DOI:10.30901/2227-8834-2016-4-18-27

УДК 633.16; 582

ОРИГИНАЛЬНАЯ СТАТЬЯ

НИЗКОЗАТРАТНЫЙ МЕТОД ДЛЯ ОПРЕДЕЛЕНИЯ ДУБЛЕТОВ КОЛЛЕКЦИИ В ГЕННЫХ БАНКАХ

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Актуальность. В настоящее время в различных генбанках мира хранятся образцы, сходные по названию и происхождению. Однако неизвестно являются ли они дублетами. Материал и методы. С целью разработки простого и недорогого метода для выявления дублетов в генбанках были выбраны образцы ячменя (*Hordeum vulgare* L.) скандинавского происхождения, хранящиеся в коллекциях ВИР им. Н. И. Вавилова и Нордического генного банка. Первый этап включал выявление образцов с одинаковыми названиями на основе паспортных баз данных в разных генных банках; второй – полевое изучение образцов, представляющих вероятные дублеты, третий – углубленное изучение с использованим более сложных методов для образцов, имеющих различия. Результаты и заключение. В двух коллекциях было выявлено 185 пар образцов с однаковыми названиями. Каждая пара была высеяны в поле рядом с друг другом на одной делянке и изучена по одним и тем же морфологическим признаки. У большинства пар изученных образцов различий не выявлено (63%) т. е. четыре из каждых шести пар предположительно являются дублетами в двух генных банках. У 13% пар образцов наблюдались значительные достоверные различия, а у 24% пар различия были недостоверны, что предполагает их более углубленное изучение. Таким образом, результаты изучения показали, что при помощи простого полевого скрининга образцов можно выявить вероятные дублеты коллекции или образцы, которые требуют более детального исследования. Результаты данного исследования важны как для работы по управлению конкретным генбанком, так и для проведения исследований между генными банками.

Ключевые слова:

ячмень, коллекция, генбанк, дублеты, скрининг, морфологические признаки

Поступление:
04.10.2016

Принято:
06.12.2016

DOI:10.30901/2227-8834-2016-4-18-27

ORIGINAL ARTICLE

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Key words:

barely, Hordeum vulgare, genebank management, morphology, screening

Received:
04.10.2016

Accepted:
06.12.2016

A LOW-COST METHOD FOR THE DETECTION OF DUPLICATE HOLDINGS AMONG GENEBANK ACCESSIONS

Background. World genebanks hold some accessions with similar names and origin. The question is whether accessions with identical or similar names preserved in genebanks are duplicates or not. Materials and methods. A study was performed in the Russian and Nordic collections of barley (*Hordeum vulgare* L.) to establish a simple, low-cost method for the detection of duplicates. The method included two steps: 1) Identify accessions with identical names based on passport data, 2) Agro-botanical screening of the accessions dividing the accessions into most likely duplicates, probably distinct accessions or accessions that would need further investigation. In a third step, more sophisticated characterization of the latter group should be conducted in future. 185 pairs of accessions subsequently cultivated side by side for a screening, one plot per accession, and scored by the same person using a set of morphological descriptors. Results and conclusion. In total, 185 pairs of accessions with the same name were identified in the two collections, cultivated, and scored using a set of morphological descriptors. Within-pair differences more than two standard deviations from the mean differences were highlighted. No differences were detected in the majority of the pairs (63%). In four out of every six pairs no differences were detected for any of the agro-botanical characters. The accessions are most likely duplicates. In 13% of the pairs, the accessions were probably distinct and another 24% fell in-between these categories and required further investigation. The results showed that a simple screening can reduce the number of duplicates or the number of accessions put into further investigations. The results are relevant for genebank management and collaboration between genebanks.

