Theor Appl Genet (2012) 125:1275–1287
DOI 10.1007/s00122-012-1912-9

ORIGINAL PAPER

Seedling development in a *Brassica napus* diversity set and its relationship to agronomic performance

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Received: 21 March 2012 / Accepted: 5 June 2012 / Published online: 11 July 2012

Abstract *Brassica napus* L. is the leading European oilseed crop and has therefore a high economical importance. The objectives of our study were to examine (1) the patterns of phenotypic diversity in a species-wide *B. napus* germplasm set of 518 inbreds with respect to various seedling development, agronomic, and seed quality traits as well as (2) the interrelationship of the examined traits and their use in selection on correlated traits. The *B. napus* germplasm set was evaluated in greenhouse and field trials for several seedling development, agronomic, and seed quality traits. The traits were highly correlated within the individual trait categories and moderately correlated between the different trait categories. We observed differences in phenotypic diversity among the examined eight germplasm types. The reduction of phenotypic diversity was on average more pronounced for the seedling development traits than for the agronomic and seed quality traits, suggesting that plant breeders need to introgress new genetic variation with respect to the former.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-012-1912-9) contains supplementary material, which is available to authorized users.

Communicated by C. Quiros.

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Introduction

Brassica napus L. is the leading European oilseed crop (European Commission 2010). Its oil is used as green fuel, for human consumption, for feeding animals, and in the chemical and pharmaceutical industry (Friedt and Snowdon 2009). *B. napus* most likely originated from interspecific hybridizations between genotypes of turnip rape (*Brassica rapa* L.; AA, $2n = 20$) and cabbage (*Brassica oleracea* L.; CC, $2n = 18$) that occurred spontaneously during medieval times times or earlier (Iñiguez Luy and Federico 2011).

The few hybridization events together with the occurrence of two bottlenecks during *B. napus* breeding led to a low level of diversity in modern elite varieties (Becker et al. 1995). Various studies examined this aspect using molecular markers. Hasan et al. (2006) characterized the genetic diversity of a set of 96 European *B. napus* genotypes based on 30 simple sequence repeat (SSR) markers distributed across the *B. napus* genome. Shengwu et al. (2003) assessed the genetic diversity revealed by random amplified polymorphic DNA (RAPD) markers of a collection of 50 *B. napus* genotypes from China and Europe. A complete understanding of the diversity patterns of a crop species requires, however, not only knowledge of genotypic but also phenotypic diversity (Gepts and Papa 2003). To the best of our knowledge, such information is not available for *B. napus*.

For plant breeders, agronomic traits as well as seed quality traits in *B. napus* are of highest importance (Friedt and Snowdon 2009). Such traits can only be assessed late during development. The selection of genotypes based on their performance during the seedling development stage, therefore, has the potential to increase the gain from selection, if these traits are correlated with the agronomic and seed quality traits of interest. Such information,

however, is not available for a species-wide germplasm set of *B. napus*.

The objectives of our study were to examine (1) the patterns of phenotypic diversity in a species-wide *B. napus* germplasm set with respect to various seedling development, agronomic, and seed quality traits as well as (2) the interrelationship of the examined traits and their use in selection on correlated traits.

Material and methods

Plant material

A set of 518 *B. napus* inbred lines, assembled to maximize genotypic variation (cf. Bus et al. 2011), was used in this study. Based on available information from gene banks, plant breeders, and our own observations, the accessions were assigned to eight different germplasm types, namely winter oilseed rape (OSR) (183), winter fodder (22), swede (75), semi-winter OSR (7), spring OSR (207), spring fodder (4), vegetable (14), and so far unspecified *B. napus* genotypes (6). From the whole set, 318 genotypes originated from Western Europe, 43 from Eastern Europe, 35 from Asia, 5 from Africa, 21 from Australia, 70 from North America, and 26 genotypes were of unknown origin (Supplementary Material S1). Furthermore, 95 winter OSR genotypes were divided into the following four subgroups according to the time period of release: 1954–1979 (11), 1980–1989 (15), 1990–1999 (28), 2000–2007 (41) (Supplementary Material S1).

Greenhouse trials and examined seedling development traits

These trials were performed with the inbred lines directly and not with test crosses as still inbred varieties of *B. napus* are commercialized and more importently, as in the case the heritabilities are expected to be higher. For assessing seedling development traits, the 518 genotypes were grown in six replicates, which were perfomed at six time points within 6 months, for 30 days in an α-lattice with 24 blocks of 24 pots in a greenhouse experiment. To reduce the effects of differencing seed qualities on seedling development, the seeds for this experiment are from two multiplication trials; one for the spring and one for the winter types. Temperature was set to 24 °C during the 16-h light exposure and 18 °C during 8-h dark exposure. Two of the 518 genotypes, namely "Express 617" and "V8", were included as common entries in each block. In a 10 × 10 cm pot filled with mini-tray soil (Balster Einheitserden, Fröndenberg, Germany), two seeds were sown and each pot was thinned to one seedling per pot at 7 days after sowing (das). The plants were fertilized twice during cultivation with Super 8 × 8 × 6 Wuxal (Wilhelm Haug GmbH & Co. KG, Ammerbuch-Pfäffingen, Germany). A large number of traits were assessed to cover a wide range of aspects as well as developmental stages during seedling growth which could be measured with high throughput methods.

From 8 to 16 das, digital infrared images of the seedlings were taken. The blue color channel of the RGB images was cut out. A mask of each *B. napus* plant was created using a color threshold, and the projected leaf areas (LA08–LA16) of the seedlings were calculated using the digital image processing software ImageJ 1.42q (http://rsb.info.nih.gov/ij/) (Table 1). In addition, various other traits were derived from the *B. napus* plant masks at 10 das (Table 1). Furthermore, the relative chlorophyll content of the leaf was mesured by Minolta Company-defined SPAD (Soil-Plant Analysis Development) value (SPD), fresh (FHM) and dry mass (DYM), as well as the H_2O content (H_2O) of the plants were collected after a growing period of 30 das.

