Comparison of Chemical and Agronomic Characteristics of Two *Brassica napus* L. Cultivars, Bronowski and Target[1,2]

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ABSTRACT

The *Brassica napus* cultivar, Bronowski, has a low glucosinolate content in the seed and thus has considerable potential as a parent stock for the production of low glucosinolate rapeseed. It also differs from other *B. napus* cultivars in fatty acid composition, but the meal amino acid analysis is similar to that of the standard cultivar, Target. The physical and chemical properties of the Bronowski seed storage proteins are similar to those from Target. While the results permit no definite conclusions about the origin of Bronowski, they show that a cross between it and Target does not change greatly the amino acid composition of meal or the protein composition in the progeny. Crosses between Bronowski and other *B. napus* cultivars have produced progeny with a glucosinolate content lower than Target and an improved agronomic performance.

INTRODUCTION

The summer rape cultivar Bronowski has been extensively used as a parent since 1967 in rapeseed breeding programs throughout the world. Like other cultivars of the *Brassica napus* species it is believed to have its origin in interspecific crosses among plants of the *B. campestris* and *B. oleracea* species. The major attribute of the Bronowski cultivar is that, within the *Brassica* genus, it is to date the only known germ plasm source capable of producing seed essentially free of glucosinolates. Although over 40 glucosinolates have been reported in plants, only three major ones occur in rapeseed and meal: gluconapin, glucobrassicanapin and progoitrin. It is these compounds, when hydrolyzed by myrosinase, that give rise to the active goitrogenic compounds 3-butenyl (BI) and 4-pentenyl isothiocyanates (PI), and 5-vinyl-2-oxazolidinethione (OZT), respectively.

These and other glucosinolate breakdown products, when fed in significant quantities, can result in metabolic upsets in nonruminant animals. Thus, although the hydrolysis of these glucosinolates is prevented in most countries by the inactivation of myrosinase prior to oil extraction, the removal of the glucosinolates through plant breeding is of fundamental as well as economic interest.

Because of the potential of the Bronowski cultivar as a parent to bring about this desired improvement, it is important to know its origin and its agronomic, morphological and chemical characteristics, which will be introduced into breeding populations as a result of its utilization. Some of the more significant of these data are presented along with comparative data for a widely grown Canadian *B. napus* cultivar, Target.

MATERIALS AND METHODS

Seed of the Bronowski cultivar was obtained from Rolimpex (P.O. Box 364, Warsaw, 1, Poland), and either the original imported seed or that increased under isolation at Saskatoon, in 1968, was utilized in the chemical and comparative trials. Seed of the cultivar Target was of certified grade, and the *B. napus* strain 105 used in crosses with Bronowski resulted from selection and inbreeding within the Target variety.

Comparative field trials of Bronowski and Target, conducted at Saskatoon in 1969, consisted of single row plots 20 ft (6.1 m) long with 0.6 m between rows. Seed was harvested with a Hege 125 combine, oil content determined on oven-dry seed by wideline NMR and protein content of the oil-free meal calculated from macro-Kjeldahl analysis of the whole seed.

For detailed chemical analysis of the oil and meal, oil extraction was affected with *n*-hexane using the Swedish tube method of Throeng (1). The fatty acid composition of the oils was determined by gas liquid chromatography after the method of Downey and Craig (2). Glucosinolate content was determined using a modified method of Youngs and Wetter (3). Meals for amino acid, sulfur and polyphenol determinations were prepared using the Swedish tube method and, following removal of the *n*-hexane solution of the oil by filtration, were kept overnight in a vacuum desiccator. Nitrogen contents averaged 6.7-6.8% (Kjeldhal). Sulfur contents were determined by the method of Shaw (4).

The amino acid compositions of the meals were made after acid hydrolysis (5), on a Beckman Model 120 C amino acid analyzer. The compositional data are the averages of triplicate determinations and for purposes of clarity we have not included the standard deviation figures (±3%).

