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Note

Genetic variation of storage compounds and seed weight in rapeseed (*Brassica napus* L.) germplasms

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One thousand five hundred rapeseed accessions were collected from gene banks in several countries and surveyed for their seed traits such as oil content, protein content, fatty acid composition and seed weight. Seed chemicals were analyzed by near-infrared reflectance spectroscopy (NIRS). An extensive genetic variation existed among accessions for all seed traits examined. The oil content ranged from 24.7 to 56.9%, and the protein content ranged from 6.7 to 36.6%. A hundred seed weight ranged from 0.13 to 0.70 g. The fatty acid compositions of germplasms also varied from 2.6 to 9.2% of C16:0 (palmitic acid), 0.3 to 70.2% of C18:1 (oleic), 10.3 to 28.9% of C18:2 (linoleic), 4.8 to 15.3% of C18:3 (linolenic), 0 to 10.5% of C20:1(eicosenoic) and 0 to 59.6% of C22:1 (erucic). The oil content had strong negative correlation with protein content ($r = -0.848$). However, it did not have any close correlations with other traits. When the genetic stability of seed traits was examined by comparison between original seeds and self-pollinated seeds (S_1 seeds), higher positive and significant correlation coefficients between original seeds and S_1 seeds were found in all seed traits.

Key Words: rapeseed, genetic variation, storage compounds, seed weight, NIRS.

Introduction

Rapeseed/canola (*Brassica napus* L.), which is cultivated in temperate zones, has become an important agricultural crop in the world over the last thirty years and is now the world's third leading source of vegetable oil. Rapeseed has become valued for its diverse range of uses. Rapeseed is used for two main products: oil and meal. The breeding for seed quality has contributed to improvement in edible oil and meal, resulting in the development of 'canola' type rapeseeds with zero or low erucic acid and low glucosinolate. Besides its use as a highly nutritional food oil and the use of residues after oil extraction for livestock feed, rapeseed oil also provides industrial lubricants, soap, biodegradable plastics, and biodiesel fuel (Kimber and McGregor 1995). For the utilization of biodiesel as a substitute for fossil diesel fuel, increasing oil content in seeds is an important object of rapeseed breeding. Genetic control of seed substances such as oil and protein content could help the breeder to improve this crop. Although some QTLs and genes involved in content of seed substances have been reported (Delourme *et al.* 2006, Li *et al.* 2006, Zhao *et al.* 2008), little is known about the key factor(s) related to oil content. On the other hand, genetic resources are the basis for the improvement of crop cultivars. *Brassica* crops pass through an extensive differentiation into an abundance of morph-, physio- and chemotypes because of the cultivation in many countries and their worldwide distribution (Becker *et al.* 1999). A better knowledge of genotypic variations of seed substances such as oil and protein content and fatty acid composition of rapeseed could help the breeder to improve this crop.

In order to improve the quantity and quality of seed oil, it is essential to be able to distinguish the genetic variation of storage compounds. However, conventional chemometric analysing procedures in oil and protein are very complicated, time-consuming, and destructive. Near-infrared reflectance spectroscopy (NIRS) has been widely used as a fast and non-destructive alternative analytical tool of both raw material and final products in the food and agricultural industries. The quantitative and qualitative traits of seeds have been analyzed using this method in many crops of cereals and oilseeds (Charron *et al.* 2005, Weinstock *et al.* 2006). In

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rapeseed, several researchers reported that NIRS using intact-seeds is useful to estimate seed components from the viewpoint of validity of NIRS analysis on seed compositions (Font *et al.* 2006, Sato 2008, Velasco and Becker 1998), single seed (Sato *et al.* 1998, Velasco *et al.* 1999, Velasco and Mollers 2002), and small seed samples (Hom *et al.*, 2007).

The objectives of this study were to clarify the variability of seed weight and seed chemicals such as oil, protein and fatty acids contents using NIRS on 1500 rapeseed accessions collected from various gene banks, and the relationships among their seed traits. In addition, the genetic stability on such seed traits was investigated by comparing collected seeds with self-pollinated next generation seeds.

