

Duplication assessments in *Brassica* vegetable accessions

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Abstract

There is extensive duplication of accessions among collection holders globally. To save costs, unwanted duplication should be avoided. This issue has been addressed internationally. In Europe, there are currently 35 *Brassica* collections located in 24 countries. Duplication may be identified not only by surveying passport data and seed transactions, but also by applying morphological or genetic characterization. Our study included two collections; one at the N. I. Vavilov Institute of Plant Genetic Resources in St. Petersburg (VIR) and one at the Nordic Genetic Resource Center (NGB). A random set of 13 accession pairs or triplets of cabbage, turnip and swede were selected on the basis of identical or similar accession names. The accessions could potentially be regarded as duplicates. Morphological characterization showed that in about 50% the pair/triplet, the accessions were identical and should thus be regarded as duplicate holding. Determining the status of the remaining accessions, which were more or less distinct but had identical or similar names, was more difficult. In this paper, possible explanations for the similarities in names are discussed, as is the need to include characterization in any duplicate assessment process.

Keywords: *Brassica napus* var. *napobrassica*, *Brassica oleracea* var. *capitata*, *Brassica rapa* var. *rapa*, cabbage, conservation, genebank management, swede, turnip

Introduction

Ex situ germplasm conservation systems (genebanks) exist to maintain crop diversity and facilitate utilization for breeding and research (Plucknett *et al.*, 1987; Hammer, 1993; Walters, 2004). Globally more than seven million accessions are conserved; however, far from all are unique, as there has been extensive duplication among collection holders (Van Hintum and Visser, 1995; Van Hintum and Boukema, 1999; Germeier *et al.*, 2003; Van Treuren *et al.*, 2009). Unwanted duplication is costly, but as long as each genebank acts on its own and fails to coordinate with other genebanks, the problem persists. The issue has been addressed (Fowler, 2007; FAO, 2010), and at European level

with a strategic framework of an integrated genebank system (AEGIS) (Engels and Maggioni, 2012). The framework contains a roadmap on how to reduce unwanted duplications as well as to ensure quality standards and long-term commitment to conservation from the participating genebanks. The *Brassica* Working Group of the European Cooperative Programme for Plant Genetic Resources has given priority to AEGIS. This means that there is an ongoing process in which potential duplicates are actively sought for (ECPGR, 2008), with the aim to include only unique accessions in the European Collection defined by AEGIS. In Europe, there are 35 *Brassica* collections located in 24 countries (Branca *et al.*, 2013); online tools have been developed to search for potential duplicate accessions (Menting and Bas, 2016). Our study includes two collections: the collection held at the N. I. Vavilov Institute of Plant Genetic Resources in St. Petersburg (VIR) and the

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collection held at the Nordic Genetic Resource Center (NGB). The genus *Brassica* includes several important vegetables, such as cabbage (*B. oleracea* L. var. *capitata*), turnip (*Brassica rapa* L. var. *rapa*) and swede [*Brassica napus* (L.) Rehb. var. *napobrassica*]. More than 15,000 accessions of *B. oleracea* are reported by the Global Gateway to Genetic Resources (GENESYS, 2017). Out of these, around 800 cabbage accessions are maintained at VIR and 190 at NGB. In addition both genebanks have significant collections of turnip and swede.

Van Hintum and Knüpffer (1995) distinguish between genetically identical accessions and common duplicates, where common duplicates derive from the same initial population. One approach has been to identify common duplicates by comparing passport data. The NGB collection was established in 1979 and accessions were acquired from universities, research stations and enterprises in the Nordic countries; however, the accessions could have been stored for years before entering the genebank. The VIR collection contains accessions from the 1920s onwards and records indicate that Nicolai Vavilov had contact with the Botanical Garden in Copenhagen and with breeders in Weibullsholm and in Svalöf, Sweden, during the inter-war period (Loskutov, 1999). Subsequent accessions have been acquired from enterprises or research institutions. While a natural and immediate assumption might be that accessions that have the same name are common duplicates, here we demonstrate that this is not always the case and argue that characterization should be included in any duplicate assessment process.