Field trials and examined agronomic and seed quality traits

The *B. napus* diversity set was evaluated in field experiments for several agronomic traits, and the harvested seeds were analysed by near infrared reflectance spectroscopy (NIRS) to extract the seed quality parameters MOI, OIL, PRT, GSL, SUL, OLA, LIA, and ERA according to the standard protocol of VDLUFA and the parameters NDF, ADF, and ADL according to Wittkop et al. (2012) (Table 1).

In the growing season 2009–2010, a subset of 217 winter *B. napus* genotypes comprising 156 winter OSR, 8 winter fodder, 51 swede, and 2 vegetable genotypes was grown in a field experiment, which is designated in the following as winter trial. The experimental design was an α-lattice with 15 blocks of 15 plots with one replication at each of the seven locations [Barley (Great Britain), Einbeck, Groß Lüsewitz, Rosenthal, Lippstadt, Rauischholzhausen, and Groß Gerau (all Germany)]. The size of one plot at the seven locations ranged from 3.75 to 4.5 m^2. Each of the two genotypes "NK-Passion" and "Favorite" were used as check three times per location. The experiment received standard cultural practices of fertilization as well as control of pests and diseases and was harvested in July 2010.

In 2010, a subset of 188 spring *B. napus* genotypes [semi-winter OSR (7), spring OSR (177), and spring fodder (4)] was evaluated at three locations in Germany, namely Rauischholzhausen, Groß Gerau, and Gießen, with two replications per location in 3.75 m^2 plots. The experiment

Table 1 Traits assessed in the *B. napus* germplasm set

Traits	Abbreviation	Unit of measurement	Obs.[a]	h^{2b}	R^{2c} (ALL-MCLUST)
Seedling development traits					
Projected leaf area at day 8	LA08	cm^2	6	0.82	0.48
Projected leaf area at day 10	LA10	cm^2	6	0.84	0.44
Projected leaf area at day 12	LA12	cm^2	6	0.81	0.43
Projected leaf area at day 14	LA14	cm^2	6	0.76	0.41
Projected leaf area at day 16	LA16	cm^2	6	0.73	0.39
Parameter *a*	PRA	cm^2	6	0.51	0.24
Parameter *k*	PRK	1/day	6	0.28	0.00
Plant perimeter length	PER	cm	6	0.80	0.41
Plant major axis of the best fitting ellipse	MAJ	cm	6	0.82	0.43
Plant minor axis of the best fitting ellipse	MIN	cm	6	0.78	0.36
Plant aspect ratio: major axis/minor axis	ASR		6	0.56	0.17
Maximum plant diameter	MAD	cm	6	0.82	0.43
Minimum plant diameter	MID	cm	6	0.81	0.38
Plant circularity: 4π(area/perimeter^2)	CIR		6	0.33	0.09
Plant roundness: 4 × area/(π × major axis^2)	ROU		6	0.52	0.16
Plant solidity: area/convex area	SOY		6	0.52	0.14
Fresh mass	FHM	g	6	0.69	0.27
Dry mass	DYM	g	6	0.72	0.35
SPAD measurement	SPD		6	0.77	0.33
H_2O content	H_2O	% of fresh mass	6	0.39	0.27

Traits	Abbreviation	Unit of measurement	Winter trial			Spring trial	
			Obs.	h^2	R^2 (WR-MCLUST)	Obs.	h^2
Agronomic traits							
Emergence	EMR	1 = bad, 9 = very good	4	0.74	0.71	2	0.57
Development after emergence	DAE	1 = bad, 9 = very good	6	0.82	0.74	4	0.56
Stem elongation before winter	SAW	1 = no, 9 = much	3	0.62	0.53		
Winter hardiness	WIH	1 = bad, 9 = very good	6	0.74	0.58		
Phoma at leaves	PHO	1 = healthy, 9 = infected	2	0.41	0.02		
Lodging before flowering	LOF	1 = low, 9 = very high	3	0.41	0.50	2	0.85
Beginning of flowering	BOF	1 = early, 9 = late	7	0.94	0.79	4	0.81
Blossom color	BLC	1 = white, 3 = dark yellow	7	0.60	0.70		
End of flowering	EOF	1 = early, 9 = late	4	0.84	0.51	3	0.62
Maturity date	MYD	1 = bad, 9 = very good	2	0.26	0.17		
Lodging at maturity	LOM	1 = low, 9 = very high	5	0.58	0.30	2	0.76
Plant height	PTH	cm	6	0.68	0.15	6	0.81
Disease status before harvest	DBH	1 = healthy, 9 = infected	3	0.56	0.32	2	0.67
Phoma at harvest	PHM	1 = healthy, 9 = infected	2	0.28	0.17		
Yield	DTH	dt/ha	2	0.70	0.68	2	0.86
Seed quality traits							
Thousand grain weight	TGW	g	6	0.87	0.65		
Average projected seed area	AVA	cm^2	3	0.81	0.59		
Moisture content	MOI	% of dry mass	4	0.61	0.34	6	0.45
Oil content	OIL	% of dry mass	7	0.86	0.53	6	0.79
Protein content	PRT	% of dry mass	5	0.81	0.46	6	0.69
Glucosinolate concentration	GSL	μmol/g	7	0.96	0.22	6	0.97

Table 1 continued

Traits	Abbreviation	Unit of measurement	Winter trial			Spring trial	
			Obs.	h^2	R^2 (WR-MCLUST)	Obs.	h^2
Sulphur concentration	SUL	% of dry mass	3	0.96	0.23	6	0.96
Oleic acid concentration	OLA	% of total fatty acid	4	0.92	0.10	6	0.90
Linolenic acid concentration	LIA	% of total fatty acid	4	0.31	0.03	6	0.70
Erucic acid concentration	ERA	% of total fatty acid	6	0.96	0.09	6	0.96
Neutral detergent fiber concentration	NDF	% of dry mass	3	0.91	0.38	6	0.95
Acid detergent fiber concentration	ADF	% of dry mass	4	0.85	0.10	6	0.91
Hemicellulose concentration	HCL	% of dry mass	3	0.79	0.00	6	0.60
Acid detergent lignin concentration	ADL	% of dry mass	4	0.82	0.16	6	0.95
Cellulose concentration	CEL	% of dry mass	3	0.64	0.17	6	0.33

[a] Obs. the number of replicates or location-replicate combinations in which the corresponding trait was recorded

[b] h^2 is the repeatability

[c] R^2 the proportion of the phenotypic variance explained by population structure

is designated in the following as spring trial. The experimental design was an α-lattice with 14 blocks of 14 plots, where the genotypes "Campino" and "Sophia" were replicated two times as well as "Pauline" was replicated three times within each replicate as checks. The experiment received standard cultural practices of fertilization as well as control of pests and diseases and was harvested in August 2010.

