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Approaches to efficient use and to increase the diversity of genetic resources for the development of prospective breeding materials of yellow-seeded *Brassica napus* L. for conditions of Siberia

Problemy użycia i poszerzania zmienności genetycznej rzepaku żółtonasiennego dla otrzymania wyjściowych materiałów hodowlanych *Brassica napus* L. dostosowanych do warunków Syberii

Key words: *Brassica napus* L., canola, yellow seed, genetic resources, genetic diversity, database

The advancement of crop quality of *Brassica napus* L., while keeping its early maturing and high yielding characteristics, requires the efficient use and diversification of genetic resources. Our breeding program utilizes the following approaches to achieve this task: (i) studies on a collection of yellow-seeded species of family *Brassicaceae*, (ii) genetic analysis of the seed coat colour and its relationship with other characters, (iii) increasing diversity of genetic resources through application of hybridization, inbreeding, in vitro propagation and selections, (iv) systematization of breeding material and efficient search for sources of desired breeding characters. The combination of applied approaches made it possible to optimize the breeding process of development of yellow-seeded *B. napus* for the conditions of Siberia, to enhance the diversity of initial material, and to increase the efficiency of search for the sources of desired breeding characters.

Słowa kluczowe: *Brassica napus* L., canola, żółte nasiona, zasoby genetyczne, różnorodność genetyczna, baza danych

Rzepak *Brassica napus* L. jest najważniejszą rośliną oleistą uprawianą w ekstremalnych warunkach klimatycznych Syberii.

Zachowanie wysokiej jakości plonu nasion w sytuacji krótszego terminu jego dojrzewania wymaga stosowania różnych metod hodowlanych oraz poszerzenia zasobów genetycznych.

Program badań został podzielony na następujące zadania:

- (I) Poznanie i gromadzenie żółtonasiennych gatunków z rodziny *Brassicaceae* (*B. campestris*, *B. juncea* i *Sinapis alba*) z pól o prowokacyjnych warunkach. Selekcję prowadzono pod kątem: wcześniejszego dojrzewania, wysokiego plonu nasion, odporności na choroby. Badania te dotyczyły form, które są aktualnie używane w programach hodowlanych.
- (II) Dla optymalizacji hodowli żółtonasiennych form zastosowano analizę genetyczną, tej cechy oraz jej współzależność z innymi cechami.

- (III) Poszerzono zakres różnicowania genetycznego stosując następujące metody: krzyżowanie, chów wsobny, rozmnażanie *in vitro* i hodowlę. Prace te zapoczątkowano, ponieważ na Syberii używano wyłącznie odmian *B. napus* o różnobarwnej okrywie nasiennej.
- (IV) Opisano systematycznie zgromadzony materiał hodowlany oraz przeprowadzono efektywne poszukiwania materiałów które mogłyby być źródłami pożądanych cech. Aby realizować wyznaczone cele, utworzono syberyjską bazę danych dla rzepaku żółtonasiennego (Siberian Yellow-seeded *Brassica* Database SBDB), która obecnie liczy 1813 dostępnych danych. Dane zapisywano w standardowych lub niestandardowych polach (7 lub 14). Zapis niestandardowy dotyczył nowych form hodowlanych.

Po użyciu wielu kombinacji w badaniach, prace przyczyniły się do wytworzenia nowych materiałów wyjściowych przeznaczonych do dalszej hodowli. Zastosowane metody pozwoliły powiększyć zakres zmienności genetycznej materiału, polepszyć skuteczność w poszukiwaniu pożądanych źródeł hodowlanych. Powyższe prace podjęto w celu wytworzenia nowych perspektywicznych żółtonasiennych form *B. napus* odpowiednich dla surowych warunków klimatycznych Syberii.

Introduction

Summer rapeseed (*Brassica napus* L.) is a primary oilseed crop grown in Siberia. The advancement of the crop quality, while maintaining its early maturing and high yielding characteristics along with resistance to the extreme climatic conditions of the region, requires the development of new, prospective breeding material. The efficient use and diversification of available genetic resources is an essential part of this research.