Material and methods

As they represent outcrossing plants, highly susceptible to crosspollination and genetic drift during regeneration, *Brassica* vegetables were selected. Selection criteria were: (1) same or similar names, (2) different (or unknown) donors or not duplicated from the other genebank, respectively, and (3) seed available for distribution. In total, 60 pairs/triplets were identified. We randomly selected 13 of these (Table 1). The following data were recorded: 'accession name', 'donor name', 'donor number' and 'acquisition year'. In most cases, the accession names provided a cultivar name, often combined with the name of a seed enterprise or a second name (most likely a selection identity). The names were compared to archives of known cultivars (Börjesson, 2015; SESTO, 2017). The accessions were grouped into potential duplication pairs/triplets based on 'accession name'.

Field assessment and characterization

The accessions were planted with a spacing of 50 cm between plants; 27 plants in total divided into three

randomized blocks were used per accession of turnip and swede, and 12 plants were used for cabbage. The growing location was Alnarp, Skandia, Sweden (55°N, 13°E), on a loamy clay soil fertilized with about 100 kg/ha PROMAGNA 11-5-18™ (Yara, Norway) at planting and 30 kg/ha YaraMila 22-0-12™ (Yara, Norway) 1 month after planting. Irrigation, biological pest control measures and fungicides were applied to safeguard plant development. Various parameters of each single plant were measured, applying SI units as given in Table 2 (cabbage) and Table 3 (swede and turnip). In addition, leaf colour, head shape and head density were scored for cabbage plants according to categories supplied by UPOV (2004). For swede, leaf type, root skin colour and root shape were scored (UPOV, 2009), as were leaf type, root skin colour, flesh colour and root shape for turnip (UPOV, 2001).

Statistical analysis

Data was processed following the guidelines defined by Jonge and van der Loo (2013) and fed into R software (R Core Team, 2014). Boxplots were used to survey the distribution of continuous numeric descriptors. Tukey multiple comparison of means with a 95% family-wise confidence level was used to examine differences among pairs/triplets (Crawley, 2009). The UPOV descriptors (categorical data) were examined by chi-squared statistics. Initially, a chi-square hypothesis test was made for each descriptor, including all data, to verify the relevance of the given descriptor. In cabbage, leaf type and leaf hairs showed no variation and were not included in the further analysis. Similarly, leaf anthocyanin and root flesh anthocyanin coloration in turnip and leaf type in swede were not included in further analysis as no variation was found. Thereafter, a comparison of accessions within a pair/triplet was done. A cluster analysis was conducted for the significant numeric descriptors only. The R function *heatmap* was used to demonstrate dendrograms for both accessions and variables in the same picture; dissimilarities are expressed as different colours. This is a two-way cluster analysis.

Results

In general, some variability was observed within accessions showing that accessions were not uniform. As expected, some clustering of accessions within pairs/triplets was detected (Figs. 1 and 2). Each figure contains two dendrograms, one for accessions and one for descriptors, shown on either side of the graph. The plotted grinds illustrate the extent of dissimilarity between each combination. Identical colour indicates the same response. In cabbage, the two Staup accessions (S1 and S2) were in different