Materials and Methods

Plant materials

One thousand five hundred and forty one rapeseed (*B. napus*) accessions were collected from 10 research institutions worldwide (Table 1). Due to contamination of other species except for *B. napus* and small amounts of seeds, of these materials, 1500 accessions were analyzed for seed traits such as oil content, protein content and fatty acid compositions, and 1457 accessions were analyzed for 100 seeds weight. Thirty-eight accessions randomly selected from the germplasm and four plants of each accession were grown in a greenhouse and self-pollinated to obtain seeds (S_1 generation). In order to understand the genetic stability of the examined traits, the same seed traits of these S_1 seeds were analyzed.

Analysis of seed traits

Oil content, protein content and six fatty acid compositions were determined by NIRS. Approximately 500 mg of seed samples of each accession were used for the NIRS analysis using NirFlex N500 (Büchi, Swiss). In order to make calibration equations in oil, protein and fatty acids of seeds, seeds of randomly selected accessions were analyzed as follows: Fatty acid compositions were analyzed by gas-chromatography according to the method of Bligh and Dyer (1959), and the oil content was measured by gas-chromatography of fatty acid methyl esters using pentadecanoic acids as an internal standard (Bening and Somerville 1992). As for protein analysis, seeds were oven-dried at 70°C and then ground to a fine powder for analysing the N concentration. Total N content of the sample was determined by Kjeldarl method. The protein content of the seed was estimated by multiplying the determined total N content by a N-to-protein conversion factor, 6.25. One hundred seed weight of each accession was measured by precision balance.

Table 1. List of genetic resources and examined number of accessions of rapeseed

Genetic resources (Country)	No. of accessions
NIAS Genebank (Japan)	665
NARCT (Japan)	49
Lab. of Plant Breeding, Tohoku University (Japan)	36
Lab. of Plant Breeding, Iwate University (Japan)	31
Dr. Sakai (Japan)	6
CGN; Centre for Genetic Resources, the Netherlands (Netherlands)	179
NBG; Nordic Gene Bank (Sweden, Denmark, Finland)	122
NPGS; USDA-National Plant Germplasm System (USA)	306
PGRC; Plant Gene Resources of Canada (Canada)	145
Monsanto SAS (France)	2
Total	1541

Statistical analysis

Statistical analysis was carried out utilizing the computer program JMP 8.0 (SAS Institute Inc.).

Results and Discussion

Seed traits

Seeds of 1500 collected accessions displayed a variation of seed colors, that is, black, brown and yellowish brown. A hundred seed weight of these accessions varied from 0.13 g of 'Austria 3' to 0.70 g of 'Quing you 2', and were normally distributed with a mean of 0.4 g of seed weight (Table 2 and Fig. 1). No correlation between seed color and weight was found (data not shown).

The oil and protein contents and fatty acid compositions of seeds measured by NIRS are shown in Table 2. The oil contents of the 1500 accessions varied from 24.7% to 56.9% and were normally distributed with a mean of 43.3% (Fig. 1). Of these accessions, eighty percent accessions were in the range between 35 and 50% of oil contents and 85 accessions (5.5% of germplasm) showed oil content of more than 50%. Such high oil content accessions might be a source for increasing oil. Bhardwaj and Hamama (2000) reported 29.6–49.2% of oil contents in 455 accessions of *B. napus*. Snowdon *et al.* (2007) mentioned that the oil content ranged from 36 to 50%. Our results also confirmed these findings, though a wider range of variation was revealed.

Protein contents of the accessions varied from 6.7% to 36.6% with a mean of 24.6% (Table 2). Our mean value is similar to the results of Becker *et al.* (1999) who reported an average of 22.5% of protein content in rapeseed seed.