The character of yellow seed coat is of high importance in the breeding of *B. napus* because it is associated with the improvement of feed value of meal and the increase of oil content in seeds (Shirzadegan, Röbbelen 1985; Simbaya et al. 1995; Marles, Gruber 2004). Naturally occurring germplasm of *B. napus* has black seed coat. A range of interspecific crosses has been used to enhance the diversity of available genetic resources. The character of yellow seed coat was transferred to *B. napus* from related species, such as *Brassica campestris* L. (syn. *Brassica rapa* L.), *Brassica juncea* (L.) Cern., and *Brassica carinata* Braun, the species had been used in different cross combinations (Chen et al. 1988; Meng et al. 1988; Rashid et al. 1994; Tang et al. 1997; Rahman 2001). By now the yellow-seeded forms of *B. napus* have been obtained. As a rule, the seed yield of these forms does not exceed that of standard cultivars but the protein and oil content of some of them display higher values (Liu, Gao 1987; Shpota, Bochkaryova 1990; van Deynze, Pauls 1994; Chen, Heneen 1992; Baetzel et al. 1999; Rahman et al. 2001; Relf-Eckstein et al. 2003; Piotrowska et al. 2003; Zhi-wen et al. 2005). In spite of ongoing research carried out for several decades in different countries, the problem of the development of stable seed colour and productivity of yellow-seeded *B. napus* cultivars remains unsolved.

The aim of the present study was to optimize the breeding process for the development of yellow-seeded *B. napus* suitable for cultivation in Siberia. Our

breeding program utilized the following approaches to achieve this task: (I) studies on a collection of yellow-seeded species of family *Brassicaceae*, (II) the genetic analysis of the seed coat colour and its relationship with other characters, (III) increasing the diversity of available genetic resources through the application of the methods of hybridization, inbreeding, *in vitro* propagation and selections, (IV) systematization of breeding material and efficient search for the sources of desired breeding characters.

Results

I. Studies on a collection of yellow-seeded species of family *Brassicaceae*

Specific agro-climatic conditions of Siberia create significant problems in the breeding of summer rapeseed. The conditions are: continental climate with large fluctuation in daily, seasonal, and annual temperatures, severe winter unfavourable for growing of winter rapeseed, short vegetation season with late-spring and early-fall frost, irregular time-distributed rainfall in the course of vegetation season, drought at the beginning and abundant humidity at the end of vegetation season that frustrate harvesting of late-maturing *B. napus* cultivars, a variety of soil types and unbalance of the elements of their fertility. Such conditions require the development of cultivars that display both high quality and stable yield of seeds.

The collection of 258 accessions of the following four species of family *Brassicaceae* has been evaluated: *B. napus*, *B. campestris*, *B. juncea* and *Sinapis alba* L. from germplasm collection of N.I. Vavilov Institute of Plant Industry in St. Petersburg, Russia and material received from other Russian and foreign breeding institutions. Light-seeded samples were selected and studied under provocative field conditions in different years and at various locations. The results of tests on 55 selected accessions of Russian and foreign origin (9 countries) indicated that among all studied species the earliest maturing species were *B. campestris* and *S. alba*. They matured 5 to 21 days earlier than *B. napus* (cv. Shpat and SibNIIK 198) but had lower yield than *B. napus*. Accessions of *B. juncea* matured 3 to 5 days later than *B. napus* but had higher seed yield. These species were employed in crosses with *B. napus* as sources of the traits of yellow seed coat colour and/or early maturing. The results of our studies on the seed yield and vegetation period of the light-seeded samples are listed in Figure 1 where the values are compared with the corresponding values for dark-seeded standards.

The main result of evaluating the accessions of the collection was the selection of several forms utilized further to transfer the yellow seed coat character to *B. napus* through interspecific crosses.