Table 1. Overview of the accessions that was included in the study

Code	Accession name and number	Genebank	Acquisition source	Year
Cabbage				
A2	Amager Høj '206', NGB13547	NGB	Denmark, Dæhnfeldt	1998
A5	Amager Høj Grøn Kalida, NGB1889	NGB	Denmark, A. Hansen	1980
B4	Blatopp Kvithamar Familie 32, K2244	VIR	Norway, Unknown	1967
B5	Blåtopp Fam. 1 (Tidlig Kvithamar), NGB4554	NGB	Norway, Kvithamar	1984
D1	Dural Resistant Winter, K2577	VIR	Denmark, Unknown	1990
D2	Dural, NGB11800	NGB	Denmark, Tystofte	1997
L4	Langendijker Vinter Dural, NGB1886	NGB	Denmark, Ohlsens Enke	1980
L5	Langendijker Vinter Vernido, NGB1893	NGB	Denmark A. Hansen	1980
OL1	Olsok, K2550	VIR	Norway, Unknown	1988
OL2	Olsok, NGB11567	NGB	Norway, Ministry of Agriculture	1995
S1	Staup, K2173	VIR	Norway, Unknown	1961
S2	Tronder Staup St., K2247	VIR	Norway, Unknown	1967
Swede				
SV1	Svalof Victoria, K597	VIR	Sweden, unknown	1958
SV2	Viktoria, NGB7176	NGB	Sweden, Svalöf	1975
WI 1	Wilhelmsburger Danila Trifolium, K691	VIR	Denmark, unknown	1964
WI2	Wilhelmsburger Danila, NGB8384	NGB	Denmark, Royal Veterinary and Agricultural University	1989
KA1	Kafifafellsrofur, K587	VIR	Iceland, unknown	1956
KA2	Kafifafellsrofur, NGB4142	NGB	Iceland, Rala	1984
Turnip				
SO1	Solanepa, NGB7799	NGB	Norway, Agricultural	1989
SO2	Solanepa, K1180	VIR	University of Norway	1960
SO3	Sola from Jotunsalget, K1186	VIR	Norway, unknown	1960
OS1	Ostersundom, K982	VIR	Sweden, unknown	1956
OS2	Svalofs Ostersundom, K1061	VIR	Sweden, unknown	1957
PT1	Purple top strap leaf, K1231	VIR	Denmark, unknown	1964
PT2	Red top globe, K1226	VIR	Denmark, unknown	1964
TO1	Topas Bortf, K1274	VIR	Sweden, unknown	1968
TO2	Topas, K1377	VIR	Sweden, unknown	1979

clusters, as were the two Amager accessions (A2 and A5) (Fig. 1). The different descriptors fell into two main clusters, where for example head height and plant diameter were close.

In swede, the Kafifafellsrofur pair (KA1 and KA2) fell into two different clusters (Fig. 2). In turnip, one of the three Sola turnip accessions (S3) was not grouped with S1 and S2. Here, one of the Purple top accessions (PT1) was in the same cluster as the two Sola accessions.

The ANOVA tests showed that all numeric descriptors were relevant for examination with Tukey multiple comparison of means. In cabbage, and based on the numeric descriptors, no clear distinction could be made for three out of six cabbage pairs (Table 2). Significant differences were detected in one character within the Staup pair, the

Langendijker Vinter pair and the Blåtopp Kvithamar pairs, respectively. Based on the qualitative, categorical descriptors only minor differences were detected among accessions for the cabbage pairs.

In turnip, three out of four pairs/triplets demonstrated significant differences in two or more numeric descriptors (Table 3). Only the Topas pair (TO1 and TO2) showed no differences in any of these characters. For the qualitative, categorical descriptors, all SO3 plants showed a lobed leaf type, while SO1 and SO2 plants both had an entire leaf type. Furthermore, all PT2 plants were lobed, while PT1 plants had entire leaves. In swede, the Kafifafellsrofur pair (K1 and K2) showed clear differences in number of lobes and lobe length but no major differences in the qualitative, categorical descriptors.