As shown in Table 2, six fatty acids, which were determined by NIRS, varied from 2.6 to 9.2% on C16:0 (palmitic acid), 0.3 to 70.2% on C18:1 (oleic), 10.3 to 28.9% on C18:2 (linoleic), 4.8 to 15.3% on C18:3 (linolenic), 0 to 10.5% on C20:1 (eicosenoic) and 0 to 59.6% on C22:1 (erucic). Of these fatty acids, erucic acid and oleic acid showed extensive variations. This is due to the fact that our germplasm contained zero and high erucic acid cultivars/lines. The accessions collected were categorized into three groups based upon amounts of erucic acid, that is, low or zero erucic acid rapeseed (less than 2% erucic acid), high erucic acid rapeseed (ca. 50% erucic acid), and intermediate type. Of the

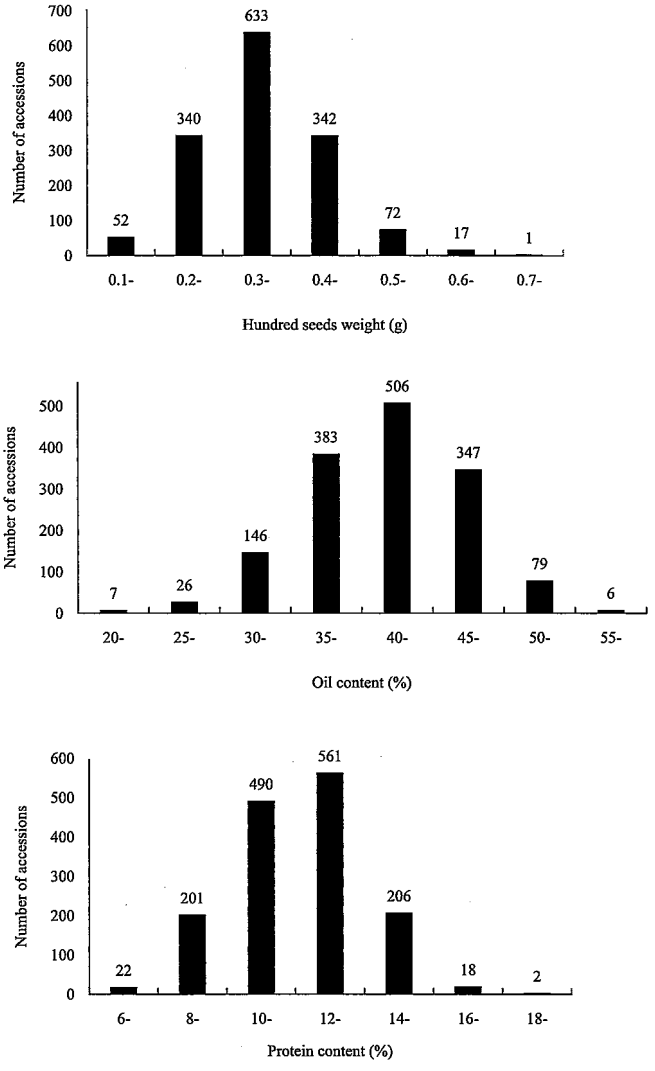

Fig. 1. Frequency distributions of seeds weight, oil content and protein content for rapeseed accessions examined.

accessions, 68.9% were high erucic acid type, 22.0% were zero or low erucic acid type, and 9.1% were intermediate type. The calibration equation showed negative erucic acid values for some accessions which were zero erucic acid cvs. These negative values were interpreted as NIRS errors defined as differences between estimated and actual values (Velasco *et al.* 1999). Zero or low erucic acid and high oleic acid cultivars have been bred for edible oil from old rapeseed cultivars, which contain high erucic acid. In contrast, higher erucic acid cultivars are used for industrial feedstock.

Table 2. Genetic variation of seed traits in 1500 rapeseed accessions

Seed traits	Range	Mean
Hundred seed weight (g)	0.13–0.70	0.4
Oil content (%)	24.7–56.9	43.3
Protein content (%)	6.7–36.6	24.6
Palmitic acid C16:0 (%)	2.6–9.2	6.7
Oleic acid C18:1 (%)	0.3–70.2	30.0
Linoleic acid C18:2 (%)	10.3–28.9	15.9
Linolenic acid C18:3 (%)	4.8–15.3	8.2
Eicosenoic acid C20:1 (%)	0–10.5	6.3
Erucic acid C22:1 (%)	0*–59.6	31.7

* Included minus value.