The polyphenol determinations were made on 1.0 g samples of meal, which were extracted with 100 ml absolute methanol for 1 hr (6). After filtration, the extract was made up to an appropriate volume and the absorption of the solution measured between 240 and 460 nm. A qualitative paper chromatographic separation (*n*-butanol/ethanol/water 40:11:19) showed there were at least eight UV absorbing compounds in the methanol extracts of the meals. This method (6) gives a reasonably accurate (±5%) estimate of the total phenolic contents of the meals; it provides results as good as those that would be obtained by the separation of the individual components and their separate quantitation.

The meal was extracted with a solution of 1 M sodium chloride, 0.01 M sodium tetraborate (pH 8.7) (50 ml solvent per 1 g meal). The extracts were prepared by 1 hr of stirring at 2 C followed by centrifugation at 10,000 g; the precipitate was washed with 25 ml solvent, recentrifuged, and the supernatants were combined and made up to 100 ml. The 12S globulin and the 1.7S protein were recovered from the supernatants as described previously (7).

The polyacrylamide gel electrophoresis was made on aliquots of the molar sodium chloride extract. The protein concentration applied to the gels (11.25% acrylamide) was ca. 5 mg/ml. The electrophoresis was made in 0.01 M acetic acid, β-alanine buffer pH 4.3, and run at 5 mA per tube (8).

RESULTS AND DISCUSSION

The Bronowski cultivar was selected in Poland before World War II by K. Moldenhawer and B. Grabiec at the plant breeding stations of Bronow and Borowo in the Poznan district of Poland. Records of parentage were lost during the war, but from morphological and chemical

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TABLE I

1969 Field Performance of Bronowski and Target Rapeseed in Western Canada

Cultivar	Seed yield, kg/ha	Oil,[a] %	Protein,[b] %	Date mature	Height, cm	Seed and plant pigmentation
Bronowski	718	41.7	47.8	Sept. 6	119	Heavy
Target	1304	42.7	46.4	Aug. 24	104	Light

[a]Moisture free.
[b]Moisture free, oil free.

characteristics Bronowski appeared to be similar to a local population of rape grown in southeast Poland under the name Brzoskiew. Although no viable seed of this suspected parent material could be located following the war, the low glucosinolate content of old nonviable seed of the Brzoskiew cultivar tends to confirm that it is probably the base stock from which Bronowski originated. The reference variety Target was bred at the University of Manitoba by B.R. Stefansson and was a single plant selection that traces back to the original seed stock imported from Argentina into Canada in 1942. The source of this material was probably Northern Europe.

Despite the nutritionally more desirable meal obtained from processing Bronowski seed (9), the cultivar has not gained acceptance in Poland, Canada or other rapeseed producing countries because of its generally poor agronomic performance (Table I). In addition to having significantly lower seed and oil yields, under Canadian conditions the variety tends to be more susceptible to seed shattering while its late maturity prohibits its successful production in Western Canada two years out of three.

The polyphenol content of Bronowski plants and seeds is also greater than that of Target by 20-25%, although there are no significant differences in the meal nitrogen contents (Table II). These polyphenol compounds have spectral characteristics that indicate they are oxygen heterocyclics and have absorption maxima at 328-330 nm. There is a general tendency for plant breeders to select against high polyphenol content, but the scientific basis for such discrimination is not well documented.

The kind and amount of glucosinolates present in commercially grown rapeseed differ widely according to species and cultivar (10). The level and pattern of glucosinolates in Target rapeseed (Table III) is similar to and within the range reported for other commercially grown spring and winter European cultivars of *B. napus* (11). Thus Bronowski is the most promising genetic base stock for the development of agronomically acceptable varieties with very low levels of glucosinolates. (Josefsson [12] has evidence for an indole glucosinolate in the leaves of Bronowski, although it has not been isolated from the seed.) It is not known at this time whether it will be possible to select cultivars with a zero level of glucosinolates, such as might be desired for the production in rapeseed flours or high protein concentrates for human consumption. However, under certain environmental conditions such as low soil availability of sulfur, no glucosinolates are detected in Bronowski seed.