Table 2. Mean value with standard deviation of the numeric descriptors in cabbage (*Brassica oleracea* var. *capitata*) accessions

	Leaf length (cm)	Plant dia-meter (cm)	Plant height (cm)	Head dia-meter (cm)	Head height (cm)	Head weight (kg)	Core length (cm)	Time to maturity (days)
Amager								
Høj pair								
A2 (NGB13547)	21 ± 4 ^A	55 ± 12 ^A	52 ± 8 ^A	15 ± 3 ^A	18 ± 4 ^A	1.2 ± 0.5 ^A	10 ± 2 ^A	170 ± 19 ^A
A5 (NGB1889)	28 ± 5 ^A	58 ± 9 ^A	53 ± 6 ^A	14 ± 1 ^A	14 ± 3 ^A	1.1 ± 0.3 ^A	12 ± 2 ^A	169 ± 14 ^A
	NS	NS	NS	NS	NS	NS	NS	NS
Blåtopp								
Kvithamar pair								
B4 (K2244)	23 ± 3 ^A	55 ± 10 ^A	41 ± 6 ^A	15 ± 2 ^A	15 ± 3 ^A	1.3 ± 0.4 ^A	9 ± 1 ^A	148 ± 10^A
B5 (NGB4554)	22 ± 2 ^A	55 ± 8 ^A	35 ± 4 ^A	16 ± 2 ^A	17 ± 2 ^A	1.4 ± 0.4 ^A	9 ± 1 ^A	128 ± 10^B
	NS	NS	NS	NS	NS	NS	NS	P < 0.05
Dural pair								
D1 (K2577)	28 ± 6 ^A	71 ± 18 ^A	51 ± 7 ^A	15 ± 3 ^A	15 ± 2 ^A	1.5 ± 0.8 ^A	12 ± 2 ^A	164 ± 12 ^A
D2 (NGB11800)	25 ± 3 ^A	62 ± 8 ^A	47 ± 6 ^A	13 ± 2 ^A	15 ± 2 ^A	1.1 ± 0.5 ^A	10 ± 2 ^A	164 ± 15 ^A
	NS	NS	NS	NS	NS	NS	NS	NS
Langendijker								
Vinter pair								
L4 (NGB1886)	27 ± 7 ^A	67 ± 11 ^A	50 ± 5 ^A	15 ± 2 ^A	14 ± 2 ^A	1.5 ± 0.5 ^A	8 ± 1^A	152 ± 10 ^A
L5 (NGB1893)	24 ± 6 ^A	71 ± 19 ^A	47 ± 6 ^A	15 ± 4 ^A	16 ± 2 ^A	1.7 ± 0.8 ^A	12 ± 2^B	156 ± 21 ^A
	NS	NS	NS	NS	NS	NS	P < 0.05	NS
Staup pair								
S1 (K2173)	27 ± 4 ^A	64 ± 15 ^A	44 ± 6^A	18 ± 2 ^A	19 ± 1 ^A	1.8 ± 0.5 ^A	11 ± 3 ^A	133 ± 7 ^A
S2 (K2247)	23 ± 5 ^A	45 ± 16 ^A	32 ± 5^B	15 ± 3 ^A	17 ± 2 ^A	1.4 ± 0.5 ^A	11 ± 2 ^A	137 ± 7 ^A
	NS	NS	P < 0.05	NS	NS	NS	NS	NS
Olsok pair								
OL1 (K2550)	21 ± 3 ^A	50 ± 7 ^A	31 ± 3 ^A	14 ± 2 ^A	19 ± 4 ^A	1.2 ± 0.4 ^A	7 ± 2 ^A	123 ± 7 ^A
OL2 (NGB11567)	23 ± 3 ^A	59 ± 9 ^A	34 ± 4 ^A	15 ± 3 ^A	13 ± 2 ^A	1.4 ± 0.7 ^A	8 ± 1 ^A	120 ± 2 ^A
	NS	NS	NS	NS	NS	NS	NS	NS

Significant differences in bold.

Petiole length, width and thickness, leaf lamina width and core width not included.

Across the three *Brassica* vegetable species, around half of the pair/triplets showed clear differences in one or more descriptors, while the other half showed no differences and could thus be regarded as duplicates.

Discussion

To maintain a high number of accessions is costly, especially for outcrossing species such as *Brassica* vegetables.