Accessions of with highest contents of oil and important two fatty acids, oleic acid and erucic acid, and heaviest seed weight identified in this study are shown in Table 3.

Relationships among seed traits

Fig. 2 shows the correlation coefficients among seed traits analyzed. Seed weight was not correlated with all seed storage components ($r = -0.200 - 0.274$). A strong negative correlation existed between oil and protein content ($r = -0.848$). Such a negative correlation between oil and protein contents in seeds has been widely reported in rapeseed (Grami *et al.* 1977) and soybean (Brim and Burton 1979, Charron *et al.* 2005), suggesting that seed oil increases at the expense of protein content causing competition of carbon source. Both oil and protein contents did not show the high positive and negative correlations with each fatty acid content, though oil content slightly positively correlated with oleic acid ($r = 0.382$). Such positively relationships between oil content and oleic acid were reported in Arabidopsis (Hobbs *et al.* 2004) and rapeseed (Zhao *et al.* 2008). Zheng *et al.* (2008) reported that oil content and oleic acid are controlled by DGAT1, which is considered a key determinant of oil and oleic acid contents in maize.

Among fatty acid composition, the highest negative correlation was found between oleic acid and C20–C22 fatty acids, eicosenoic acid ($r = -0.935$) and erucic acid ($r = -0.948$). On the other hand, the highest positive correlation coefficient was found between eicosenoic acid and erucic acid ($r = 0.900$). Palmitic acid was positively correlated with C18 fatty acids (oleic, linoleic and linolenic acids) ($r = 0.659 - 0.775$). Generally, there were negative correlations which

Table 3. List of top five accessions with high contents of three seed compositions and heavy seed weight in rapeseed germplasm examined

Oil content	Oleic acid content	Erucic acid content	100 seed weight
Heimer (NBG)	Andor (NIAS)	Weibulls 506 (NIAS)	Quing you 2 (NIAS)
Teddy (NBG)	Doubleol (CGN)	Ka 322 (NIAS)	Everest (NBG)
Barsica (NPGS)	Lergo (NIAS)	Minami Kyushu 5 (NIAS)	Ning you 8 (NIAS)
Chang (NBG)	Booster (NBG)	Regal (NIAS)	Tohoku 79 (NIAS)
Impala (NBG)	Collo (CGN)	Nu51627 (NPGS)	202-23 (NIAS)

( ): Genetic resources. They were referred to Table 1.

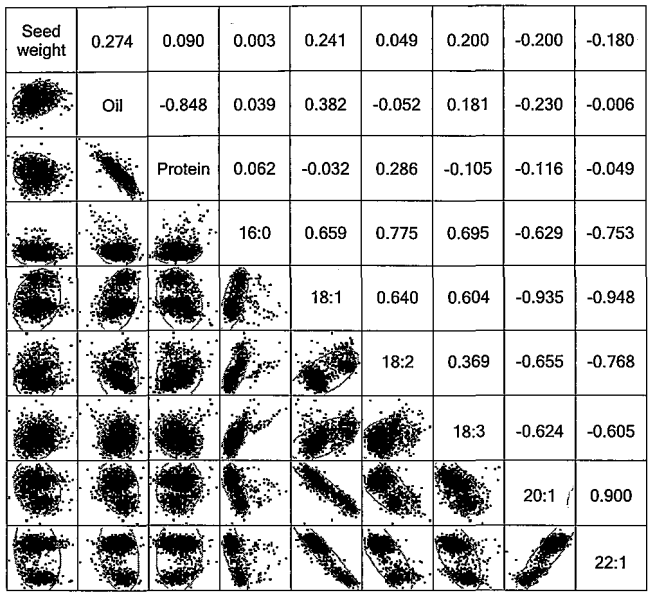

Fig. 2. Relationships among 9 seed traits examined in 1500 rapeseed accessions. Figures of the upper right show correlation coefficients among seed traits. The lower left shows 36 scatter diagrams of 1500 accessions among seed traits. The correlation coefficients between 100 seeds weight and other seed traits were examined in 1457 accessions.