Statistical analyses

For the traits projected leaf area at 8–16 das (LA08, LA10, LA12, LA14, and LA16), we fitted the following growth curve to the data of each replication-genotype combination:

$$y_d = a * e^{k*t_d} + e_d,$$

where y_d was the projected leaf area of the dth das, a the intercept (PRA), k the growth factor (PRK), t_d was the dth das, and e_d the residual. PRA and PRK were included as regular seedling development traits in further analyses.

For the statistical analyses of the performed experiments, different mixed models were fitted. Model (1) was used to analyse the seedling development traits from the greenhouse experiment. The agronomic and seed quality traits from the winter trial were analysed using model (2) and the agronomic and seed quality traits from the spring trial were analysed using model (3):

$$y_{ikm} = \mu + g_i + r_k + b_{km} + e_{ikm} \quad (1)$$

$$y_{ijm} = \mu + g_i + l_j + b_{jm} + e_{ijm} \quad (2)$$

$$y_{ijkm} = \mu + g_i + l_j + g_i{}^* l_j + r_{jk} + b_{jkm} + e_{ijkm}, \quad (3)$$

where y_{ikm} was the observation of the ith genotype in the mth block of the kth replication, μ an intercept term, g_i the genotypic effect of the ith genotype, r_k the effect of the kth replicate, b_{km} the effect of the mth block in the kth replicate, e_{ikm} the residual, y_{ijm} the observation of the ith genotype in the mth block at the jth location, l_j the effect of the jth location, b_{jm} the effect of the mth block at the jth location, e_{ijm} the residual, y_{ijkm} the observation of the ith genotype in the mth block of the kth replication at the jth location, $g_i^* l_j$ the interaction effect of the ith genotype and the jth location, r_{jk} the effect of the kth replicate at the jth location, b_{jkm} the effect of the mth block in the kth replicate of the jth location, and e_{ijkm} the residual.

For estimation of the genotypic variance σ_g^2, all effects were regarded as random. For calculating an adjusted entry mean M for each genotype, which was the basis for all further analyses, g_i was regarded as fixed and all other effects as random. The repeatability h^2 was calculated for the various traits according to Emrich et al. (2008). All mixed-model calculations were performed with ASReml (Gilmour et al 2006).

The Shannon index H (Shannon 1948) was calculated to measure phenotypic diversity of each trait. For this analysis, all traits were transformed into categorical traits by grouping the observed trait values into ten frequency classes according to Pecetti et al. (1992). The following resampling procedure was performed to correct for the different sizes of the examined germplasm groups (Stich et al. 2005). A subset of N_s genotypes was randomly selected from the original germplasm groups, and H was calculated. This procedure was repeated 1,000 times and the results were averaged. Due to the small sample sizes of the germplasm types spring fodder ($N = 4$) and unspecified inbred lines ($N = 6$), the size of the subset of genotypes was chosen to be $N_s = 7$, and the resampling procedure was not performed for the aforementioned two germplasm types. The same analyses were performed with $N_s = 75$ for

the seedling development traits and with $N_s = 51$ for the agronomic and seed quality traits. For the analyses of the four different release periods, N_s was set to 11.

Pearson's partial correlation coefficient r was calculated among all pairs of traits (Fisher 1924). To visualize these trait correlations, network analyses were performed. According to Ursem et al. (2008), only correlations with an absolute value ≥ 0.3 were drawn. Based on the evaluated phenotypic traits, associations among genotypes and traits were revealed by principal component analyses (PCA).

A total of 509 of the 518 *B. napus* genotypes of our study were assigned to three clusters (ALL-MCLUST) based on SSR marker data (Bus et al. 2011). Furthermore, according to these marker data, the genotypes of the winter trial were assigned to two clusters (WR-MCLUST groups 1 and 2), whereas no distinct clusters were observed for the genotypes from the spring trial.

To identify the set of seedling development traits that predicts best the agronomic and seed quality traits assessed in the winter and spring trial, we performed the following multiple stepwise regression analysis for each agronomic and seed quality trait, where variables were selected based on the Bayesian Information Criterion (Schwarz 1978):

$$y_i = \mu + \sum_{p=1}^{o} b_p x_{pi} + e_i,$$

where y_i was the observation of the ith genotype, for which the best set of seedling development predictors should be identified, μ an intercept term, o the number of selected variables, b_p the regression coefficient of the pth seedling development trait, x_{pi} the adjusted entry mean for the pth seedling development trait of the ith genotype, and e_i the residual. The analyses were performed for each agronomic and seed quality trait with the adjusted entry mean of all genotypes as well as separately for the two WR-MCLUST groups to reduce the influence of population structure on the set of selected predictors as well as for the 188 genotypes from the spring trial. If not stated differently, all analyses were performed with the statistical software R (R Development Core Team 2011).

Results

The repeatability h^2 of the seedling development traits ranged from 0.28 to 0.84 (Table 1). For the agronomic and seed quality traits, the heritabilities were on average slightly higher and ranged for the winter trial from 0.26 to 0.96 and for the spring trial from 0.33 to 0.96. The proportion of the genotypic variance of seedling development traits which was explained by population structure R^2 ranged from 0.00 to 0.48. For the agronomic and seed quality traits collected in the winter trial, R^2 ranged from 0.00 to 0.79.

