GENETIC AND ENVIRONMENTAL FACTORS INFLUENCING GLUCOSINOLATE CONTENT IN RAPESEED IN SOUTHERN AUSTRALIA

Phillip Salisbury[1], Joseph Sang[2] and Ron Cawood[1]
Department of Agriculture and Rural Affairs, Victoria
[1]Victorian Crops Research Institute, Private Bag 260, Horsham, Victoria 3400, Australia
[2]State Chemistry Laboratory, 5 Macarthur Street, East Melbourne, Victoria 3002, Australia

Rapeseed (Brassica napus and Brassica campestris) is sown in a wide range of environments in southern Australia, with sowing from April (autumn) to October (spring), and harvest from mid-November to January (summer). As a consequence, large variations occur in meal quality and oil content of harvested seed. In particular, glucosinolate content in "double low" varieties sometimes reaches twice the acceptable Canola standard of 30 µmoles/g of meal. Such variable quality has been of major concern to the industry which must be able to guarantee Canola quality products in order to compete effectively on world markets. This paper summarises four years of trials aimed at elucidating the genetic and environmental factors which influence glucosinolate content in rapeseed in southern Australia.

GENETIC EFFECTS

Time of sowing experiments in 1983 (2 sites x 5 sowing times) and 1984 (1 site x 10 sowing times) using both low and high glucosinolate lines have shown large variation in glucosinolate contents. Some examples are given in Table 1.

Table 1. Range of total glucosinolate contents with time of sowing*

Variety	Total glucosinolates (µmoles/g)#							
	1983				1984			
	Mean	Min.	Max.	Range	Mean	Min.	Max.	Range
Midas	138	107	173	66	166	129	210	81
Primor	111	79	151	72	168	122	219	97
Tower	39	18	57	39	+			
Marnoo	38	12	78	66	49	20	75	55
Tatyoon	21	13	33	20	49	25	67	42
Wesroona	46	15	76	61	47	34	60	26
Wesbrook	+				35	30	44	14

*Sowing times varied from April to September in both years
#All glucosinolates are quoted as µmoles/g of oil free, moisture free meal
+Variety not sown

There were marked differences between the lines in mean and range of total glucosinolates, with some low glucosinolate lines more stable than others. Varieties with similar flowering and maturity times did not necessarily respond in the same way to the different times of sowing. The time of sowing which produced the highest glucosinolate content for one variety sometimes produced the lowest glucosinolate content for another variety of similar maturity. The highly significant positive correlation between time of sowing and glucosinolate levels in Marnoo found by Sang et al. (1984) did not occur with most of the varieties in this trial.

The total glucosinolate levels were derived from the summation of eight individual glucosinolates measured by HPLC (Sang and Truscott, 1984). They were the Canola glucosinolates (3-butenyl-, 4-pentenyl-, 2-hydroxy-3-butenyl- and 2-hydroxy-4-pentenyl-), the indole glucosinolates (3-indolymethyl- and 4-hydroxy-3-indolylmethyl-) and other glucosinolates present in small amounts (2-phenylethyl- and 2-propenyl-).

The relative contributions of these different groups to the total can be seen in Table 2.

Table 2. Minimum and maximum contents of different glucosinolate groups

Variety	Glucosinolates (min.→max.) (umoles/g)							
	1983				1984			
	Canola	Indole	Other*	Indole/ total (%)	Canola	Indole	Other	Indole/ total (%)
Midas	98-164	2-7	3-5	2-6	119-196	5-10	4-6	3-6
Primor	71-142	4-7	2-4	3-7	112-208	6-10	3-6	3-6
Tower	13-47	4-8	0-3	9-27				
Marnoo	9-71	3-6	0-2	6-27	14-64	6-10	0-2	11-29
Tatyoon	9-31	2-5	0-1	5-33	23-57	1-8	1-3	3-18
Wesroona	13-67	2-8	1-2	8-16	26-50	5-9	1-3	10-22
Wesbrook		+			22-32	2-11	1-2	7-31

*2-phenylethyl- and 2-propenyl- glucosinolates only +Not sown

All low glucosinolate varieties exceeded the Canola limit at some time, with varying frequency and degree. The amount of indole glucosinolates was relatively constant for all lines in all sowings, usually 4-9 umoles/g. This was a significant proportion (up to 33%) of the total for low glucosinolate lines. It is important therefore, as Fenwick (1985) suggested, to include indole (and other non-Canola) glucosinolates in quoted values unless it can be proven that these glucosinolates have no anti-nutritional properties. This would require redefinition of the Canola standard.

Major emphasis is now being given in Australian breeding programmes to selecting lines which have total glucosinolates as low as possible and which never drift over the Canola limits in any environment. The progress in the Victorian breeding programme is given in Table 3 as an example.

Table 3. Glucosinolate contents in Victorian varieties and breeding lines

Variety/ line	Year of release	Total glucosinolates (μmoles/g) 1985 Mean	1985 Min.	1985 Max.	1986 Mean	1986 Min.	1986 Max.
Marnoo	1980		+		35	27	45
Tatyoon	1984	44	36	64	34	18	44
RX 9	1987*	28	18	38	25	17	32
RY 6	1988*	17	13	20	16	9	27

*Anticipated release dates +Not sown

ENVIRONMENTAL EFFECTS

Attempts to understand the effects of temperature and moisture stress on glucosinolate contents of different varieties in 1985 glasshouse trials were hampered by the extreme variation between replicates of any given variety and treatment. To minimise the genetic variability within varieties, seed from bagged, selfed single plants (s.p.) was used in some 1986 experiments.

