Selection of rapeseed (*B. napus* L.*)* mutants with low antinutritional compounds

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ABSTRACT

The high nutritional value of oilseed rape meal has been thwarted by presence of anti-nutritional factors such as sinapine and glucosinolates. Furthermore developing of standardised methods for selection and quantification of these compounds is very interesting from breeding point of view.

A large collection of mutant lines (9052 plants) obtained from *Brassica napus* DH line 0120 after treatment with chemical mutagens (SA, MNUA) were evaluated in the field in 2000 and 2001. The first step for biochemical analyse was performed by near infrared reflectance spectroscopy (NIRS) for oil, protein, glucosinolates and sinapine content. A great variation of analysed traits was found among mutants compare to initial line. A few of selected mutants based on NIRS method were additionally HPLC analysed for glucosinolates (GSL). GSL content in mutants ranged from 12,3 to 3,0 цmol/g as compared to initial form, 9,7±0,5 цmol/g. Some of analysed mutants showed also decrease of sinapine content, which ranged from 5,6 to 13,5 µg/g seeds. Moreover, a few analysed mutants showed improved oil quality (higher content of oleic acid) and tocopherol compare to DH line 0120. Further testing of the selected material is necessary to confirm the obtained results.

Key words: mutation breeding, NIRS, glucosinolates, sinapine

INTRODUCTION

Mutagenesis has been used successfully to develop mutants with improved quality in Brassica species (Röbbelen 1990) but without leading to altered anti-nutritional compounds (sinapine and gucosinolates). Efficiency of conventional plant breeding method depends on genetic variability of analysed compounds in initial material and developing suitable and fast analytical methodology. Recently, a non-destructive seed method such e.g. NIRS has been described for sinapine (Velasco & Möllers 1998) and glucosinolates (Velasco & Becker 1998). The objective of the present study was to investigate variability for sinapine and glucosinolate content in a large collection of mutant lines determined by different methods (NIRS, HPLC and GLC).