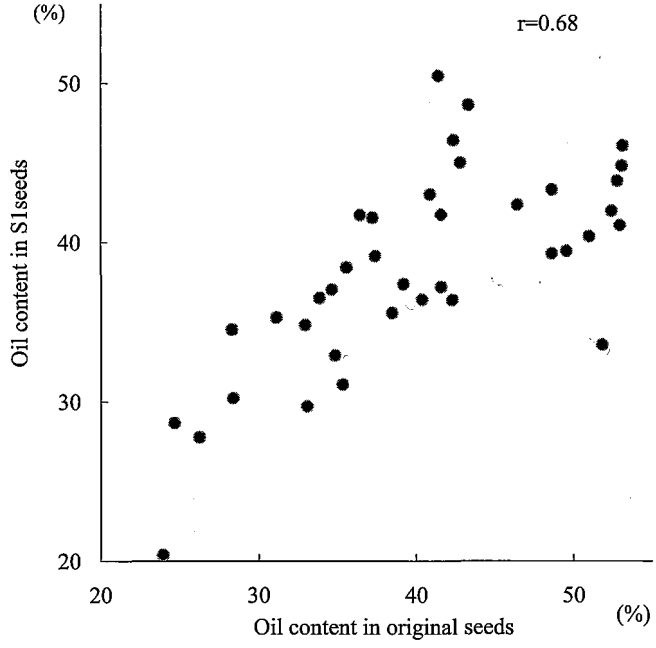

Fig. 3. Relationship of oil content between original seeds and S_1 seeds in 38 rapeseed accessions.

existed between C16-18 fatty acids and C20-22 ones. Kumar and Tsunoda (1980) found negative correlations between the long-chain fatty acids and the C16-18 fatty acids. It is known that oleic acid is the main precursor for erucic acid biosynthesis via an elongation process in the developing seed of rapeseed. Our results showed no contradiction with the pathway in fatty acid biosynthesis.

Genetic stability of seed traits

Using thirty-eight accessions randomly selected from the germplasm collected, the genetic stability of seed traits was examined by comparing the original seeds with the S_1 seeds. Higher positive and significant correlation coefficients between the original seeds and S_1 seeds are shown in all seed traits (Table 4). Correlation coefficients of seed weight ($r = 0.611$), oil content ($r = 0.677$) and protein content ($r = 0.689$) were generally lower than those of fatty acids ($r = 0.976 - 0.680$). These results are due to the fact that the former traits are quantitative traits, which are affected by not only genetic factor(s) but also environmental effects (Mendham and Salisbury 1995). For example, high oil content lines of original accessions did not show such a high oil content in S_1 generation, although low oil content lines showed low oil content (Fig. 3). It was reported that oil content of the plants grown in the greenhouse was lower than that in the field (Weselake *et al.* 2008). Our results are in agreement with this report. Higher correlations of each fatty acid are considered to be due to the large effect of genetic control on biosynthesis of fatty acids.

A large genetic variation in storage compounds and seed weight was revealed in 1500 accessions of rapeseed germplasm. Maximum and minimum values of seed storage compound were sometimes beyond those of previous reports. This may be interpreted as due to NIRS errors between estimated and actual values or considered to be a species-identification error of accessions collected as *B. napus*. When we grew a part of these accessions, we found misidentified materials as other species such as *B. rapa* and *B. juncea*. Of course, we excluded such materials from our analysis, however, it could be not denied that a very few other species exist besides *B. napus* in germplasm analyzed. Although there is some incorporation of such materials, the present data should be useful for rapeseed breeding in improving quantity and quality of seeds as well as genetic studies of traits examined. In order to identify the functional differences between high oil content and low oil content lines, gene expression analysis using microarray analysis is currently being carried out.

Table 4. Correlation coefficients between original seeds and S_1 seeds in each trait

Seed weight	Oil	Protein	Palmitic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid	Erucic acid
0.611**	0.677**	0.689**	0.804**	0.864**	0.788**	0.718**	0.680**	0.976**

** Significant at 1% level.

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