Introduction

Genebanks for orthodox seed germplasm have to maintain seed viability and to facilitate use in breeding and research (Vertucci, Roos, 1990; Walters, 2004; FAO, 2010). The germplasm collection at the Nordic Genetic Resources Center at Alnarp, Sweden, (here abbreviated as NGB) consists of approx. 35,000 accessions. One of the world's oldest and largest collections, with more than 325,000 accessions, is held at the N. I. Vavilov Institute of Plant Genetic Resources (VIR) in St. Petersburg. The idea of establishing *ex situ* collections spread during the twentieth century and today 1500 collections can be found globally with a total number of 7.4 million accessions (FAO, 2010). Far from all of these accessions are unique as there have been extensive duplications among collection holders (Plucknett et al., 1987; van Hintum, Visser, 1995; van Hintum, Boukema, 1999; Germeier et al., 2003; van Treuren et al., 2009). However, according to FAO (1998) many collections are in an unsatisfactory state due to excessive expansion combined with inadequate resources. In Europe, the European Genebank Integrated System (AEGIS) aims to minimize duplications (ECPGR, 2008; Engels, Maggioni, 2012; Veteläinen, 2012). One approach has been to search for samples with the same or similar names and to start a process to reduce duplicates from there. This was also our motivation. We know that seeds have been exchanged between the Nordic countries and Russia. The records indicated that Nicolai Vavilov had for example contact with the Botanical Garden in Copenhagen in the period between World War 1 and World War 2 and with the breeders in Weibullsholm and in Svalöf (Loskutov, 1999). The question is whether accessions with identical or similar names preserved in genebanks are duplicates or not.

Material and methods

For this study, barley (*Hordeum vulgare* L.) was selected as a model crop. A two step method was suggested where the first step was to identify accessions with identical names based on passport data. A second step was an agro botanical screening of the identified accessions, resulting in a division of the pairs into most likely duplicates, probably distinct accessions or accessions that would need further investigation.

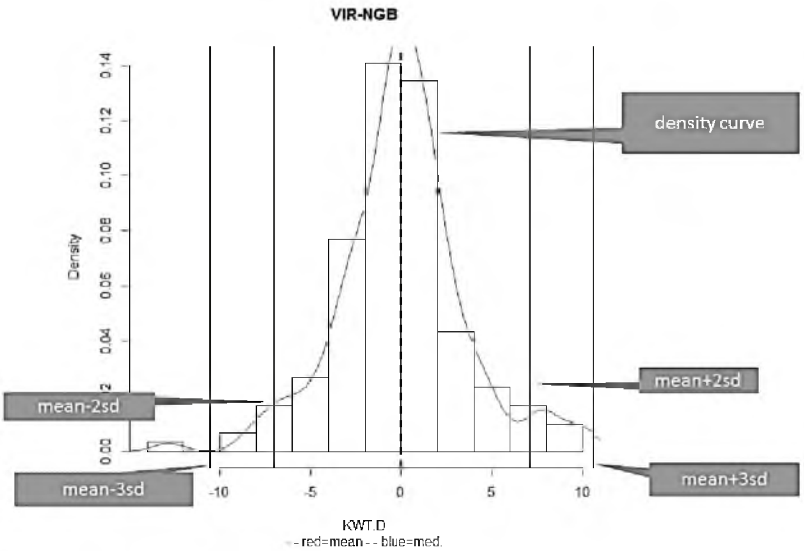

График распределения различий по значению показателя масса 1000 зерен у изученных 185 пар потенциальных дублетов
Graph showing the distribution of the differences in values for the character 1000 kernel weight (KWT) when comparing the two values in the value pairs of 185 potential duplicates

Passport data were extracted from the NGB and VIR databases. We searched for accessions with same (or very similar) names in the two collections and were able to detect more than 200 such pairs. Pairs where accessions were donated from the other genebank or pairs where seeds were not available were discarded from the study resulting in 185 pairs of potential duplicates. The agro botanical screening was carried out in a field at the Pushkin branch of VIR, located in the Leningrad region. Pairs were seeded next to each other, one plot per accession. Each plot was scored once by the same person and and according to standard characterization guidelines for barley developed at VIR (Loskutov et al. 2012, table 1). Statistical analysis was performed using R software (R Core Team, 2014). For each character we calculated a mean difference between VIR and NGB, and standard deviations of the difference. Figure illustrates a histogram for one of the characters; 1000 kernel weight (KWT), showing a two tailed graph with a normal distribution when comparing the two values in 185 potential duplicates. The R function *hist* was used for surveying the distribution pattern and exclude characters with no or very little variation were removed from the further analysis (Glume width, Awn roughness, and Kernel covering; data not shown). Rachilla hair length was scored but not included in the analysis due to uncertainties in the scoring method (short or long category only). After analysis, the accession pairs were divided into three categories based on the following criteria: (1) Probably different accessions: more than two of the characters show differences exceeding two standard deviations from mean difference, (2) Doubtful duplicates: one or two of the characters show differences exceeding two standard deviations from mean difference, and (3) Similar accessions: none of the characters show differences exceeding two standard deviations from mean difference.