From a management point of view, any reduction in the number of accessions should be welcomed. Our study clearly highlights the need to include characterization in the duplicate assessment process. In cabbage, plant height, core length and time to maturity were the most useful characters to distinguish between the cabbage pairs. Root length and leaf width were most useful characters for turnip and swede, but leaf type, number of lobes, and lobe length were also useful. In cabbage, we can summarize that the Olsok pair and the Dural pair are true duplicates. In

Table 3. Mean value with standard deviation of the numeric descriptors in turnip and swede accessions

		Number of lobes	Lobe length (cm)	Lobe width (cm)	Leaf length (cm)	Leaf width (cm)	Root length (cm)	Root diameter (cm)
Swede	Kafifaellsrofur pair							
	KA1 (K587)	6 ± 1^A	19 ± 3^A	18 ± 3 ^A	51 ± 5 ^A	16 ± 3 ^A	12 ± 2^A	11 ± 3 ^A
	KA2 (NGB4142)	7 ± 1^B	14 ± 3^B	16 ± 2 ^A	50 ± 5 ^A	18 ± 2 ^A	14 ± 2^B	12 ± 2 ^A
		P < 0.05	P < 0.05	NS	NS	NS	P < 0.05	NS
	Wilhelmsburger pair							
	WI1 (K691)	7 ± 1 ^A	16 ± 3 ^A	17 ± 3 ^A	53 ± 6 ^A	17 ± 3 ^A	11 ± 3 ^A	10 ± 3 ^A
	WI2 (NGB8384)	8 ± 1 ^A	18 ± 2 ^A	17 ± 4 ^A	56 ± 4 ^A	18 ± 3 ^A	13 ± 2 ^A	12 ± 2 ^A
		NS	NS	NS	NS	NS	NS	NS
	Viktorija pair							
	SV1 (K597)	7 ± 2 ^A	12 ± 3 ^A	14 ± 2 ^A	39 ± 5 ^A	16 ± 3 ^A	10 ± 2 ^A	10 ± 2 ^A
SV2 (NGB7176)	7 ± 1 ^A	14 ± 2 ^A	15 ± 3 ^A	45 ± 5 ^A	18 ± 4 ^A	8 ± 1 ^A	10 ± 2 ^A	
	NS	NS	NS	NS	NS	NS	NS	
Turnip	Topas pair							
	TO1 (K1274)	27 ± 7 ^A	67 ± 11 ^A	50 ± 5 ^A	47 ± 8 ^A	18 ± 3 ^A	8 ± 2 ^A	6 ± 2 ^A
	TO2 (K1377)	24 ± 6 ^A	71 ± 19 ^A	47 ± 6 ^A	47 ± 4 ^A	17 ± 2 ^A	9 ± 1 ^A	5 ± 1 ^A
		NS	NS	NS	NS	NS	NS	NS
	Purple top pair							
	PT1 (K1231)	0 ± 0^A	NA	NA	40 ± 4 ^A	14 ± 2^A	5 ± 1^A	10 ± 1 ^A
	PT2 (K1226)	9 ± 2^B	13 ± 2	14 ± 2	45 ± 6 ^A	17 ± 3^B	8 ± 2^B	10 ± 2 ^A
		P < 0.05	NA	NA	NS	P < 0.05	P < 0.05	NS
	Ostersundom pair							
	OS1 (K982)	10 ± 2 ^A	13 ± 1^A	17 ± 2^A	42 ± 7^A	19 ± 3^A	17 ± 1^A	7 ± 1 ^A
	OS2 (K1061)	10 ± 1 ^A	17 ± 2^B	19 ± 1^B	53 ± 3^B	23 ± 2^B	13 ± 1^B	8 ± 1 ^A
		NS	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05	NS
	Solanepe triplet							
	SO1 (NGB7799)	0 ± 0^A	NA	NA	42 ± 5^A	14 ± 2^A	4 ± 1^A	10 ± 2 ^A
	SO2 (K1180)	0 ± 0^A	NA	NA	40 ± 6^A	14 ± 3^A	5 ± 3^A	8 ± 3 ^A
SO3 (K1186)	8 ± 1^B	15 ± 2	23 ± 3	47 ± 3^B	17 ± 2^B	10 ± 1^B	9 ± 1 ^A	
	P < 0.05	NA	NA	P < 0.05	P < 0.05	P < 0.05	NS	