The Shannon index H ranged for the seedling development traits ($N_s = 7$) from 1.25 (swedes, semi-winter OSR) to 1.48 (vegetable) (Table 2). In the winter trial, H ranged for the agronomic traits from 0.60 (vegetable) to 1.29 (winter fodder) and for the seed quality traits from 0.42 (vegetable) to 1.37 (winter fodder). For the spring trial, H ranged for the agronomic traits from 1.23 (semi-winter OSR) to 1.43 (spring OSR), whereas for the seed quality traits the opposite trend was observed. H calculated for the subgroups of winter OSR based on the release periods resulted in the tendency of a lower H value for more recently released genotypes (Table 3). The reduction of H was strongest for the seedling development traits and lowest for the agronomic traits.

In the PCA of the 518 *B. napus* inbreds and the seedling development traits, the first two principal components explained 64.61 and 14.39 % of the variance (Fig. 1a). With respect to these two principal components, all inbreds were assigned to three slightly overlapping clusters. The examined seedling development traits MAJ, MAD, PER, FHM, DYM, MIN, MID, SPD, PRA, LA08, LA10, LA12, LA14, and LA16 had loadings mainly on PC1, whereas ASR, CIR, SOY, and ROU contributed to PC1 and PC2. For the 217 genotypes from the winter and the 188 genotypes from the spring trial based on all 50 traits, the first two principal components explained between 10.52 and 45.20 % of the variance. These PCA revealed distinct clusters for the inbreds examined in the winter trial (Fig. 1b), whereas no obvious clusters were observed for the inbreds of the spring trial (Fig. 1c).

The partial correlations observed for the genotypes from the spring and WR-MCLUST group 1 of the winter trial were high among the traits of the same trait category but low among traits of different trait categories (Fig. 2). In contrast, for the genotypes from the WR-MCLUST group 2 of the winter trial, high partial correlations were observed among all traits.

The proportion of the phenotypic variance of the agronomic and seed quality traits assessed for the 188 genotypes from the spring trial which could be explained by a selected set of the seedling development traits was on average 0.11. For the WR-MCLUST group 1 and 2, the average R^2 for the agronomic and seed quality traits explained by the selected set of seedling development traits was 0.09 and 0.17, respectively (Table 4).

Discussion

We observed across all seedling development traits an average repeatability of 0.66 (Table 1). In contrast,

Table 2 Shannon index measuring phenotypic diversity of 518 *B. napus* inbreds assigned to eight germplasm types

Germplasm type	N^a	Seedling development traits (average)	Winter trial			Spring trial		
			N	Agronomic traits (average)	Seed quality traits (average)	*N*	Agronomic traits (average)	Seed quality traits (average)
Winter OSR	183	1.96	156	1.51	1.75			
Winter OSR sampling	75	1.92	51	1.46	1.69			
Winter OSR sampling	7	1.40	7	1.11	1.27			
Winter fodder	22	1.61	8	1.35	1.44			
Winter fodder sampling	7	1.30	7	1.29	1.37			
Swedes	75	1.69	51	1.65	1.62			
Swedes sampling	7	1.25	7	1.26	1.22			
Semi-winter OSR	7	1.25				7	1.23	1.42
Spring OSR	207	1.85				177	2.02	1.96
Spring OSR sampling	75	1.81				51	1.96	1.89
Spring OSR sampling	7	1.32				7	1.43	1.37
Spring fodder	4	1.23				4	1.23	0.85
Vegetable	14	1.77	2	0.60	0.42			
Vegetable sampling	7	1.48	2	0.60	0.42			
Unspecified	6	1.44						
Entire set	518	2.11	217	1.90	1.96	188	2.03	1.96
Entire set sampling	188	2.09	188	1.90	1.95	188	2.03	1.96

[a] *N* is the sample size

Basunanda et al. (2010) observed for seedling biomass traits of *B. napus*, which were assessed in greenhouse experiments, h^2 values of 0.43. This difference could be attributed to the fact that our study was based on a germplasm set, whereas Basunanda et al. (2010) studied segregating populations derived from biparental crosses. This in turn is expected to reduce the genotypic variance and to result in a lower repeatability.

The h^2 values observed for the seedling development traits were lower compared to those observed for the seed quality traits. Our observation might be due to that the seedling developments were assessed on a single plant basis, whereas seed quality traits were recorded on a plot basis. This in turn reduces the error variance.

We observed for the winter as well as the spring trial higher h^2 values for the seed quality traits compared to the agronomic traits (Table 1). Our observation can be partially explained by the higher number of locations in which the seed quality traits have been assessed. Furthermore, our finding is in good accordance with results from earlier QTL mapping studies suggesting that the genetic complexity of seed quality traits is lower than that of agronomic traits (cf. Würschum et al. 2012). In summary, we observed with few exceptions (PRK, CIR, MYD, PHM, and LIA) moderate to high h^2 values for all traits. This finding indicates that the collected data can be successfully used to improve our understanding of the patterns of phenotypic diversity in *B. napus*.

Phenotypic diversity of the *B. napus* germplasm set

We observed for the entire germplasm set based on the seedling development traits a total phenotypic diversity of 2.09. In contrast, the agronomic and seed quality traits showed a slightly lower diversity index for the entire set of 1.93 in the winter trial and 2.00 in the spring trial (Table 2). The absolute values of phenotypic diversity indices across different traits have to be interpreted with caution because of the potential influence of scaling effects. Therefore in the following only the trends over the time are discussed.

We observed for the winter OSR germplasm type a reduction of the phenotypic diversity index across all traits of 9.7 % when considering the different release period subgroups (Table 3). This finding might be due to the selection during the breeding process and the occurred bottlenecks such as the development of zero seed erucic acid and low seed glucosinolate content varieties (Hasan et al. 2008; Sharpe and Lydiate 2003). However, we observed a reduction of the phenotypic diversity for the agronomic and seed quality traits of 5.6 % (Table 3) and a two and a half times higher reduction of phenotypic diversity for the seedling development traits. Our observation might be due to the fact that when plant breeders introgressed new genetic material this was done with respect to agronomic and seed quality traits but not seedling development traits.