Results and discussion

The results are summarized in table 2. No differences were detected in the majority of the pairs and these were regarded as duplicates (accession details not shown). In 13 and 24% of the pairs, accessions were seemingly different or doubtful duplicates. The details are shown in table 3 and table 4, respectively. Worth noting is that four pairs include accession of different sub-type; 6-rows or 2-rows barley, respectively.

Таблица 1. Результаты полевого изучения в баллах (Аббр.), средняя разница между данными по 185 парам образцов из коллекции ВИР и Нордического генного банка, и значения стандартных отклонений

Table 1. Examined agro-botanical traits with code (Abbr.) and mean difference between VIR and NGB accessions (185 value pairs) with the standard deviations of the difference

Character Признак	Abbr. Аббр.	Mean difference (VIR – NGB) Средняя разница	Standard deviation of the difference Стандартное отклонение
Continuous, numerical			
Spike lenght (cm) Длина колоса (см)	SL	– 0.20	1.46
Seeds per spike (in number) Число зерен в колосе, шт.	SNS	– 1.08	7.82
Spikelet per spike (in number) Число колосков в колосе, шт.	SGS	– 0.92	7.84
Days from heading to maturity Дни от колошения до созревания	DHM	– 0.08	0.74
Days from seeding to heading Дни от всходов до колошения	DHE	– 0.05	0.73
Days from seeding to maturity Дни от всходов до созревания	DMA	– 0.02	0.44

Character Признак	Abbr. Аббр.	Mean difference (VIR – NGB) Средняя разница	Standard deviation of the difference Стандартное отклонение
1000 kernel weight (g) Масса 1000 зерен, г	KWT	0.06	3.79
Plant height (cm) Высота растений, см	PLH	0.00	0.52
Categorical, numerical[a]			
Resistance to powdery mildew Устойчивость к мучнистой росе	RPM	0.23	0.21
Hoodedness-awnedness[b]	H_A	0.01	0.15
Resistance to lodging Устойчивость к полеганию	LOD	– 0.02	0.37
Spike density Плотность колоса	SDE	– 0.04	1.39
Resistance dark-brown spot blotch Устойчивость к темно-бурой пятнистости	RDBB	0.01	0.50
Categorical, non-numerical			
Lemma colour[c] Цвет колосковых чешуй	LCO	0.05	0.61
Spike type (1 six row, 2 two row) Тип колоса (1 6-рядный, 2 2-рядный	RNO	– 0.01	0.23
Colour of caryopsis[d] Цвет зерновки	CCO	0.01	0.31

[a] Categorical for resistance characters and density were 1-very low, 3-low, 5-intermediate, 7-high, 9-very high (very good). [b] Hoodedness-awnedness categories were: 1-sessile hoods, 2-elevated hoods, 3-awnless or awned (<2 cm), 4-awned on central rows only for two rowed forms, on all 6 rows - for six-rowed forms, 5-awned on central rows only, lateral rows awnless or awned for 6-row forms only. [c] Lemma colour; 1-white/brown, 2-yellow, 3-white, 4-brown, 5-black, 6-purple. [d] Colour of caryopsis; 1-white, 2-blue, 3-black. [a]Баллы по устойчивости и плотности колоса: 1 – очень низкая, 3-низкая, 5-среднее, 7-высокая, 9-очень высокая. [b]

Таблица 2. Возможно разные образцы, сомнительные дублеты и одинаковые образцы, выделенные в процессе сравнительного анализа результатов агроботанической оценки 185 пар потенциальных дублетов Hordeum vulgare из коллекций ВИР и Нордического генного банка

Table 2. Probably different accessions, doubtful duplicates and similar accessions based on comparing agro botanical characterization results in 185 pairs of potential duplicates between VIR and NordGen holdings of Hordeum vulgare

Category	Number of accession pairs	% of accession pairs
Probably different accessions	24	13
Doubtful duplicates	44	24
Similar accessions	117	63
Total	185	100

Таблица 3. Пары образцов со значительными различиями между ними (различия в два стандартных отклонения обозначены – X и в три стандартных отклонения – XX)

