Analysis of glucosinolate components of different DH populations in *Brassica napus*

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Abstract

Glucosinolate components of 4 DH populations from different donors derived from different breeding programs were investigated by high performance liquid chromatography (HPLC). Compared with the donors, the segregation oftotal glucosinolate and its components in DH progeny were investigated. The variations of main glucosinolate components were different among 4 populations. And the variances of total glucosinolate concentration in the DH lines derived from the F1s with low glucosinolates parents were the most remarkable. All the DH populations were also used to analyze the partial correlations among different glucosinolate components. Significant positive correlation ($P<0.01$) was found between progoitrin and gluconapin as well as between 4-hydroxyglucobrassicin and gluconasturtiin, respectively. It indicated that there were common precursors or intermediates in their synthesizing pathway. Genotypes with high glucosinolates were more readily to form embryos. However, in the 4 DH populations, progeny with lower glucosinolate concentration still could be selected , which indicated microspore culture was useful to low-glucosinolate breeding while suitable population size should be considered.

Key words: *Brassica napus*, Microspore culture, Glucosinolate components
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Introduction

Oilseed rape (*Brassica napus* L.) is one of the most important oilseed crops. Glucosinolates are important secondary metabolites in *B.napus*. They are classified into three classes, depending on the amino acid from which they are derived: aliphatic/alkenyl glucosinolates derived from methionine, aromatic glucosinolates derived from phenylalanine and tyrosine, and indoly glucosinolates derived from typtophan and so on (Rosa 1999, Mikkelsen et al.2000, Wittstock and Halkier 2000.). In the plant, glucosinolates and their breakdown products are thought to play an important role in many respects, such as the anti-microbial, anti-fungicidal, anti-bacterial and thyroidal properties, which create the natural protection of the plant itself (Zukalová and Vašák, 2002). At the same time, as the secondary metabolites in seeds, some glucosinolates are hydrolyzed to a set of products poisonous to livestock (Bjerg et al., 1989, Hansen et al., 1997). Therefore, high concentration of glucosinolates restrict the value of rapeseed meal. Conversely, some specific glucosinolate components are suggested being positive for human body, t (Bjergegaard et al., 1994, Buskov et al., 2000a, b, and refs. cited therein) It is not therefore a question of reducing glucosinolate content to a minimum, but of developing horticultural and agricultural cultivars with an appropriate glucosinolate components, (Bagger et al., 1998).

Plant breeding, based on knowledge of glucosinolate biosynthesis, play an important role in the development of double-low (low erucic acid, low glucosinolates) oilseed rape cultivars. (J. Hill, 2003). Genetic control of glucosinolate biosynthesis was revealed by studies using ^{14}C-acetate, which resulted in ^{14}C-labelling of aliphatic but not indol-3-ylmethylglucosinolates. (Bjerg et al., 1987, Haughn et al., 1991). Recent advances in plant genomics as gene mapping and cloning have helped to better understand the mechanism of glucosinolate biosynthesis, which was proven intractable via a purely biochemical approach (Richard Mithen 2001). QTL analysis for different glucosinolate components in seeds of *B.napus* suggested that the synthesis of different glucosinolates were controlled by some common genes. For example, the aromatic glucosinolate (2-phenylethyl glucosinolate) was involved in the aliphatic glucosinolate pathway (Zhao and Meng 2003). Here, we detected the glucosinolate components in 4 double-haploid (DH) populations derived from microspore culture. This paper presented the effect of seed glucosinolate components on the microspore culture, and the association between specific glucosinolate components.