Under normal environmental conditions the presence and amount of the three major glucosinolates in spring *B. napus* have been shown to be under genetic control. The low levels or absence of these compounds in Bronowski seed are recessive characteristics and are controlled through the genotype of the maternal plant rather than the genetic make-up of the developing embryo. Kondra and Stefansson (13) report that in progeny of crosses between plants of Bronowski and Target the content of BI is controlled by three loci, while four to five loci probably controlled the level of PI. Four loci are indicated for high levels of OZT. However in their crosses OZT was always absent when the amounts of BI were low and its homolog was absent. These findings suggest that the blocks in the biosynthesis of

TABLE II

Some Analytical Data from Seed Samples[a]

Measurement	Target	Bronowski	B x 105
Nitrogen (meal), %	6.7	6.8	6.8
Saline soluble N, mgN/gm meal	46.0	47.0	46.0
Sulfur content, mgS/gm meal	20.0	6.0	16.5
Polyphenol content[b]	1.20	1.50	1.20

[a]Oil-free, 5% moisture.
[b]Based on relative absorptivity at λ_{max} 328 nm.

TABLE III

Thioglucosides in Oil-Free Meals of Target and Bronowski Cultivars[a]

Cultivar	Isothiocyanate			
	Allyl	3-Butenyl	4-Pentenyl	OZT
Bronowski	0.0	0.03	0.0	0.57
Target	0.0	1.90	0.70	13.60

[a]Given in mg/g meal of their hydrolysis products, allyl, 3-butenyl, 4-pentenyl isothiocyanates and 5-vinyl-2-oxazolidinethione (OZT). Average of four determinations.

TABLE IV

1972 Agronomic Performance of *B. napus* Varieties and Low Glucosinolate Strains at Saskatoon

Variety or strain	Yield, cwt/a	Oil, %	Maturation days	Erucic, %	Glucosinolates, mg/g		
					3BI	4PI	OZT
Target	26.9	42.7	101	40.0	3.0	0.2	10.0
Zephyr	23.3	39.6	103	0.9	2.8	0.1	8.2
Midas	27.4	44.2	100	1.5	2.5	0.2	8.5
SZN71-1788	23.9	42.4	98	2.2	0.2	0.0	0.8
-1787	22.8	41.7	101	9.4	0.1	0.0	0.3
-1785	24.7	42.3	98	6.2	0.1	0.0	0.4
-1784	23.2	41.1	98	0.6	0.1	0.0	0.8

TABLE V

Per Cent Fatty Acid Composition of Seed Oil from Cultivar Target Compared with Polish Grown Bronowski and That Produced in Two Multiplications at Saskatoon

	Target		Bronowski		
Fatty acid	1966	1970	Poland	1966	1970
16:0	3.6	3.1	3.4	3.6	3.2
16:1	0.3	tr	0.3	0.3	0.3
18:0	1.4	1.4	1.5	1.5	1.5
18:1	22.0	21.4	39.3	40.2	38.7
18:2	15.4	13.6	14.6	14.0	14.6
18:3	7.3	5.8	7.7	9.3	8.0
20:0	tr	tr	0.5	tr	0.4
20:1	13.2	13.2	15.2	15.3	15.1
22:1	36.7	39.6	17.4	15.8	18.2

glucosinolates in Bronowski may occur further back in the pathway than is suggested by Josefsson (14) for the *B. campestris* species. They are supported by unpublished results from the Saskatoon Research Station (CDA). This linkage (13) has speeded identification of *B. napus* strains combining low glucosinolate content of the seed meal and low erucic acid content in the oil and early maturity (Table IV).

Selection of low glucosinolate strains of *B. campestris* has not been as successful, partly because of the self-incompatibility of most *B. campestris* cultivars and because the blocks in the glucosinolate biosynthesis that have been identified (14) have been located near the end of the pathway. For example, progeny of seeds selected for zero or low concentrations of BI and PI have accumulated the precursors methylthiobutyl- and methylthiopentyl isothiocyanates. It now appears that interspecific crosses between Bronowski or its out-crossed progeny with *B. campestris* will yield the first agronomically accetable low glucosinolate strains of *B. campestris*.