Table 3. These 24 accession pairs showed considerable differences among the two samples. They had within-pair differences of more than two standard deviations from mean difference in all pair comparisons (marked as x) or three standard deviations from mean value (marked as xx)

Accession pair information					**Continous, numeric characters**								**Categorical, numeric**					**Non-numeric**		
Name	Year	Country [a]	VIR #	NGB #	SL	SNS	SGS	DHM	DHE	DMA	KWT	PLH	RPM	H_A	LOD	SD	RDBB	LCO	RNO	CCO
Akta	1984	DK	k30491	2665				XX	XX						X			(X)		
Alf	1978	DK	k26909	4707						XX								(X)		
Arabische	L	-	k20923	8245		XX				XX							XX			(X)
Åsa	1949	SE	K18502	1487													XX		(X)	(X)
Caminant	1994	DK	K30374	15054						X				XX				(X)		
Camir	1986	DK	K29776	10710			XX	XX												
Chevalier	1830	UK	K18045	9443													XX	(X)		
Dore	1932	SE	K15536	6272		X									X	XX				
Etu	1970	FI	K21834	332						XX								(X)		
Frida	1984	SE	K29420	1519	X												XX			
Gula	1976	DK	K26806	13681				X	XX		XX									
Jadar II	1947	No	K19035	457		X											XX			
Jotun	1930	No	K19037	466		X											XX	(X)		
Laari	L	FI	K29877	273											X		XX			
Magda	1989	SE	K29761	9949	XX															
Mari	1960	SE	K19354	4694										XX			XX			
Mie	1995	SE	K19494	12285		XX						XX			X	XX			(X)	(X)
Pamina	1981	SE	K20466	2675		X	XX	XX	X							XX			(X)	(X)
Patrik	1980	SE	K29421	2676													XX			
Polar	1933	No	K30048	2277						X								(X)		
Riegel	1941	DK	K21879	8818			XX		XX		XX	XX		XX		XX				
Ringve	1972	No	K23664	2078											XX			(X)		
Silja	1979	FI	K26913	9280	XX	X									X					(X)
Suvi	1973	FI	K21992	296	X	XX				X					X			(X)	(X)	(X)

[a] DK is Denmark, SE is Sweden, FI is Finland, NO is Norway, DE is Germany, UK is United Kingdom, L is landrace

Таблица 4. Пары образцов с незначительными различиями между ними (различия в два стандартных отклонения обозначены – X и в три стандартных отклонения – XX)

Table 4. These 44 accession pairs showed differences among the two samples and represent doubtful duplicates. They had differences in one or two characters. Within-pair differences greater than two standard deviations from mean difference in all pair comparisons (marked as x) or three standard deviations from mean value (marked as xx)

Accession pair information					Continous, numeric characters								Categorical, numeric					Non-numeric		
Name	Year	Country[a]	VIR #	NGB #	SL	SNS	SGS	DHM	DHE	DMA	KWT	PLH	RPM	H_A	LOD	SD	RDBB	LCO	RNO	CCO
Archer	1927	DK	ꝁ3537	6933											X					
Anita	1962	No	ꝁ19447	15250														(X)		
Annika	1983	SE	ꝁ30052	9929	X															
Apex	1982	NL	ꝁ28001	13689	X													(X)		
Arena	-	-	K28947	13687																
Arla	1962	SE	K20508	2681														(X)		
Arra	1984	FI	K28189	4011											X					
Arvo	1966	FI	K19958	303						X										
Bente	1982	SE	K28948	9289												XX				
Bingo	1984	DK	K29234	9933														(X)		
Bomi	1966	DK	K21887	5096						X										
Bonus	1950	SE	K17017	1489						X					X					
Canut	1987	DK	K29775	13381													XX			
Danpro	1969	DK	K22139	9659	X															
Denso	-	DK	K18816	8826											X					
Donnes	L	No	K4249	456														(X)		
Duks	1975	DK	K25108	9651														(X)		
Foma	1961	SE	K19353	1492												XX				
Griar	-	No	K24005	15153		X									X					
Haaraniemi	L	FI	K29878	320											XX					
Harry	1978	SE	K26916	2666						X								(X)		
Hellas	1967	SE	K20320	1495						X					X					