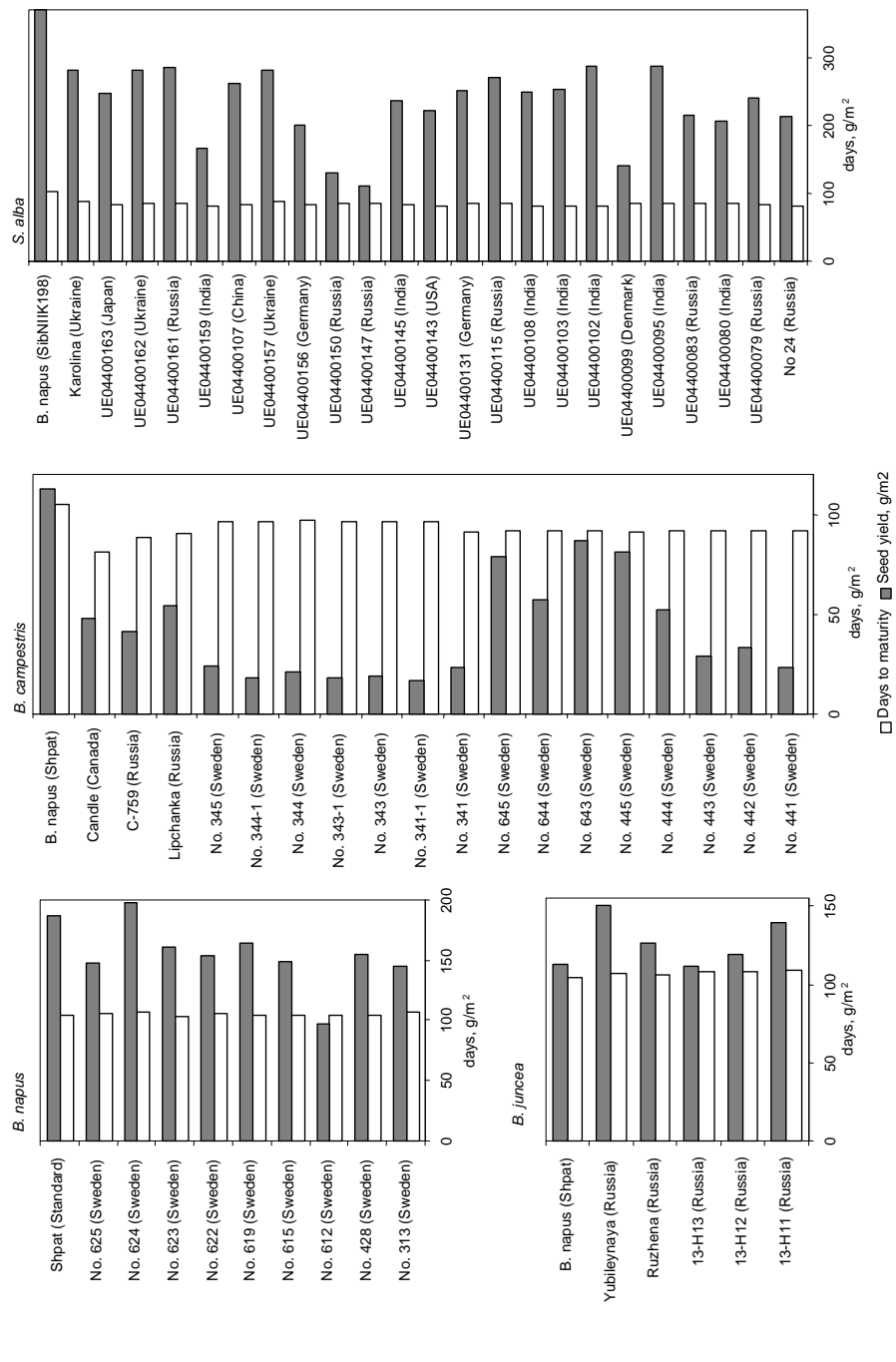


Fig. 1. Results of studies on a collection of yellow-seeded species of family Brassicaceae — Liczba dni do dojrzalosci roślin oraz plon nasion z 1 m² dla żółtonasiennych form Brassicaceae

II. Genetic analysis of the seed coat colour and its relationship with other characters

Our studies showed that the inheritance of the seed coat colour is controlled by two or three genes. The colour of light-pigmented seeds may vary from yellow to light-brown, including yellow with darker coloured spots, shades and hilum. To some extent these colour variations may be caused by meteorological factors. However, the major role is most probably played by genes-modifiers whose influence varies and changes with environmental conditions (Osipova and Potapov 1996). Some hybrid combinations were revealed with the segregation of seed colour not corresponding to the theoretically expected ratios of 15 : 1 and 63 : 1; it was impossible to determine the type of inheritance in these combinations. The material in which seed coat colour is controlled by three genes was found to be more stable through a number of generations than a material in which seed coat colour is controlled by two genes. For example, in line No. 39 seed coat colour was controlled by three genes and the variability of the character in six generations of inbreeding was lower in comparison with other lines (No. 283, 361) in which two genes were involved in the determination of seed colour. In general, the inheritance and variability of seed coat colour substantially depended on individual features of the initial material.