Significant differences in bold.

swede, the Wilhelmsburger pair and the Viktoria pair should be regarded as true duplicates and in turnip, the Topas pair. Across species, around half of the pairs/triplets did not fully match. In most of these, one or two traits that did not match. However, in the Ostersundom turnip pair, the Sola turnip triplet, and the Kafifaellsrofur swede pair, there were more characters that differ. The overall result is in line with Axel Diederichsen's observations (2009) on oat (*Avena sativa*) accessions in the Canadian genebank. Indeed, having the same name does not necessarily mean that the holdings are duplicates. Numerous causes may account for the differences. Genetic drift caused by random forces, and genetic shift, caused by selection due to errors or improper regeneration protocols, have been showed to cause changes in material conserved *ex situ*

(Soleri and Smith, 1995; Gomez *et al.*, 2005; Negri and Tiranti, 2010). This risk is particularly relevant in small populations during regeneration (Ellstrand and Elam, 1993; Solberg *et al.*, 2017). Van Hintum *et al.* (2007) showed that genetic changes under standard genebank regeneration were of a magnitude comparable to the differences among white cabbage accessions with the same or similar names. Van Hintum's team studied genetic diversity applying AFLP markers and questioned conservation of a large number of similar accessions if accessions are in any case changed by regeneration. Van Treuren *et al.* (2009) proposed a strategy to structure genebank collections to avoid under- and over-representation of accessions within each of its different components, building on the concept of core collection, to facilitate a relevant collection for

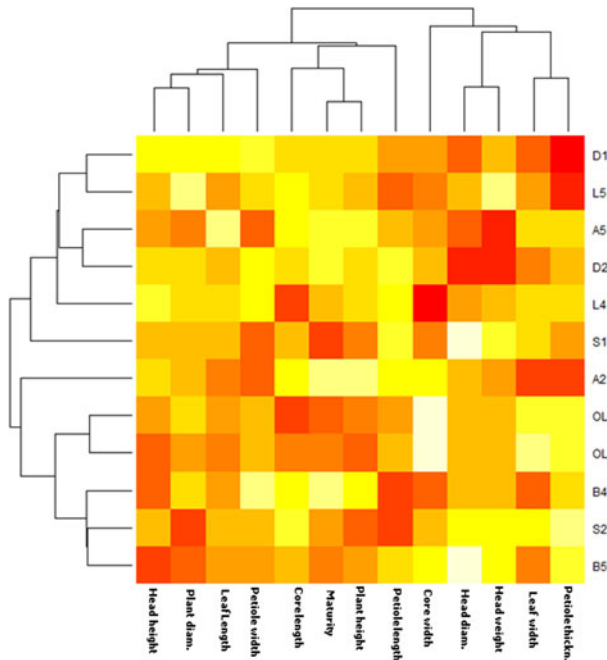


Fig. 1. Two-way cluster diagrams (heatmap) of cabbage (see text for explanation).