Materials and methods

L1815 and P28 were double low polima CMS restores developed by recurrent selection (RS) and by pedigree method respectively. L1745 was a polima CMS restores with *Sclerotinia* resistance selected by marker assistant selection (MAS). And B11 was another *Sclerotinia* resistant line selected by pedigree method. all the materials were from the rapeseed laboratory of Huazhong Agricultural University (HAU). . Two F1s were obtained from 'P28×L1815′ (L650) and 'P28×B11' (FL) respectively. Microspores from L1815, L1745 and the two F1s were cultured to produce 4 DH populations in 2002 spring. And each genotype was self-pollinated to produce enough seeds in 2003 spring. In 2003 autumn, all the populations along

with the respective parents were sowed in the field experiment station, Huazhong Agricultural University. Self-pollinating seeds were harvested and prepared for glucosinolate analysis in 2004 spring. Populations were described in Table 1. The total seed glucosinolates and components were detected by HPLC. The HPLC protocol was referred as the national standard of P.R.China, 'GB*****-98 HPLC'. Quantities in seeds were given in μmol/g. SAS 6.12 was exploited to data analysis.

Results

Variation in 4 DH populations

Table 1,2 presented the total glucosinolate content of four **donors.** L1745 was high in glucosinolate(82.6 μmol /g in seeds), while L1815, L650 and FL were low glucosinolate materials . Seven, nine, thirteen and eleven glucosinolate components were identified infour DH populations derived from L1745, L1815, FL and L650 F1 **donors** , respectively. Among the glucosinolates, only 4 components were found common in all the 4 DH populations. They are progoitrin, gluconapin, 4-hydroxyglucobrassicin and gluconasturtiin.

For all the DH populations, aliphatic glucosinolates was the dominant components in seeds. In the population derived from high glucosinolate donor L1745′, aliphatic glucosinolate was the main content of total glucosinolates (91.16% in total). But in the low glucosinolate populations (from 'P28×L1815′, 'L1815′ and ' P28×B11'), the content of aliphatic glucosinolates was very lower (Table 2). Especially progoitrin and gluconapin were sharply decreased (Table 2). At the same time, for the indoly glucosinolates in the low glucosinolate populations, although the percentage in total sharply increased, the absolute content increased not too much (Table 2).

Thirteen glucosinolate components were detected in the DH population from the FL. The dominant component is progoitrin, with the mean 4.82μmol/g (33.30% of the total glucosinolates). Relatively lower level of gluconapin and 4-hydroxyglucobrassicin were also detected (2.37 and 2.34μmol/g, respectively). The total aliphatic glucosinolates was the major content (63.41% in total), which was higher than indoly glucosinolates (25.75%) and aromatic glucosinolates (8.89%). Except for a little higher population mean value, the FL DH population had the similar glucosinolate profiles as the female parent 'P28'. Both had the similar ratio to total glucosinolate concentrations (Table 2). Eleven components were identified in DH population from 650 and the results were similar to those of FL DH population (Table 2).

Altogether seven glucosinolates were identified in L1745 population. Progoitrin was the dominant component, having 47.59μmol/g in population mean (and 55.49% in total glucosinolate concentration). The mean content of gluconapin was lower than that of progoitrin (Table 2). For the main glucosinolates, the DH population had the similar mean contents as the donor 'L1745′. As expected, aliphatic glucosinolates constructed the main components, which had the similar ratio as donor (about 91% in total, Table 2).

Compared with the L1815 population, the profile of main glucosinolates in the L1815 kept similar. Also the aliphatic glucosinolates constructed the main components (Table 2).

Variances of glucosinolate in different populations

For coefficients of variation (CV) of each component, there were great differences in different populations. The DH populations derived from F1 plants as donors (FL and 650) had the greatest CVs, followed by recurrent parent and MAS parent as donors. Such regulation was found when compared the CVs of total glucosinolate in the four DH populations. Further more, the means of total glucosinolates concentration of all the DH populations were higher than that of their donors. (Table 2)

Partial correlations of the glucosinolate components in different populations

Partial correlation analysis can decrease the interferences from other variables. So it is suitable to analyze any two variables in multi-variables. We detected the relationships between different glucosinolate components. The results were shown in Table 3. Significant positive correlations were identified between progoitrin and gluconapin as well as between 4-hydroxyglucobrassicin and gluconasturtiin in the four populations (α=0.01).