Table 3 Shannon index measuring phenotypic diversity of 95 winter oilseed rape inbreds divided into four release period subgroups

Traits	1954–79	1980–89		1990–99		2000–07	
	$N = 11$	$N = 15$	$N^* = 11$	$N = 28$	$N^* = 11$	$N = 41$	$N^* = 11$
Seedling development traits							
LA08	1.86	1.87	1.77	1.49	1.31	1.85	1.58
LA10	1.86	1.54	1.45	1.67	1.45	1.77	1.54
LA12	1.89	1.54	1.45	1.55	1.37	1.87	1.56
LA14	1.94	1.45	1.35	1.40	1.26	1.86	1.54
LA16	1.69	1.53	1.47	1.46	1.32	1.82	1.53
PRA	1.72	2.07	1.95	1.70	1.52	2.19	1.87
PRK	1.76	1.89	1.76	1.30	1.18	1.96	1.60
PER	1.55	1.40	1.32	1.56	1.34	1.50	1.31
MAJ	1.85	1.36	1.32	1.32	1.22	1.44	1.26
MIN	1.42	1.45	1.38	1.48	1.35	1.55	1.40
ASR	1.47	2.00	1.87	2.18	1.88	1.09	1.60
MAD	1.67	1.49	1.42	1.37	1.27	1.51	1.32
MID	1.42	1.27	1.21	1.31	1.21	1.56	1.36
CIR	1.52	1.66	1.57	1.69	1.47	1.71	1.48
ROU	1.52	1.58	1.49	1.73	1.52	1.72	1.51
SOY	1.29	1.84	1.73	1.71	1.54	1.83	1.57
FHM	1.85	1.43	1.36	1.44	1.30	1.72	1.47
DYM	2.19	1.34	1.26	1.32	1.22	1.87	1.58
SPD	2.24	1.54	1.45	1.66	1.49	1.76	1.49
H_2O	2.11	1.49	1.42	1.54	1.37	1.82	1.57
Average	1.74	1.59	1.50	1.54	1.38	1.76	1.51
Agronomic traits							
EMR	1.03	1.24	1.19	1.16	1.06	1.28	1.10
DAE	1.10	0.99	0.94	1.20	1.09	1.36	1.20
SAW	1.41	1.42	1.34	1.35	1.21	1.67	1.44
WIH	0.60	1.19	1.15	1.08	0.98	1.28	1.13
PHO	1.63	1.49	1.43	1.80	1.55	1.85	1.54
LOF	1.16	1.40	1.33	1.10	0.97	0.77	0.70
BOF	1.21	0.99	0.97	1.25	1.11	1.19	1.06
BLC	0.45	0.43	0.40	0.15	0.12	0.11	0.08
EOF	1.28	1.36	1.30	1.53	1.36	1.67	1.47
MYD	1.10	1.08	1.03	1.64	1.47	1.45	1.28
LOM	1.33	1.62	1.53	1.02	0.92	0.98	0.86
PTH	1.23	1.49	1.42	1.76	1.55	1.55	1.32
DBH	1.46	1.77	1.66	1.54	1.38	1.52	1.32
PHM	1.54	1.59	1.50	1.17	1.06	1.30	1.10
DTH	0.98	1.18	1.14	1.43	1.30	1.27	1.08
Average	1.17	1.28	1.22	1.28	1.14	1.28	1.11
Seed quality traits							
TGW	0.95	0.99	0.96	1.32	1.15	1.27	1.11
AVA	1.03	0.88	0.86	1.40	1.19	1.46	1.30
MOI	1.24	1.36	1.32	1.47	1.33	1.86	1.59
OIL	1.58	1.40	1.32	1.87	1.64	1.46	1.26
PRT	1.21	1.64	1.55	1.52	1.35	1.39	1.20
GSL	1.16	1.49	1.42	1.05	0.88	0.79	0.72
SUL	1.10	1.45	1.38	1.18	1.00	0.79	0.72

Table 3 continued

Traits	1954–79	1980–89		1990–99		2000–07	
	$N = 11$	$N = 15$	$N^* = 11$	$N = 28$	$N^* = 11$	$N = 41$	$N^* = 11$
OLA	1.46	1.66	1.57	1.64	1.47	1.44	1.27
LIA	1.71	1.78	1.67	1.65	1.43	1.87	1.59
ERA	0.98	0.95	0.90	0.41	0.35	0.31	0.24
NDF	1.33	1.59	1.49	1.86	1.64	1.60	1.41
ADF	1.54	1.53	1.43	1.99	1.70	1.81	1.51
HCL	1.54	1.71	1.61	1.94	1.71	1.55	1.35
ADL	1.54	1.49	1.42	1.71	1.49	1.82	1.56
CEL	1.24	1.81	1.69	2.13	1.81	1.81	1.54
Average	1.31	1.45	1.37	1.54	1.34	1.41	1.23
Total average	1.44	1.45	1.38	1.46	1.30	1.51	1.30

Data of agronomic and seed quality traits were from the winter trial. For release period subgroups with more than $N = 11$ genotypes, a resampling procedure was applied (N^*)

N is the sample size

Nevertheless, we observed for the agronomic traits BLC, LOF, LOM, and PHM a strong reduction of the phenotypic diversity index between 82 and 29 %, and for the seed quality traits ERA, GSL, SUL, and OIL a reduction between 76 and 20 % (Table 3) when comparing the oldest and the most recent release period subgroups. Our finding can be explained by the high importance of these traits during the last centuries of *B. napus* breeding (cf. Friedt and Snowdon 2009; Becker 2011). In addition, our observation might be explained by the oligogenic inheritance of these traits and, thus, the rapid change due to selection.

We observed for the seedling development traits with one exception the same diversity trends across the different germplasm types (Table 2) as those observed by Bus et al. (2011) based on 89 SSR markers. The only difference was that the inbreds of the winter OSR germplasm type showed a high phenotypic diversity but a low gene diversity (Table 2). This observation might be due to that almost 50 % of the SSR markers studied by Bus et al. (2011) were developed for spring OSR material which could lead to an underestimation of the gene diversity of winter OSR inbreds. However, this explanation was not supported by our finding of a good accordance of the diversity trends across the different germplasm types for agronomic and seed quality traits as well as SSR marker data.