The cluster analysis of 99 light-seeded lines evaluated during 1996–1999 allowed us to find a relationship among 17 agronomic and morphological characters and to identify several groups of related characters (Fig. 2). The analysis was based on maximum coefficients of correlation between the characters. At a low level of correlation ($r = 0.3$) three groups were revealed (I–III, Fig. 2). At a higher level of correlation ($r = 0.7$) group I split into four groups (A–D, Fig. 2) and group II included only two characters: weight of 1000 seeds and volume of seed. Thus, a pleiotropic effect of genes on genetic control of the studied characters cannot be excluded.

III. Increasing the diversity of available genetic resources

In our studies, increasing the diversity of genetic resources was achieved by applying several methods including interspecific hybridization, inbreeding, *in vitro* propagation, and selections. As a result, diversified initial and breeding material of *B. napus* was created with a variety of seed coat colours (Potapov, Osipova 2003).

The tests of this material at different stages of the breeding process revealed a wide range of volumes within basic agronomic and morphological characters. Studies on the variability of these characters demonstrated higher variability of yellow-seeded lines as compared to black-seeded lines. Some yellow-seeded forms had higher values of seed yield and the seed yield components than black-seeded forms (Table 1). From the developed material, early maturing forms

Table 1

Agronomic and morphological characters for light- and dark-seeded *Brassica napus* lines (1996–1999)
Agronomiczna i morfologiczna charakterystyka linii jasno i ciemnonasiennych rzepaku Brassica napus L. (1996–1999)

Characters <i>Charakterystyka</i>	Light-seeded lines (116 lines) <i>Linie jasnonasienne (116 linii)</i>				Dark-seeded lines (84 lines) <i>Linie ciemnonasienne (84 linii)</i>			
	average <i>średnia</i>	range <i>zakres</i>	coefficient of variation <i>współczynnik zmienności</i> [%]		average <i>średnia</i>	range <i>zakres</i>	coefficient of variation <i>współczynnik zmienności</i> [%]	
Seed yield per plant — <i>Plon nasion z rośliny</i> [g]	46.6 ± 5.5	2.4 ÷ 272.1	127		49.6 ± 2.6	9.4 ÷ 133.5	48	
Number of pods per plant — <i>Ilość łuszczyń z rośliny</i>	367 ± 42	62 ÷ 1767	80		387 ± 17	88 ÷ 785	40	
Number of seeds per pod — <i>Ilość nasion z łuszczyzny</i>	25 ± 1*	12 ÷ 39	25		28 ± 1	18 ÷ 37	14	
Number of pods per mainstem <i>Ilość łuszczyń na gałązce</i>	225 ± 17*	21 ÷ 809	80		148 ± 6	45 ÷ 314	39	
Weight of 1000 seeds — <i>Masa 1000 nasion</i> [g]	2.7 ± 0.1*	1.4 ÷ 5.6	40		4.3 ± 0.1	2.3 ÷ 6.0	16	
Length of pod — <i>Długość łuszczyzny</i> [cm]	5.8 ± 0.1*	4.1 ÷ 8.0	16		6.7 ± 0.1	5.1 ÷ 8.1	12	
Length of beak — <i>Długość dzioba</i> [cm]	0.7 ± 0.1	0.4 ÷ 1.0	20		0.8 ± 0.1	0.5 ÷ 1.2	22	
Volume of seed — <i>Objętość nasion</i> [mm ³]	2.2 ± 0.1*	1.2 ÷ 4.2	37		3.8 ± 0.1	2.4 ÷ 4.9	23	
Number of first-order branches per plant <i>Liczba pierwszych rozgałęzień na roślinie</i>	6 ± 0.1*	2 ÷ 9	28		5 ± 0.2	2 ÷ 8	29	
Number of second-order branches per plant <i>Liczba następných rozgałęzień na roślinie</i>	14 ± 0.4*	5 ÷ 24	29		12 ± 0.3	7 ÷ 23	31	