users. In cases with extensive overlap in diversity, duplicates could be removed or bulked (Van Hintum *et al.*, 2002; Cruz *et al.*, 2006). In our case, most putative duplicate accessions are maintained by different genebanks and bulking is not an option, but only one of three similar accessions might be selected to become part of the European Collection. Our study demonstrated that relying on passport data exclusively is fraught with pitfalls. A common

challenge is passport data quality; how accessions are named and how information is organized in genebanks. Here, the past century's cultivar naming practice proved to be a challenge. For any given cultivar, a number of strains could be present, as seed companies or research stations frequently made different selections, but retained the original cultivar name or added a second or third name, for example in the case of the cabbage cultivar 'Blåtopp Kvithamar'. Names given in this period often indicated a 'strain' or selection within a cultivar type. Furthermore, in many cases a third name was involved. In the Blåtopp Kvithamar case, time to maturity was the only trait that varied between B4 (Blåtopp Kvithamar Familie 32) and B5 (Blåtopp Fam. 1 Tidlig Kvithamar), where the latter accession was 20 d earlier than the other. This was indicated in the selection name, where the Norwegian 'Tidlig Kvithamar' translates as 'Early Kvithamar'. The two accessions are definitely not duplicates but morphologically closely related. Another example is the Ostersunddom pair. The accessions differ in the majority of the descriptors. Both accessions are maintained in the VIR genebank, and both were received from Sweden in the late 1950s. We have no good explanations why the two accessions are so different, we could only speculate. In the Nordic countries, national variety lists were established in the 1950s (NPVB, 1952) and the UPOV convention came into force in 1969 (NPVB, 1960; Jördens and Button, 2011). Some strains or selections were no longer able to fulfil the DUS-criteria (distinct, uniform and stable) of UPOV, which may be one reason why many cultivars were removed from the variety lists between 1970 and 1980 (Solberg and Breian, 2015). Nevertheless, major agro-botanical variation, as detected in this study, demonstrates the value of agro-botanical

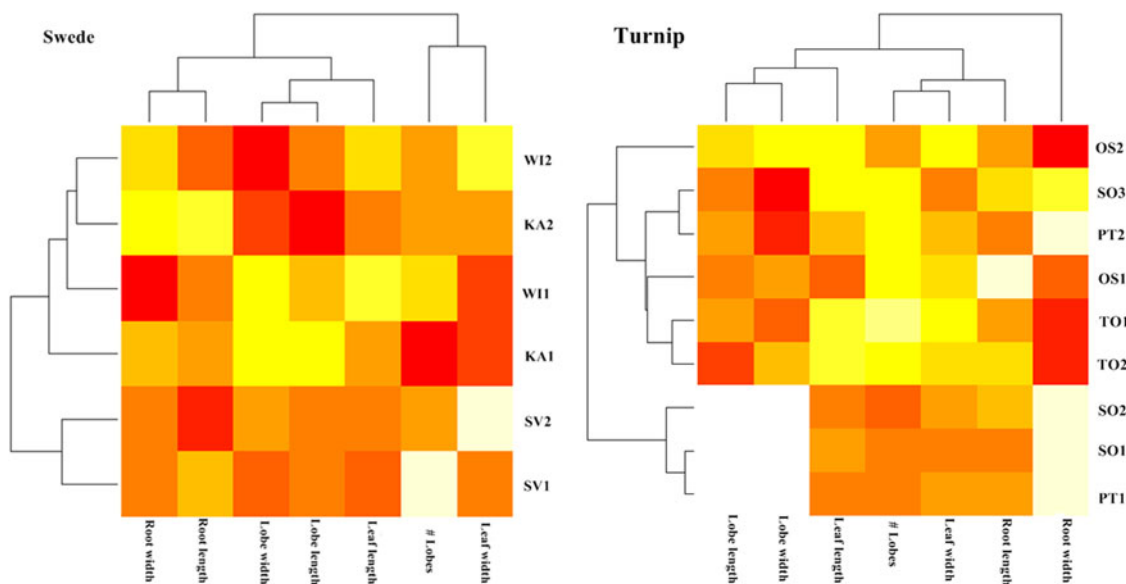


Fig. 2. Two-way cluster diagrams (heatmap) of turnip and swede (see text for explanation).

characterization. Indeed, accessions with the same or similar names do not necessarily represent the same material. AEGIS has suggested a road-map for duplicate assessment of accessions at a European level. We would stress that elimination of accessions should only take place in situations where solid data are available.

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