For the agronomic and seed quality traits of the winter trial, the most diverse germplasm type was winter fodder, and, in contrast, the winter OSR germplasm type showed a low diversity (Table 2). This finding might be due to a weak selection for germplasm types with low economic importance but a strong selection of modern germplasm types during the intensive breeding process in the last decades. Thus, the germplasm types with low economic importance might be useful to increase phenotypic diversity of certain agronomic and seed quality traits in the low diversity winter OSR germplasm.

Population structure of the *B. napus* germplasm set

Based on the seedling development traits, we observed for the PCA of the 518 *B. napus* inbreds a slight overlapping of the three major germplasm types, namely winter OSR, swede, and spring OSR (Fig. 1a). This finding is in good accordance with the results based on SSR data of the same germplasm set (Bus et al. 2011). The clustering and separation could be explained by the breeding history as well as the adaptation of the different germplasm types to distinct environments.

We measured the relationship between population structure calculated from 89 SSR markers and the examined phenotypic traits by calculating the proportion of the phenotypic variance explained by the population structure information inferred from SSR markers (ALL-MCLUST). We observed for the seedling development traits an average R^2 value of 30.9 % (Table 1). This value is about three times higher than that reported by Flint-Garcia et al. (2005) for a set of 101 maize inbreds genotyped with 89 SSR markers and phenotyped for 60 traits. This difference in the phenotypic variation explained by population structure might be due to the exclusion of sweet corn and popcorn lines in the study of Flint-Garcia et al. (2005). Our finding suggests that the population structure information has to be taken into account for association analyses of most examined traits to decrease the proportion of false-positive associations, as was already indicated by Hasan et al. (2008) and Rezaeizad et al. (2010).

We observed an assignment of the germplasm types winter OSR and swede to distinct clusters in the PCA when

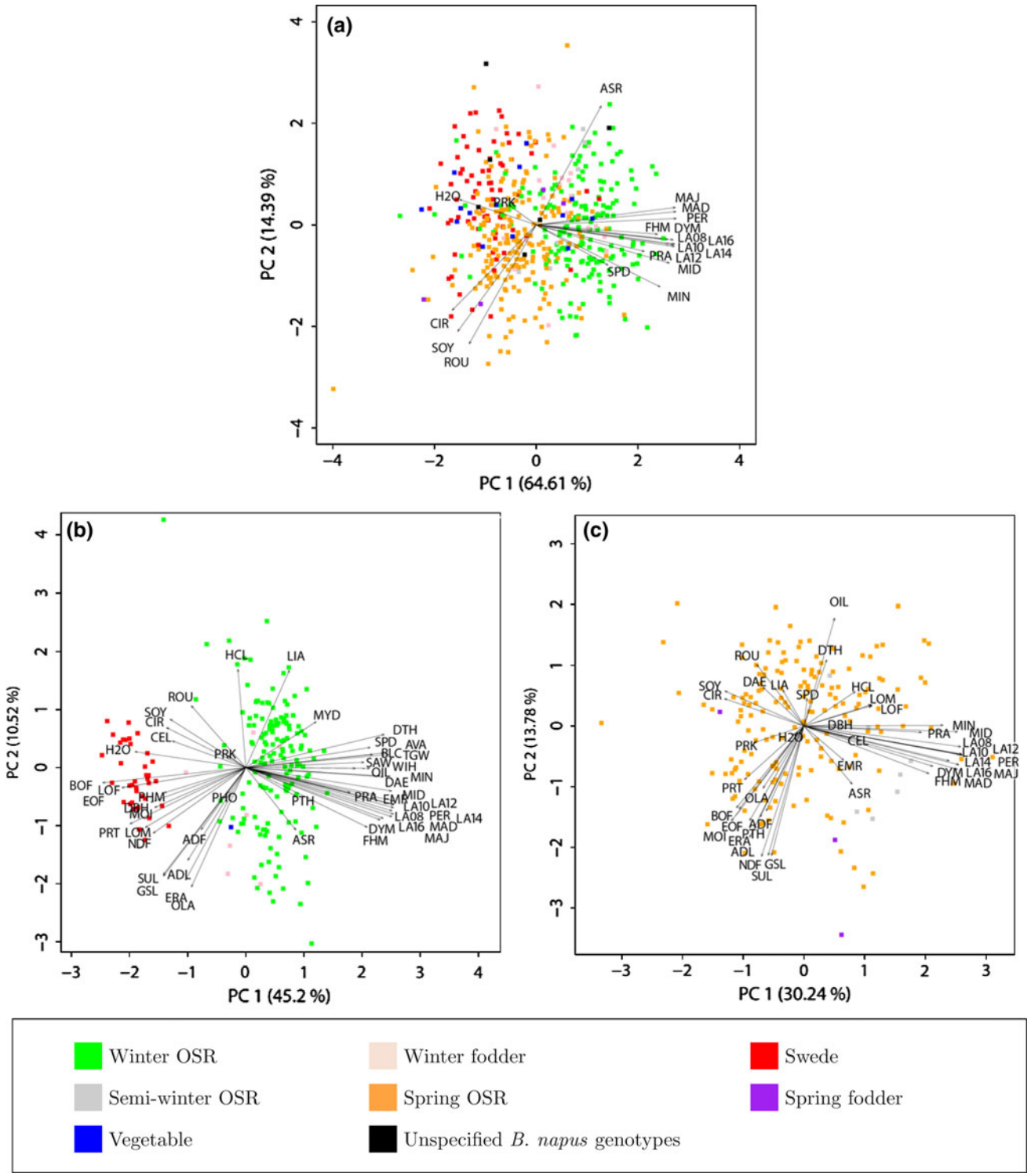

Fig. 1 Principal component analysis of *B. napus* inbreds and the seedling development, agronomic, and seed quality traits. PC 1 and PC 2 are the first and second principal component, respectively. The proportion of variance explained by the principal components is given in *parentheses*. Oilseed rape is abbreviated as OSR. For abbreviations of the traits see Table 1. The analyses were performed for all 518 inbreds based on the seedling development traits (**a**), 217 genotypes from the winter trial based on the seedling development, agronomic, and seed quality traits (**b**), as well as 188 genotypes from the spring trial based on the seedling development, agronomic, and seed quality traits (**c**)