The fatty acid composition of Bronowski oil is unique (Table V). With the exception of Bronowski and the Liho cultivar, all *B. napus* cultivars, prior to 1968, had erucic acid contents greater than 36%. Selection within the Liho cultivar yielded plants whose seed oil contained no erucic acid. Similar selections within Bronowski gave strains with a 7% erucic acid content, but no zero erucic acid plants could be obtained without first crossing a Bronowski plant with a zero erucic acid plant originating from the Liho source (15). It was subsequently shown that instead of four E^a alleles each contributing about 10% erucic acid, Bronowski

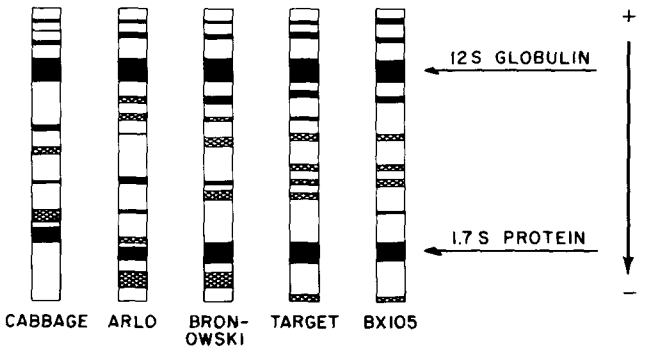

FIG. 1. Reproductions of gel electrophoresis (1 M NaCl, 0.01 M sodium borate extracts of meals) of the saline soluble proteins from *B. oleracea*, cabbage cv. golden acre; *B. campestris*, cv. Arlo; three *B. napus* cultivars, Bronowski, Target and B x 105. Gels were run for 1.5 hr in 0.01 M acetic acid, β-alanine buffer, pH. 4.3.

contained in the *B. oleracea* genome E^d alleles, which contributed 3.5-4% erucic acid to the seed oil. It has been postulated that the remaining 10% of the eruicic acid, in the unselected variety is contributed by a E^a allele present in a heterozygous condition in the *B. campestris* genome (15). This kind of segregation suggests that there has been little or no pairing of chromosomes from the two genomes and that Bronowski or its parent cultivar has been derived from a relatively recent interspecific cross between plants of *B. oleracea* (n=9) and *B. campestris* (n=10). This postulation is supported by the ease with which controlled hybridization may be achieved between plants of the *B. campestris* species and Bronowski. Over 100 such crosses have been made at the Saskatoon Research Station (CDA) and in each case seed was produced; of over 250 seeds sown, all germinated and subsequently produced hybrid plants.

A number of studies (16,17) have been started concerning the physical and chemical properties of the soluble seed proteins in attempts to establish the phylogenetic relationships in plants and, more specifically, to derive information about the protein relationships in the *Brassica* species. For instance, Boulter and coworkers (17) have shown that species within the genus *Vicia* have similar disc gel electrophoresis patterns for the globulin fractions indicating that the proteins in the seed extracts may be closely related although not identical. In the genus *Brassica* similar relationships exist within the proteins. The three *B. napus*

TABLE VI

Amino Acid Compositions of Rapeseed Meals[a]

Amino acid	Arlo	Target	Bronowski	BX 105
Aspartic	0.220	0.180	0.220	0.195
Threonine	0.130	0.140	0.150	0.130
Serine	0.150	0.145	0.170	0.160
Glutamic	0.400	0.605	0.605	0.470
Proline	0.240	0.220	0.230	0.200
Glycine	0.240	0.280	0.270	0.240
Alanine	0.195	0.200	0.195	0.170
Valine	0.170	0.160	0.180	0.120
1/2 Cystine	0.015	0.020	0.035	0.040
Methionine	0.015	0.020	0.020	0.030
Isoleucine	0.130	0.120	0.130	0.105
Leucine	0.195	0.200	0.220	0.185
Tyrosine	0.055	0.050	0.050	0.050
Phenylalanine	0.075	0.090	0.100	0.080
Ammonia	0.570	0.600	0.600	0.495
Lysine	0.160	0.130	0.150	0.210
Histidine	0.080	0.055	0.060	0.070
Arginine	0.110	0.110	0.140	0.140
Total N	3.800	3.895	4.055	3.860
% Recovery	79	81	83	80

[a]mmoles amino acid per gram meal (6.7% N).