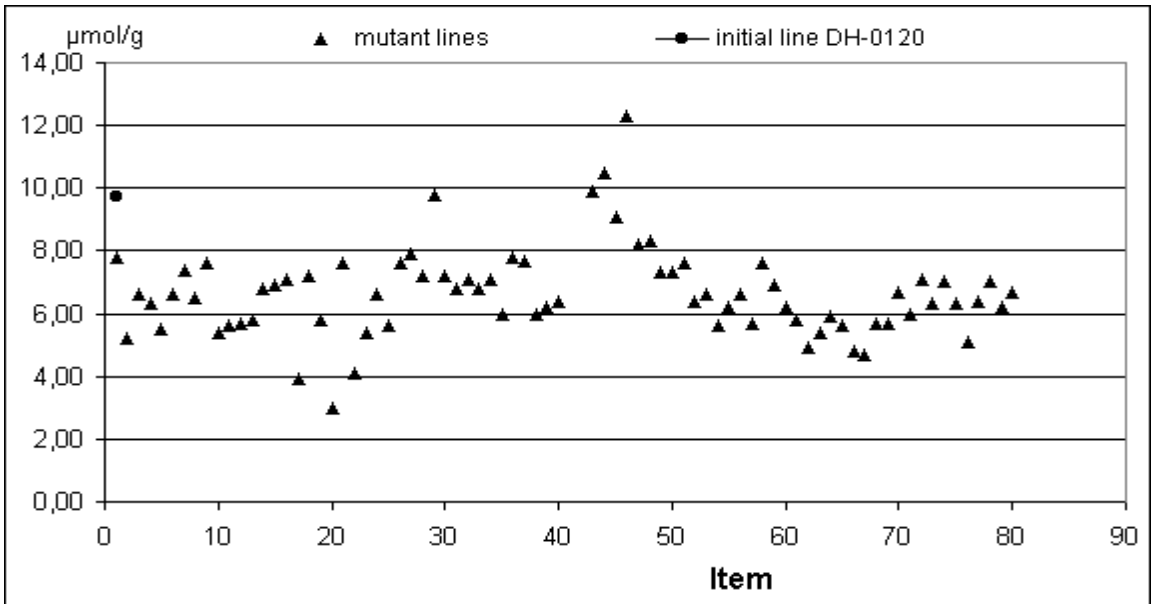
MATERIAL AND METHODS

Initial winter rapeseed line DH-0120 (00 – quality) was received from the Institute of Plant Breeding and Acclimatization in Poznań. A large number of mutant lines M_4 – generation (9052 lines) derived at Institute of Plant Genetics in Poznan, Poland from material originally treated with the chemical mutagens sodium azide (SA) and N-nitrozo-N-metylo-urea (NMU) were analysed in the laboratory of George-August University, Göttingen, Germany. The field experiment was carried in 2000-2001 at the Experimental Station Cerekwica near Poznan. 300 mg intact seeds were analysed by near-infrared reflectance spectroscopy (NIRS, FOSS 6500) using a special adapter for the ring cups. Using suitable calibration the oil, protein, glucosinolate and sinapine content and composition of fatty acids were calculated. Based on NIRS analyses some mutant lines with low glucosinolate and sinapine content were selected for more accurate analyses using HPLC. Some of mutant lines were also analysed by HPLC for tocopherols content and by GLC for fatty acid composition.

RESULTS AND DISCUSSION

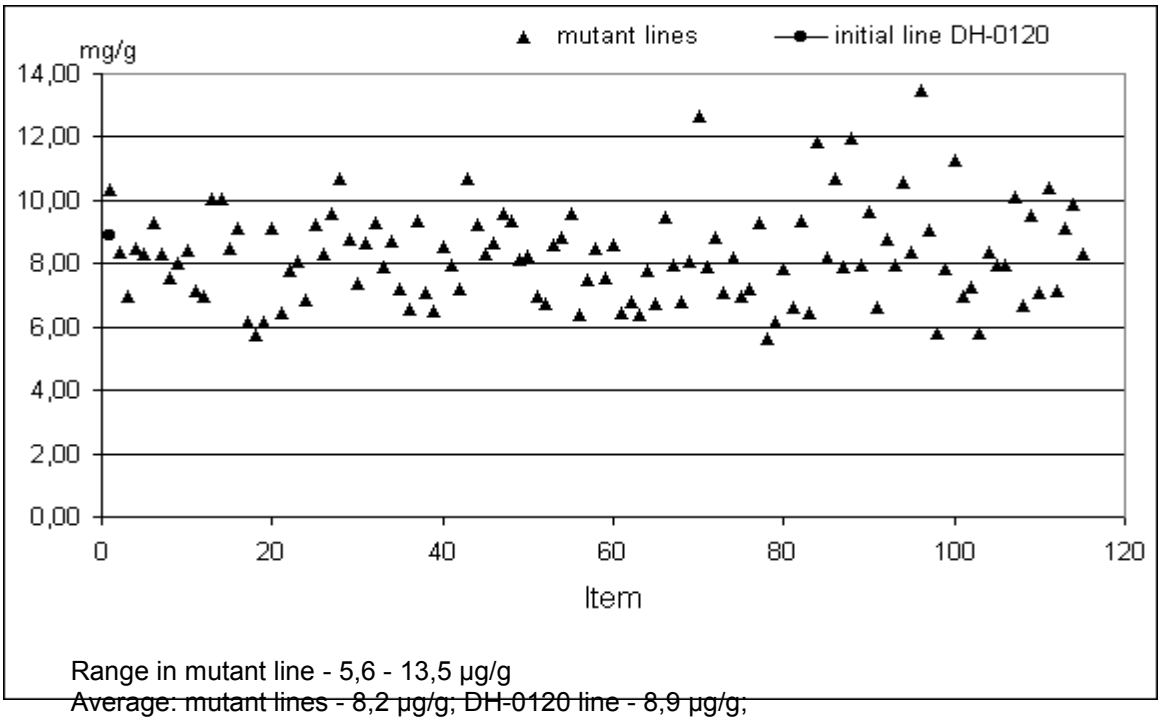
Analyses of oil, protein, glucosinolate and sinapine content determined by NIRS showed variation for the traits that ranged from 23,1 to 54,6 %, 15,0 – 32,1 %, 0,2 – 20,2µmol/g and 4,4 – 14,6 %, respectively. The selected mutant lines showed GSL content of 2,5 -12,3 µmol/g as determined by