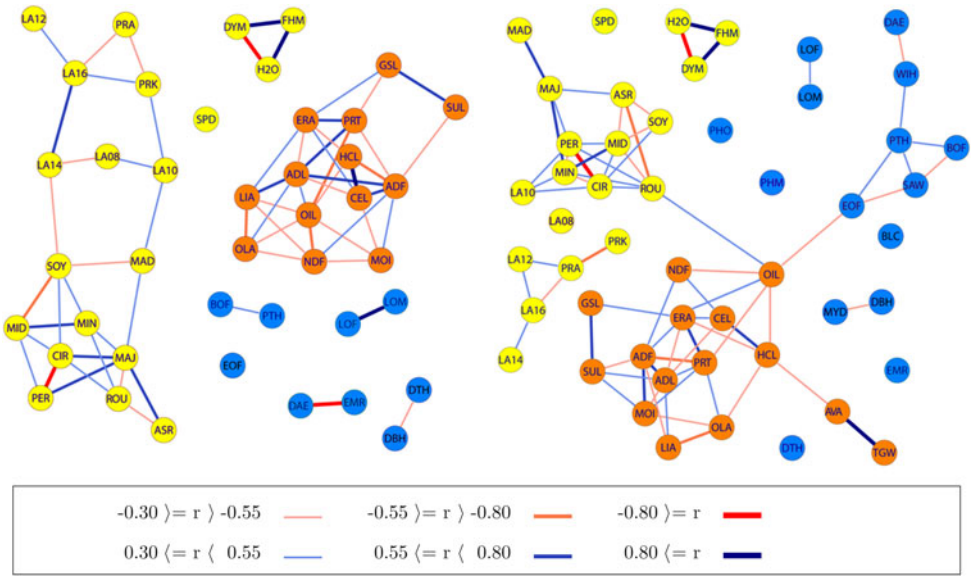

Fig. 2 Networks constructed based on the partial Pearson's correlation coefficients among all pairs of traits. The *nodes* represent the examined traits, whereas the *lines* represent the corresponding pairwise partial correlations of the seedling development, agronomic, and seed quality traits assessed in the 188 inbreds of the spring trial (*left*) and the 165 inbreds of the WR-MCLUST group 1 from the winter trial (*right*). All traits from the same category of seedling development, agronomic, and seed quality were *colored identically*

adding the data from the winter trial to the seedling development traits (Fig. 1b). This observation is in accordance with the finding that the proportion of variance explained by population structure (WR-MCLUST) was for the agronomic and seed quality traits assessed in the winter trial with 38 % (Table 1) on average higher than that of the seedling development traits. The finding that population structure is more pronounced for agronomic and seed quality traits than for seedling development traits might be due to a germplasm-specific selection which was stronger for the former than for the latter.

In contrast, neither based on phenotypic nor on marker data distinct clusters were observed for the inbreds of the spring trial (Fig. 1c). This is in accordance with our observation that the proportion of variance explained by population structure was on average below 3 % for the agronomic and seed quality traits assessed in the spring trial (data not shown). This finding might be on one hand due to a high genotypic but also phenotypic diversity among the genotypes of the spring trial and on the other hand due to the small number of non-spring OSR genotypes in this part of the germplasm set.

Correlations among traits and the identification of phenotypic predictors

The correlations among all pairs of traits were examined to identify phenotypic predictors that can be used during the breeding process.

We observed for the 188 genotypes of the spring trial as well as for the 165 winter genotypes of the WR-MCLUST group 1 that the correlation coefficients were higher between traits from the same category of seedling development, agronomic, and seed quality traits than between traits from different categories (Fig. 2). This observation can be explained by genetical linkage, pleiotropy, or by co-selection of traits.

We observed negative correlations between oleic acid (OLA) and linolenic acid concentration (LIA) as well as between oil (OIL) and protein content (PRT) in the 188 inbreds of the spring trial and the 165 inbreds of the WR-MCLUST group 1 from the winter trial (Fig. 2). This finding is an example for pleiotropy, because these pairs of traits compete for the same basic substrates in the biochemical pathways (Hu et al. 2006; Zhao et al. 2006).

Furthermore, we observed a positive correlation between erucic acid (ERU) and glucosinolate concentration (GSL) in the 188 inbreds of the spring trial and the 165 inbreds of the WR-MCLUST group 1 from the winter trial (Fig. 2). This observation might be due to the co-selection for these seed quality traits during the breeding process of modern spring and winter OSR germplasm types (Friedt and Snowdon 2009).

In contrast to the 188 genotypes of the spring trial and the 165 winter genotypes of the WR-MCLUST group 1, considerably higher correlations within and between the trait categories were observed for the genotypes of WR-MCLUST group 2 of the winter trial. This observation might be due to the fact that the WR-MCLUST group 2 was represented by a small number of genotypes compared to the genotypes of the spring trial or the WR-MCLUST group 1.

Independent of the reason for the correlation, correlated phenotypic traits can potentially be used to increase the gain from selection, because the seedling development traits measured in an early stage of development could be used to predict agronomic traits. We calculated for the agronomic and seed quality traits the proportion of the phenotypic variance explained by sets of selected seedling

Table 4 Agronomic and seed quality traits of the two WR-MCLUST groups evaluated in the winter trial and the genotypes of the spring trial predicted by linear combinations of the seedling development traits