TABLE VII

Amino Acid Compositions of 12S Globulins and 1.7S Proteins[a]

	12S Globulin		1.7S Protein	
Amino Acid	Target	Bronowski	Target	Bronowski
Aspartic	0.700	0.740	0.180	0.195
Threonine	0.340	0.330	0.330	0.270
Serine	0.390	0.410	0.550	0.350
Glutamic	1.260	1.490	2.660	2.110
Proline	0.435	0.500	0.915	0.675
Glycine	0.675	0.750	0.560	0.440
Alanine	0.480	0.510	0.485	0.410
Valine	0.435	0.505	0.490	0.430
1/2 Cystine	0.040	0.070	0.110	0.010
Methionine	0.110	0.090	0.080	0.030
Isoleucine	0.330	0.400	0.315	0.250
Leucine	0.585	0.660	0.680	0.500
Tyrosine	0.160	0.150	0.070	0.050
Phenylalanine	0.270	0.290	0.165	0.140
Ammonia	1.130	1.180	1.760	1.750
Lysine	0.270	0.280	0.650	0.540
Histidine	0.120	0.150	0.310	0.260
Arginine	0.350	0.390	0.400	0.315
Total N	9.635	10.635	11.960	10.730
% Recovery	80	92	95	93

[a]mmoles per gram protein (17% N).

cultivars examined (Fig. 1) appear to be similar, having gel patterns more like those of the *B. campestris* proteins than that of *B. oleracea*. There are a number of protein bands found in *B. campestris* that are not present in the *B. oleracea* cultivar; thus the *B. napus* species apparently has inherited these from the *B. campestris* species. There are only minor differences between the three *B. napus* cultivars, and the 12S globulins and 1.7S proteins isolated from them have the same electrophoretic mobilities (Fig. 1). It appears that, if Bronowski originated from a relatively recent cross between *B. oleracea* and *B. campestris* cultivars, the gel patterns of Bronowski would probably approximate an average between the two species and be different from Target. However the two *B. napus* cultivars appear more closely related to each other than to the *B. oleracea* used in this comparison. Hence the gel observations are inconclusive as far as the origin of Bronowski is concerned.

Table VI contains the amino acid compositions of the rapeseed meals used in this study. There is a general similarity between all of them, although there are some significant differences. For instance, the cystine contents of Bronowski x Target cross (B x 105) are greater than that of Target. However the general similarity of the analyses indicate that the meal amino acid analyses are of limited value for differentiating between cultivars, since other analyses (18) have shown that it is difficult to establish trends in meal amino acid compositions. An examination of the amino acid compositions of the 12S globulins and 1.7S proteins isolated from both Target and Bronowski (Table VII) shows there are differences between the amino acid compositions of the two proteins recovered from each cultivar. For instance, the 1.7S protein from Target contains ca. 10 times more cystine than its Bronowski analog. However the 12S globulins have their cystine contents reversed; Bronowski contains more than Target. Other differences are apparent but not so marked. Because of the general similarities in the compositions of proteins that have similar physical properties (the 1.7S proteins from Bronowski and Target), the analyses are of limited value for differentiating between cultivars except where significant differences exist in the amounts of certain amino acids. Furthermore the amino acid compositions of the proteins may show variations that are not reflected in the meal amino acid compositions (Tables VI, VII). Thus the meal amino acid analyses are not accurate indicators of changes in protein amino acid compositions that may have occurred as a result of crossing experiments. It appears, then, that sequencing will be required to establish differences in the structures of seed storage proteins from plants produced in species or cultivar crossing experiments.

It is still not clear whether Bronowski is a chance selection or a result of an intensive breeding effort to improve the seed. The source of the *B. campestris* genes that allows the expression of the low glucosinolate content is also unknown, as is the function of the glucosinolates in the plant.

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