ciąg dalszy tabeli 1

Characters <i>Charakterystyka</i>	Light-seeded lines (116 lines) <i>Linie jasnonasienne (116 linii)</i>			Dark-seeded lines (84 lines) <i>Linie ciemnonasienne (84 linie)</i>		
	average <i>średnia</i>	range <i>zakres</i>	coefficient of variation <i>współczynnik zmienności</i> [%]	average <i>średnia</i>	range <i>zakres</i>	coefficient of variation <i>współczynnik zmienności</i> [%]
Height of plant — <i>Wysokość roślin [cm]</i>	92 ± 1.7*	54 ÷ 129	20	104 ± 2	76 ÷ 140	12
Height of mainstem — <i>Długość gałązki [cm]</i>	68 ± 1*	40 ÷ 110	22	75 ± 2	45 ÷ 120	18
Height to the first branching [cm] <i>Długość pierwszego odgałęzienia [cm]</i>	30 ± 1*	8 ÷ 60	42	34 ± 1	20 ÷ 55	21
Seedling-flowering [days] <i>Od siewki do kwitnącej rośliny [dni]</i>	40 ± 1	36 ÷ 45	7	39 ± 1	32 ÷ 47	10
Seedling – maturing [days] <i>Od siewki do dojrzałej rośliny [dni]</i>	94 ± 1*	73 ÷ 111	12	98 ± 1	88 ÷ 106	6
Flowering – maturing [days] <i>Od kwitnącej rośliny do dojrzałej [dni]</i>	53 ± 1*	30 ÷ 75	20	61 ± 1	51 ÷ 79	20
Flowering [days] — <i>Kwitnienie [dni]</i>	25 ± 1*	16 ÷ 33	21	29 ± 1	17 ÷ 45	38

* The t-test indicates that the averages for light- and dark-seeded lines are different at 1% level
T-test, różny na poziomie 1% dla linii jasno- i ciemnonasiennych