HPLC. The original DH line 0120 contain as an average 9,7 µmol/g (Fig. 1). The sinapine content in the seeds of 114 selected mutant lines as analysed by HPLC showed a large variation from 5,6-13,5 µg/g seeds (Fig. 2). According to Wang et al. (1998) and Wojciechowski et al. (1994) a similar great variation of sinapine content was also observed in different forms of white mustard and oilseed rape. The oil, protein, GSL, tocopherol content as well as the fatty acids composition of some of the selected mutants is presented in table 1. The range of tocopherols content was markedly wide, from very low values (207,0 µg/g seeds, J1-59) to very high values (483,0 µg/g seeds, J31-51). A little variability for a fatty acid composition was found in the analysed lines. Future testing of the selected lines to confirm the obtained results are necessary. Backcrossing experiments are in progress to investigate the nature of the genetic changes in selected lines.



Range in mutant line - 3,0 - 12,3 µmol/g
Average: mutant lines - 6,6 µmol/g; DH-0120 line - 9,7 µmol/g;
SD=1,4

Fig. 1. Variability of glucosinolate content in M_4 - lines and in the original line DH-0120



Range in mutant line - 5,6 - 13,5 µg/g
Average: mutant lines - 8,2 µg/g; DH-0120 line - 8,9 µg/g;
SD=1,5

Fig. 2. Variability of sinapine content in M4 - lines and in the original line DH-0120

Table 1. Variability of tocopherols (T) composition connected with another biochemical traits in *Brassica napus* mutant lines and in initial line DH-0120

Item	Total –T µg/g seed	α – T µg/g seed	γ – T µg/g seed	Ratio α/γ	Fatty acids (%)				Oil content (%)	Protein content (%)	GSL content (µmol/g)
					18:01	18:02	18:03	22:01			
DH-0120	324	131	192	0,7	58,9	21,1	10,3	0,0	44,6	22,8	9,7
J-31-280	371	144	228	0,6	66,9	15,1	10,6	0,0	48,1	24	5,2
J31-40	413	160	252	0,6	63,2	19,1	9,6	0,0	46,2	26	6,6
J31-51	484	174	310	0,6	63,4	18,5	9,8	0,0	48,4	23,2	6,3
J6-190	396	157	273	0,4	66,4	15	10,3	0,0	47,3	23,4	5,5
J4-96	344	111	233	0,5	65,6	17,4	9,4	0,0	43,6	27	6,6
J7-45	301	106	195	0,5	65,6	17,1	9,1	0,0	36,5	32,7	7,4
J-4-167	353	110	243	0,4	65,3	18	9	0,0	42,3	29,1	6,5
J22-168	401	149	252	0,6	60,6	21,3	10,5	0,0	48,6	21,5	7,6
J22-342	390	142	248	0,6	63,7	19	10	0,0	50,4	21	5,4
J24-127	366	164	202	0,8	58,3	21,3	10,5	0,0	39,7	32,8	5,6
J1-59	207	103	104	1	58,1	21,4	11,4	0,0	40,9	25,4	5,7
J12-259	404	168	237	0,7	60,5	21,2	9,3	0,0	47,9	23	6,9
J19-16	411	147	264	0,5	60,5	21,1	10,1	0,0	50,3	21,7	7,1
Average	372,4	141,2	233,9	0,6	62,9	18,9	10,0	0,0	45,4	25,4	6,3
SD	65,8	25,2	48,8	0,2	3,0	2,3	0,7	0,0	4,4	4,0	0,8

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