Water stress

The effect of water stress on glucosinolate content of these single plant derived lines was investigated in a glasshouse trial. Plants (in 50 L pots) were subjected to 9 cycles of water stress between flowering and maturity, each cycle allowed plants to reach water potentials of at least -15 bar for 24 hours before being re-watered. Such stress cycles during the post-flowering period resembled field conditions where gradual development of stress is relieved by intermittent rainfall. Despite a uniform 75% yield reduction, the effects of stress on glucosinolate levels of each line were variable (Table 4).

Table 4. Effect of water stress on glucosinolates in 1986 glasshouse trial (4 replicates per treatment)

Line	Glucosinolates (μmoles/g) Unstressed	Stressed
Marnoo s.p.	15.5	12.3*
Tatyoon s.p.	13.3	18.0
Wesbrook s.p.	13.3	7.0
L.S.D.* (P=0.05)	6.20	

*Line x treatment

There was a significant effect only for the Wesbrook selection, where glucosinolate content was reduced by water stress. The Tatyoon line tended to produce higher glucosinolates under stress, while the Marnoo line was relatively unaffected.

Glucosinolate contents in this trial were relatively low compared with results obtained from field trials. Environmental conditions in the glasshouse may have been conducive to lower levels, and the single plant selections may have been genetically lower than the original varieties.

The effect of water availability on glucosinolates was also investigated in a field trial in 1986 with four varieties (Marnoo, Tatyoon, Wesroona and Wesbrook). Three watering regimes were imposed at flowering: (1) rainfed only, (2) a single irrigation at flowering, (3) twice weekly irrigations (55 mm each time) from flowering to physiological maturity. Unseasonally high rainfall (75 mm - three times the monthly average) during late seed filling minimised the effect of water stress in the rainfed only treatment. No significant differences in glucosinolate levels were found between the watering regimes in any variety, although significant yield increases of 10-20% (one irrigation) and 30-60% (twice weekly irrigation) in excess of the rainfed treatment were evident in all varieties.

These glasshouse and field results do not give any clear indication that water stress is a major contributing factor to the high glucosinolate levels sometimes observed in commercial crops.

Temperature

A controlled environment study with single plant derived lines showed that temperature during seed development influenced glucosinolate content and that significant temperature x variety interactions were evident (Table 5). All plants were grown under 15°/10°C (day/night) conditions until flowering and then transferred into 15°/10°, 24°/19°, 27°/22° or 30°/25°C until maturity.

Table 5. Effect of temperature on glucosinolate content (8 replicates per treatment)

Line	Glucosinolates (umoles/g)			
	15°/10°C	24°/19°C	27°/22°C	30°/25°C
Marnoo s.p.	25	21	27	64
Tatyoon s.p.	33	34	48	60
Wesbrook s.p.	32	28	24	-*
L.S.D.* (P=0.05)		9.5		

*No seed produced *Line x treatment

The Tatyoon line was most susceptible to the effects of temperature, with glucosinolate content being significantly higher at 27°/22° than at the lower temperatures. In contrast, only the highest temperature increased glucosinolate content in the Marnoo selection, while Wesbrook tended to have decreasing glucosinolates with moderate temperature increases. Further work is necessary to establish a causal relationship between temperature and glucosinolate levels. Day temperatures in the field regularly reach 30°C or higher during seed ripening (late spring

or early summer), but night temperatures are generally much lower. The most important factor influencing glucosinolate synthesis - day temperature, day-night difference, or night temperature - remains unknown. It seems likely, however, that temperature has a significant influence on the glucosinolate variability seen in the field.

Disease and nutrition

A preliminary glasshouse trial in 1985, using bulk seed samples from several plants, indicated that infection with blackleg increased seed glucosinolates compared with uninfected plants in two lines, Wesroona (45 μmoles/g uninfected; 81 μmoles/g infected) and RX 9 (23,36), but not in Tatyoon (50,43). This is being further investigated.

The potential importance of variability in soil sulphur and nitrogen levels and applied S and N fertilisers in influencing glucosinolates is also being evaluated. Results from one of these trials have shown that rates of applied nitrogen higher than 100 kg/ha increased glucosinolate content in Tatyoon by 3-5 μmoles/g. However, such high levels of applied N are not currently used in southern Australia. They are not therefore, a major contributing factor to the present variability in glucosinolate contents.

CONCLUSION

This study has shown that glucosinolate content is subject to major interactions between varieties and environmental factors. The differential response of varieties of similar maturity to time of sowing may be explained by their variable reactions to the same environmental factors. This is not entirely unexpected given the inherent variability within Australian B. napus varieties. It is likely that each consists of several genotypes and that each of these may have a unique environmental response pattern.

The inability to clearly define general environmental factors which contribute to high glucosinolate contents has complicated the selection of low glucosinolate types. However, genotypes which have very low and stable glucosinolate contents are being identified, and these will form the basis of the future Australian rapeseed industry.

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