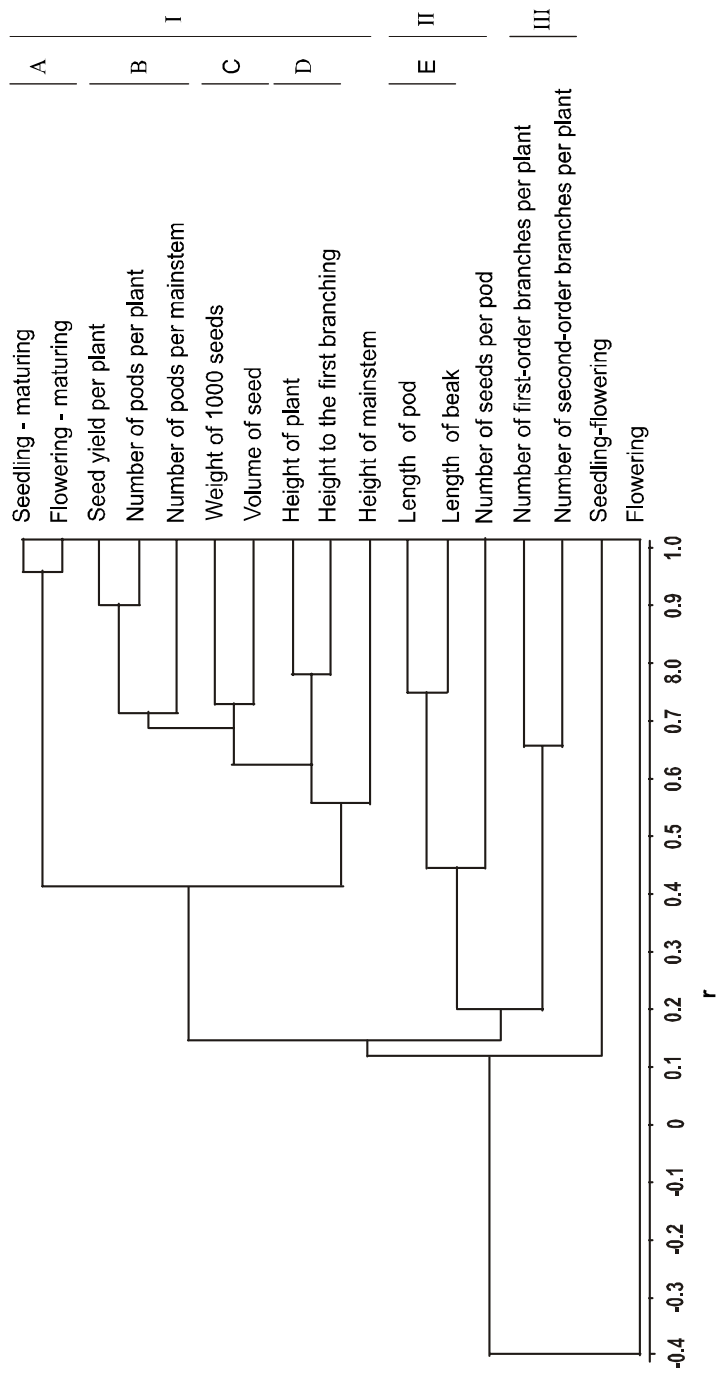


Fig. 2. Cluster dendrogram of quantitative characters of inbred lines of yellow-seeded *B. napus* (r is the coefficient of correlation)
Dendrogram ilościowych cech ilościowych dla badanych cech linii żółtonasiennego rzepaku (r — współczynnik korelacji)

suitable for cultivation in the extreme conditions of West Siberia were selected; some of these forms had higher seed yields than a black-seeded check cultivar SibNIIK 198 (Potapov, Osipova 2004).

Cluster analysis based on minimum euclidean distances was conducted on 99 lines for 17 agronomic and morphological characters. The analysis confirmed enhanced diversity of the material designed in our studies and made it possible to allocate eight groups of phenotypically homogeneous lines (Fig. 3).

In general, the results of clustering were in good agreement with known pedigree relationships. However, some of the groups comprised phenotypically indistinguishable lines which originated from different genetic sources. Group I consisted of inbred lines derived from Swedish forms No. 615 and No. 624. Groups II and V contained inbred lines derived from crosses between *B. napus* and *B. campestris* as well as the inbred line derived from the form No. 615. Group III comprised inbred lines from crosses *B. napus* × *S. alba*. Groups IV, VI and VIII included inbred lines from No. 615. Group VII was represented by inbred lines of hybrid pedigree *B. napus* × *B. juncea*.

Thus, the applied set of methods allowed us to create a diversified germplasm of summer rapeseed with various colours of seed coat and suitable for the conditions of Siberia.

IV. Systematization of breeding material and efficient search for the sources of desired breeding characters

Selection, evaluation and comprehensive analysis of the initial and breeding material may be greatly facilitated by the use of information technologies. Databases proved to be especially useful as a part of successful breeding programs (Stavelikova et al. 2002; Boukema et al. 2003). They intensify information retrieval, improve systematization and rationalization of available data and, eventually, make it possible to substantially optimize the breeding process.

The development of Siberian Yellow-seeded Brassica Database (SBDB) was started at the Siberian Research Institute of Fodder Crops in 2002 (Osipova and Potapov 2003). By now, two passport databases have been created that account for the acquired and developed breeding material at the Institute.

The Breeding Material Database and the Initial Material Database comprise data listed in 7 and 14 fields of passport descriptors, respectively. The descriptors comply with a standard multi-crop passport descriptor list developed by IPGRI (2001). In addition, non-standard descriptors have been developed in order to account for the specificity of the breeding material. The current version of SBDB retains 1813 accessions (Table 2).