Discussion

Selection materials with lower glucosinolates by microspore culture

One of our projects was to evaluate whether microspore culture could be used to further improve the quality of oilseed rape varieties derived from different breeding programs. The frequency distributions of all the dominant glucosinolates were continuous. Only one peak was found for each glucosinolate, which indicated that minor genes had effect on the content of components. The result indicated that it was possible to select DH offsprings with decreased glucosinolate content. DH population was very suitable to be identified quality trait because each offspring was homogeneous. For glucosinolates, although the phenotype of parents kept stable, segregation still could be found in DH populations. Some special genotypes were successfully selected out from the DH populations. For example, a genotype had 139.67μmol/g total glucosinolate concentration, much higher than its donor (82.60μmol/g). And selection for lower total glucosinolate concentration was efficient in the DH populations, too. A genotype in FL population had 4.35μmol/g total glucosinolate concentration, lower than that of both parents. Another genotype in L1815 DH population had 1.01μmol/g total glucosinolate concentration (L1815 with 3.2μmol/g). We hypothesized that the segregation of minor genes controlling the glucosinolates in the DH population leading the efficiencies.

The CVs of glucosinolate components in 4 DH populations were different from each other. Background in F1 lines was more complicated than that in RS or MAS lines. Therefore, the segregations in F1 derived DH populations were most obvious. Although L1815 was original from RS, it was selfed over 5 times. Background was dealt with by MAS in the backcross breeding for L1745. So the background was simpler than RS.

We found the distribution of dominant glucosinolates in 4 DH populations were continuous. It indicated that the major genes controlling the biosynthesizing pathway of glucosinolates were homogenous. The segregations came from the minor genes. So it is still efficient to select special lower glucosinolates by microspore culture.

Glucosinolates biosynthesis pathway

According to the R group, glucosinolates are classfied into three types (aliphatic, indoly and armoratic). Glucosinolate content and glucosinolate profiles exhibited diversity in *B.napus* (Liu, 2000; Qian et, 1996).

The **biosynthesis** pathway has been puzzled the biologists. Until now it was ambiguous for some components. In the three kinds of glucosinolates only the biosynthesis of aliphatic glucosinolates is well known. Generally speaking, glucosinolates are biosynthesized from a variety of typical protein amino acids (alanine, leucine, isoleucine, valine, phenylalanine, tyrosine, tryptophan, methionine and their chain–elongated analogs). The first step is oxidation of the amino group to an oxime moiety. After a thiohydroximate intermediate is formed, sequential transfer of glucose and sulfate residue creates the basic glucosinolate skeleton.The initially formed glucosinolate can undergo a variety of subsequent transformations that modify the side chain. (Danidl et al., 2001; Mikkelsen et al., 2002)

By comparing of the partial correlations of glucosinolate components in different DH populations, significant associations were found between progoitrin and gluconapin as well as between 4-hydroxyglucobrassicin and gluconasturtiin. It is a hint that there are common precursors in their metabolic pathways. The former was coincident with the previous reports (Mikkelsen et al 2002,Richard et al 1995 and Danidl et al2001) and the later will be testified in our further research.

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Table 1 Four donors for microspore culture

Populations	Origin of donors	Population size	Glucosinolate concentration parents/donor(μmol/g)
L650(P28×B11)	F1 hybrid	85	12.84
	P1(P28)	-	13.18
	P2(B11)	-	12.50
FL(P28×L1815)	F1 hybrid	68	9.95
	P1(P28)	-	13.18
	P2(1815)	-	3.20
L1815	RS	47	3.20
L1745	MAS	89	82.60

Table 2 The variation of the glucosinolate components in different DH populations