Traits	Genotypes of winter trial ($N = 217$)			
	WR-MCLUST group 1 ($N = 165$)		WR-MCLUST group 2 ($N = 49$)	
	Optimum linear model	R^{2a}	Optimum linear model	R^2
Agronomic traits				
EMR	$\mu + 5.15$ FHM	0.27	$\mu + 2.91$ LA16	0.20
DAE	$\mu + 8.89$ LA16 + 0.07 LA10 − 0.21 PRK − 3.70 SPD	0.20	$\mu + 2.76$ FHM	0.12
SAW	$\mu + 1.42$ MIN	0.06	$\mu - 0.94$ CIR + 4.39 DYM	0.20
WIH			$\mu + 57.74$ SPD + 0.18 H_2O	0.22
PHO			$\mu + 9.10$ ROU	0.08
LOF			$\mu + 7.99$ DYM	0.11
BOF	$\mu + 6.43$ SPD	0.04	$\mu - 54.02$ H_2O + 0.66 LA10 + 0.84 LA14 − 0.61 LA12	0.41
BLC	$\mu + 1.92$ MIN	0.03	$\mu + 0.07$ SPD	0.09
EOF	$\mu + 11.81$ SPD − 0.13 MIN	0.09	$\mu - 25.32$ H_2O	0.09
MYD			$\mu + 0.12$ LA14	0.09
PTH	$\mu + 129.89$ MAD + 20.48 PER	0.08	$\mu + 50.42$ SPD	0.11
DBH			$\mu + 12.54$ PRK	0.12
DTH	$\mu - 0.58$ SPD	0.08		
Seed quality traits				
TGW	$\mu + 3.81$ LA16	0.14		
AVA	$\mu + 3.97$ LA16	0.12		
MOI			$\mu + 5.25$ LA16	0.10
OIL			$\mu + 126.88$ H_2O	0.08
PRT	$\mu + 27.43$ SPD	0.07		
GSL	$\mu + 128.07$ SPD − 3.43 DYM	0.11		
SUL	$\mu + 1.03$ SPD − 0.02 FHM	0.11		
OLA	$\mu + 61.91$ FHM	0.04	$\mu + 254.79$ H_2O	0.12
LIA			$\mu + 10.97$ FHM	0.12
ERA	$\mu + 83.55$ SPD	0.06	$\mu + 479.65$ H_2O	0.08
NDF	$\mu + 12.16$ MAJ + 0.78 CIR	0.09	$\mu + 16.21$ LA16	0.32
ADF	$\mu + 10.62$ MAD	0.03	$\mu + 10.65$ LA16	0.21
HCL	$\mu + 4.94$ FHM − 0.08 LA12	0.08	$\mu - 15.36$ PRK − 4.92 H_2O	0.29
ADL	$\mu + 4.07$ FHM + 0.07 ASR	0.09	$\mu + 6.28$ LA16 + 0.24 LA10	0.29
CEL	$\mu + 6.23$ FHM − 0.04 LA08	0.10	$\mu + 5.69$ LA12	0.19

Traits	Genotypes of spring trial ($N = 188$)	
	Optimum linear model	R^2
Agronomic traits		
EMR	$\mu + 3.32$ MAJ	0.07
DAE	$\mu + 4.95$ LA16	0.07
LOF	$\mu - 0.62$ MID	0.13
BOF	$\mu + 8.23$ MIN	0.14
EOF	$\mu + 9.03$ MIN	0.14
LOM	$\mu + 1.83$ MID	0.10
PTH	$\mu + 148.43$ MID − 27.88 MAD	0.08
DBH	$\mu + 1.66$ MIN + 3.04 FHM	0.07
DTH	$\mu - 4.99$ SPD	0.14

Table 4 continued

Traits	Genotypes of spring trial ($N = 188$)	
	Optimum linear model	R^2
Seed quality traits		
MOI	$\mu + 6.47$ LA08 $- 1.06$ LA10	0.14
OIL	$\mu + 34.38$ SPD	0.09
PRT	$\mu + 34.66$ SPD $- 0.19$ MID	0.24
GSL	$\mu - 51.47$ PRK	0.07
SUL	$\mu + 0.01$ PRK	0.07
OLA	$\mu + 47.10$ SOY	0.08
LIA	$\mu + 18.45$ DYM $- 1.61$ SOY	0.09
ERA	$\mu + 44.50$ MID $- 28.48$ FHM	0.17
ADF	$\mu + 7.42$ SPD $+ 0.13$ LA08 $- 2.57$ LA10	0.15
HCL	$\mu + 7.14$ SOY $- 2.19$ SPD $- 0.03$ MID	0.15
ADL	$\mu + 4.33$ LA08 $- 1.78$ LA12 $+ 0.44$ SPD	0.13
CEL	$\mu + 5.96$ LA16	0.08

[a] R^2 is the proportion of the phenotypic variance explained by the selected independent variables

development traits. We observed for the genotypes from the spring and WR-MCLUST group 1 and 2 of the winter trial an average R^2 value of 13 % (Table 2). This proportion of explained variance is considerably lower than the values reported in earlier QTL mapping studies. Our result indicates that the seedling development traits examined in this study are of moderate value as indirect selection parameters in *B. napus* breeding.

Summary

The moderate to high h^2 values observed for most traits of our study suggested that the collected data can be successfully used to improve our understanding of the patterns of phenotypic diversity in *B. napus*. Furthermore, the results of our study indicated that phenotypic diversity was more severely reduced across the different release period subgroups of winter OSR for the seedling development traits than for the agronomic and seed quality traits indicating that targeted introgression of diversity is required for the former. Nevertheless, our results suggested that this might also be required for some of the agronomic and seed quality traits for which a reduction of phenotypic diversity of up to 82 % has been observed. Finally, the proportion of variance of agronomic and seed quality traits explained by sets of selected seedling development traits was with about 13 % rather low, indicating that the seedling development traits examined in our study are of moderate value as indirect selection parameters in *B. napus* breeding.

Acknowledgments The authors thank the breeding companies Deutsche Saatveredelung AG, KWS SAAT AG, Limagrain GmbH, and Norddeutsche Pflanzenzucht for performing the winter trials. Furthermore, we would like to thank Wolfgang Ecke (University of Göttingen, Germany), the Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben (Germany), Nordic Gene Bank, Alnarp (Sweden), the Centre for Genetic Resources (Netherlands), and Warwick Horticulture Research International Genetic Resources Unit (UK) for providing the seeds of the examined germplasm. This research was funded by the Deutsche Forschungsgemeinschaft and the Max Planck Society. It was performed in the framework of the ERA-NET PG project "ASSYST". We are deeply grateful to Andrea Lossow, Nele Kaul, Annika Legerlotz, Bent Müller, Frank Eikelmann, and Andreas Lautscham for excellent technical assistance. Finally, the authors thank the associate editor C. Quiros and three anonymous reviewers for their valuable suggestions.

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