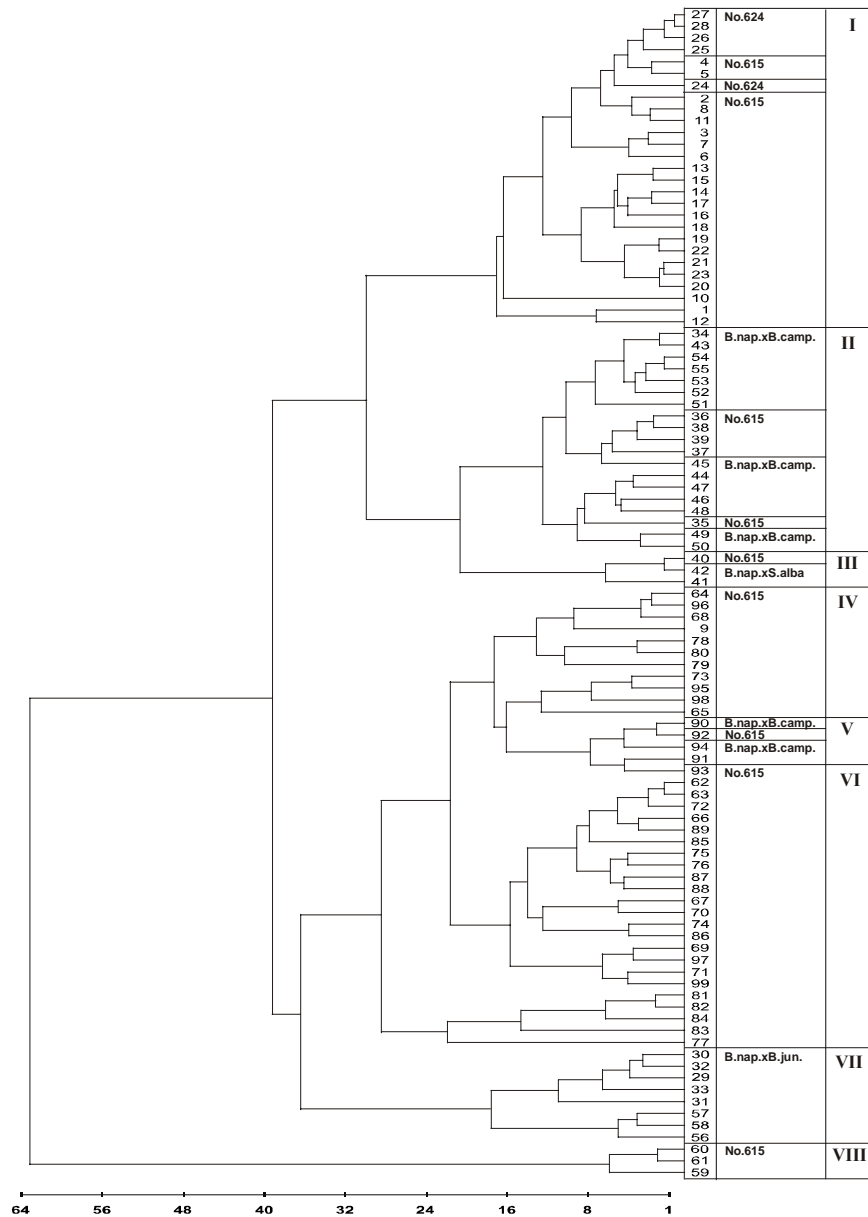


Fig. 3. Cluster dendrogram of inbred lines of yellow-seeded *B. napus* evaluated during 1996–1999 for 17 quantitative characters. On the horizontal axis the normalized distances are given; vertical columns – groups (Roman numbers), pedigrees, lines (Arabic numbers) — *Dendrogram linii żółtonasiennego rzepaku B. napus dla 17 cech ilościowych (ocena w latach 1996-1999). W kolumnach pionowych zapisano: grupy (rzymskie numery), rody lub linie (arabskie numery)*

Table 2
The contents of Siberian Yellow-seeded Brassica Database subdivided by the seed coat colour of the samples — *Zawartość bazy danych dla syberyjskich żółtonasiennych form Brassica*

Seed coat colour — <i>Kolor okrywy nasiennej</i>	Number of accessions <i>Liczba obiektów</i>
Light-yellow — <i>Jasno-żółte</i>	29
Yellow — <i>Żółte</i>	734
Light-brown — <i>Jasno-brązowe</i>	711
Brown — <i>Brązowe</i>	41
Dark-brown — <i>Ciemno-brązowe</i>	14
Light-ochre — <i>Jasna ochra</i>	10
Ochre — <i>Ochra</i>	36
Dark-ochre — <i>Ciemna ochra</i>	10
Black — <i>Czarne</i>	228
Total — <i>Razem</i>	1813

Conclusion

The combination of approaches applied in our research proved to be fruitful for the development of new breeding material and for the optimization of the breeding process.

The utilized strategy made it possible to increase the diversity of initial material, to improve the efficiency of search for the sources of desired breeding characters and to create new prospective yellow-seeded forms of *B. napus* suitable for specific conditions of Siberia.

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