Variable	Donor (%)(μmol/g)	Mean (%)(μmol/g)	SD(μmol/g)	CV(%)
FL	CK	DH		
Proitrin	1.99(20.00)	4.82(33.30)	4.55	94.50
Gluconapin	1.23(12.36)	2.37(16.39)	2.15	90.66
4-HydroxyGlucobrassicin	2.35(23.62)	2.34(16.18	1.56	66.83
Aliphatic	5.15 (51.82)	9.17 (63.41)	6.83	74.48
Aromatic	1.14(11.44)	1.29(8.89)	1.15	89.68
Indoly	3.49(35.05)	3.72 (25.75)	2.01	53.98
Total glucosinolates	9.95(100)	14.46(100)	8.34	57.68
L650	CK	DH		
Proitrin	2.73(21.26)	10.17(41.16)	7.54	74.15
Gluconapin	1.45(11.29)	5.39(21.81)	4.92	91.21
4-HydroxyGlucobrassicin	1.50(11.68)	4.16(16.84)	2.34	56.20
Aliphatic	6.10(47.51)	17.05(68.98)	12.50	73.35
Aromatic	1.56(12.15)	2.555(10.33)	2.24	87.72
Indoly.	5.18(40.34)	5.115(20.69)	3.03	59.20
Total glucosinolates	12.84(100)	24.71(100)	14.05	56.85
LL1815	CK	DH		
Proitrin	0.18(5.63)	0.61(10.75)	0.39	64.15
Gluconapin	0.15(4.69)	0.65(11.44)	0.43	66.17
4-HydroxyGlucobrassicin	1.03(32.19)	1.64(28.9)	0.74	44.97
Aliphatic	1.47(45.97)	2.81(49.35)	1.38	49.03
Aromatic	0.25 (7.95)	0.32 (5.67)	0.14	44.86
Indoly	1.47 (46.08)	2.16(37.53)	0.93	43.50
Total glucosinolates	3.20(100)	5.69(100)	2.87	50.38
L1745	CK	DH		
Proitrin	47.40(57.38)	47.59(55.49)	14.70	30.88
Gluconapin	24.14(29.23)	26.2730.63()	7.70	29.33
4-HydroxyGlucobrassicin	3.14(3.80)	1.91(2.23)	0.83	43.22
Aliphatic	75.30(91.16)	78.65(91.71)	22.97	29.20
Aromatic	3.99(4.83)	5.12(5.97)	1.77	34.64
Indoly.	3.14(3.80)	1.91(2.23)	0.83	43.22
Total glucosinolates	82.60(100)	85.76(100)	24.51	28.57

Table3 Correlations and partial correlations of the DH populations

Components	Progoitrin	Gluconapin	4-HydroxyGlucobrassicin	Gluconasturtiin
FL DH				
Progoitrin	1	0.89**	-0.15	-0.07
Gluconapin	0.86*	1	0.14	0.04
4-HydroxyGlucobrassicin	0.15	0.30**	1	0.60**
Gluconasturtiin	0.04	0.13	0.60**	1
650 DH				
Progoitrin	1	0.55**	-0.07	0.32
Gluconapin	0.78**	1	0.05	0.12
4-HydroxyGlucobrassicin	0.07	0.05	1	0.27*
Gluconasturtiin	0.12	0.17	0.16	1
1815 DH				
Progoitrin	1	0.89**	-0.38*	0.41**
Gluconapin	0.93**	1	0.34	-0.28
4-HydroxyGlucobrassicin	0.69**	0.72**	1	0.83**
Gluconasturtiin	0.66**	0.70**	0.88**	1
1745 DH				
Progoitrin	1	0.73**	0.04	-0.27*
Gluconapin	0.86**	1	0.06	0.33**
4-HydroxyGlucobrassicin	0.29**	0.37**	1	0.33**
Gluconasturtiin	0.34**	0.54**	0.46**	1

Note: **:significant correlation at $\alpha=0.01$;*:significant correlation at $\alpha=0.05$;The numbers in the upper dexter corners are correspouding partial correlations;The numbers in the lower sinister corners are correspouding correlations