Accession pair information					**Continous, numeric characters**								**Categorical, numeric**					**Non-numeric**		
Name	Year	Country[a]	VIR #	NGB #	SL	SNS	SGS	DHM	DHE	DMA	KWT	PLH	RPM	H_A	LOD	SD	RDBB	LCO	RNO	CCO
Jonna	1980	DK	K27568	4719													XX			
Kilta	1981	FI	K28190	301														(X)		
Kristina	1968	SE	K20321	1500														(X)		
Maskin	1918	No	K8506	459											X			(X)		
Mette	1984	SE	K29163	9275											X					
Nordlys	1962	No	K20478	2076											X					
Numaen	-	-.	K15623	15147		X				X										
Paavo	1959	FI	K19360	13661		X														
Pendo	-	DK	K19912	9635														(X)		
Rauto	L	FI	K29876	265	X	X														
Reform	2000	DK	K25926	1521														(X)		
Robert	1985	DK	K28944	6310													XX	(X)		
Romi	1983	DK	K28912	6307									XX							
Senat	1974	SE	K21931	1503													XX			
Simon	-	SE	K27972	2668														(X)		
Siri	1969	DK	K22007	9637												XX				
Stallar II	1952	SE	K30053	2661														(X)		
Stella	1935	SE	K16497	1484														(X)		
Svanhals	1903	SE	K6496	9997												XX				
Tikkurilla	-	-	K17557	15354														(X)		
Varde	1941	No	K17013	8861		X														
Vigdis	1964	No	~~K~~20316	2083		X														

[a] DK is Denmark, SE is Sweden, FI is Finland, NO is Norway, DE is Germany, UK is United Kingdom, L is landrace

There are several possible explanations for the differences found in accessions with identical names. Different cultivars could have the same name. Among the material this was the case for the pair Bonus. Bonus is a Swedish variety released in 1950 but there also exists a Norwegian variety with the same name released by a local research station in the 1930s. Misspellings could be another reason to error, especially with different languages involved. In the case of *Akta*; the NGB accession NGB2665 was misspelled to *Akka* and by mistake paired wrongly. Other mistakes could be caused by the fact that some Cyrillic letters look like certain Latin letters but have different character encodings. For example; Frida which is a Swedish variety from 1984 but Frieda is a German variety. Erroneous handling of seed lots in the genebank can also bias the pairs. A more biological explanation to differences is that forces are continuously acting on the genetic make-up of the populations (Ellstrand, Elam, 1993; Gomez et al., 2005, Ouborg et al., 2006; Negri, Tiranti, 2010). The relationship between population size and genetic diversity has been well described (Ellstrand, Elam, 1993; Dittbrenner et al., 2005; Hensen, Oberpieler, 2005; van Treuren et al., 1991). In genebanks, regeneration is a critical step (Leino et al., 2013; Solberg et al., 2015) and standards have been developed to reduce genetic drift and erroneous germplasm handling (FAO, 2014). Combined field assessment with more a sophisticated method was suggested by Diederichsen (2009) as the most efficient way for determination of internal duplication holdings. Our study highlights the cost-effectiveness of including an initial field screening before more extensive assessments with sophisticated analysis. By adding such a screening the number of accessions included in the more sophisticated analysis could be reduced by two third. For genebanks such cost reductions are of great value. A screening method using one plot per accession represents a low-cost method that could potentially be useful for the extraction of a subset of accessions for more detailed examination. This second examination could be performed using for example molecular markers (Lund et al., 2003), other biochemical analysis (Perchuk et al., 2016) or more detailed morphological characterization based on multi-locational experiments (Diederichsen, 2009). Our study furthermore demonstrates the need of including agro botanical characterization in duplicate assessment process. Rejection of accessions based on accession names only could lead to the loss of diversity. Including passport data analysis in the process would be better (van Hintum, Knupffer, 1995) however including agro botanical screening should be part of a standard procedure to reduce duplicate holdings. Our case study in barley showed that one of the accessions in more than 60% of the pairs we could be eliminated. From a genebank management perspective this would reduce the long-term maintenance costs. The European Genebank Integrated System has tried to suggest a road-map from which of the collections such elimination should take place, however, elimination can only take place when long-term commitment and collaboration among collection holders and nations are present.

Acknowledgements

The work is dedicated to Sergey M. Alexanian, Vice Director for Foreign Relations of VIR, who passed away in 2014.

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