromatic and indolyl glucosinolates in *B.napus* leaves

The profile of aliphatic, aromatic and indolyl content in *B.napus* leave was showed in Fig.2. The content of aliphatic glucosinolates was from 0.51 to 14.42 μmol·g$^{-1}$, which was the dominant glucosinolates accounting for 23.05 to 68.13 percent of the total content. The aromatic gluocosinolates content was from 0.50 to 3.64 μmol·g$^{-1,}$ accounting for 8.51~68.00 percent of the total content. The concentration of indolyl glucosinolates was from 0.20 to 4.94 μmol·g$^{-}$ accounting for 8.94%~36.26 percent of the total glucosinolates content.

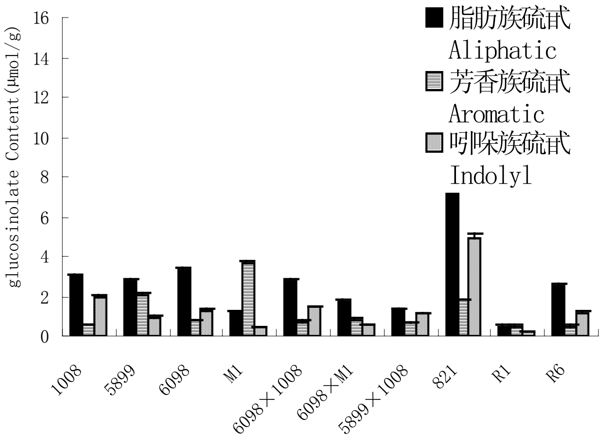


Fig. 2 Profile of aliphatic, aromatic and indolyl glucosinolates content in *B.napus* leaves

**Table 2 showed correlations among all three types of glucosinolates. The correlations between aliphatic,indolyl and all glucosinolates components were significant with coefficient 0.98 and 0.95. The correlation between aliphatic and aromatic ones was also significant with correlation coefficient 0.97.**

**Table 2 Correlations between aliphatic, aromatic, indolyl and total glucosinolates content in *B.napus* leaves**

| Item | All individuals | Aliphatic | Aromotic |
|---|---|---|---|
| Aliphatic | 0.98** | | |
| Aromatic | 0.32 | 0.16 | |
| Indolyl | 0.95** | 0.97** | 0.05 |

*Significant ($P \leqslant 0.01$)
**Very significant($P \leqslant 0.05$)

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Glucosinolates content differences in leaves of *B.napus* between double low and double high materials were mainly from aliphatic glucosinolates variation. Progoitrin, gluconapin,,4-hydroxyglucobrassicin, glucobrassicanapin and glucobrassicin are the main components of glucosinolates in leaves. It would be beneficial to plant diseases resistance when the parents material selected with high concentration of those glucosinolates above were used for crossing. The heterosis of leaf glucosinolates were significant and negative in stem elongation and floral period, suggesting that it is difficult to increase leaf glucosinolates in these periods for high disease resistance and it would be the best time for harvesting shoots as vegetable.

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