

SELECTION OF RESISTANT SPRING BARLEY ACCESSIONS AFTER NATURAL INFECTION WITH LEAF STRIPE (*PYRENOPHORA GRAMINEA*) UNDER ORGANIC FARMING CONDITIONS IN GERMANY AND BY SANDWICH TEST

K.J. Mueller¹, G. Valè², D. Enneking³

¹ Cereal Breeding Research, Darzau Hof, D-29490 Neu Darchau, Germany

² Experimental Institute for Cereal Research, Via S. Protaso 302, I-29017, Fiorenzuola d'Arda (PC), Italy

³ Genebank, Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Corrensstrasse 3, D-06466 Gatersleben, Germany

SUMMARY

Six-hundreds-twelve barley (*Hordeum vulgare* L.) accessions were tested for resistance to *Pyrenophora graminea* under natural infection in an organic farm in Lower Saxony, Germany, during 1999-2002. More than 30% of these accessions were observed to be resistant in the field and are listed in this report. Varieties with previously documented 'Vada'- and 'Betzes'- resistance, were observed without infection, but those with 'Lion'-resistance and with 'Thibaut'-resistance were found to be susceptible. Many new sources of resistance from East Asia and also samples with deviating symptoms of sensitivity were found. A small group of accessions was infected artificially with two aggressive isolates of the pathogen (I2, I5) and tested by the sandwich method. This revealed both differences and agreement with the results obtained under natural infection. Accessions BGRC 5592, HOR 333, HOR 11475 and OU J362 showed resistance in all experiments.

Keywords: *Pyrenophora graminea*, partial resistance, organic farming, *Hordeum vulgare*, genetic erosion.

INTRODUCTION

Seed multiplication of barley under organic farming conditions requires that attention is paid to stripe disease caused by the fungus *Pyrenophora graminea* (Ito et Kuribay) [*Drechslera graminea* (Rabenh. ex Schlech.) Shoemaker]. It is an important seed transmitted disease in Germany. The cool and humid weather conditions required for infection during germination also occur frequently in Scandinavia and Italy.

Infection is generally favoured by temperatures below 12°C during germination. Twenty-one days at 2°C increase the level of infection (Teviotdale and Hall, 1976). At temperatures of more than 10°C almost no infection will take place (Obst, 1993). For *in vitro* culture on agar plates the optimum temperatures for fungal development were found to be higher, but the susceptibility of

barley is decreased at such temperatures. Very dry and very wet conditions seem to be unsuitable for fungal development (Prasad *et al.*, 1976). The susceptibility of barley to leaf stripe disease is favoured by cool and humid climatic conditions during germination and flowering (Obst, 1993).

In conventional farming systems the disease is controlled by applications of chemical seed dressings. Under certified organic farming conditions in countries of the European Union, only hot water treatment of seed is currently permitted for control of stripe disease since no accepted alternative seed treatments are presently available. Breeding for resistance is therefore the first and most efficient choice to deal with stripe disease in an organic farming context.

MATERIALS AND METHODS

Under natural infection conditions resistance screening can only be made after replanting of seed harvested from a field with infected plants (one cycle). To get a good reliability of the information about the resistant accessions the testing under natural infection took place for at least two growth cycles.

In the years 1999 to 2002, 612 accessions were evaluated for leaf stripe disease. The tested accessions included samples of diverse origin. In particular, the Barley Core Collection (BCC) was targeted with an emphasis on East Asia, a collection from Scandinavia (NGB) and varieties of different origin with resistances described in literature were included in the study. Material was obtained from the following genebanks (with accession prefix): Genebank Braunschweig Genetic Research Center, Germany (BGRC); Genebank IPK Gatersleben, Germany (HOR); Breeding Station Kromeric, Czech Republik (KM); Nordic Genebank, Alnarp, Sweden (NGB); Barley Germplasm Center Okayama, Japan (OU); National Small Grains Collection, Aberdeen, Idaho, USA (PI and CIho); Genebank N.I. Vavilov-Institute, St. Petersburg, Russia (VIR and K).

Accessions were drilled in 6 rows per plot (= 1 m²). Adjacent to this an equally sized plot was sown with an infected barley mixture as a source of inoculum. In these spreader plots 25-50 plants m⁻² showed the typical symptoms of leaf stripe disease and they confirmed also that

the weather conditions were suitable for the infection process. The fields for testing are located near Darzau [53 14 N, 010 53 E], about 70 km South-East of Hamburg, Germany. They are managed according to controlled organic farming principles of the European Union. The soil is a loamy sand with winter-rye as previous crop. No manure was given to the barley. Annual precipitation approximates 600 mm. After ripening of the ears, a bunch from each accession was harvested and threshed. In the following year, the seeds of each accession were sown in 1 m² plots, between two long plots with naturally infected plants. In this second season, all plants m² were counted after germination at DC 11 (Zadocks *et al.*, 1974). The infected plants m² were first counted at DC 31 (end of tillering/beginning of shooting) and then for a second time at DC 61 (ear emergence/flowering). The highest number of infected plants was then used to calculate the percentage of infected plants m². Samples with less than 0.75% infected plants under natural infection were considered as resistant and tested for a second time. There were two reasons for using 0.75% as a border to distinguish resistant accessions. The first most important reason was the logarithmic scale used for scoring plant diseases in the European project, of which this evaluation for stripe disease was a part (http://barley.ipk-gatersleben.de/methods/GENRES-CT98-104-evaluation_methods_1999.pdf). The second reason was that, from a practical point of farming, up till 0.75% infection has no measurable effect to the harvest.

A small group of samples was infected artificially at the "Istituto Sperimentale per la Cerealicoltura", Fiorenzuola, Italy, following the sandwich-test procedure (Pecchioni *et al.*, 1999) using the single conidium isolates I2 and I5 (Gatti *et al.*, 1992). Taking into account that artificial infection leads to a higher expression of the disease, samples with less than an average of 4% infected plants in sandwich-test with both isolates were classified

as resistant. The discrimination was used according the logarithmic scale for scoring from 1 (resistant) to 9 (susceptible) in the European evaluation project.

RESULTS AND DISCUSSIONS

The percentage of infected plants in the field trials ranged from 0 to 68%. Two-hundreds-twelve of the accessions tested at Darzau showed less than 0.75% infected plants. The majority of resistant accessions originates from Asia, in particular Nepal, Bhutan, China, Korea and Japan (Table 1). In contrast to this, accessions from Ethiopia were highly susceptible.

Darzau races of *P. graminea* showed on a set of varieties the same virulence observed in Denmark (Table 2) (Skou *et al.*, 1994; Skou and Haahr, 1987; Knudsen 1986).

Varieties with 'Vada'-resistance, like 'Vada' and 'Zita', or with 'Betzes'-resistance, like 'Betzes', 'Erbet' and 'Scout', showed no symptoms at Darzau. But 'Vijaya', a genotype reported resistant in India (Atheya, 1974), was found to be susceptible. The varieties with 'Lion'-resistance, like 'Lion', 'Plains', 'Velvet' and 'Glabron', reported resistant in India (Mathur and Bhatnagar, 1992), Carolina, Georgia, Virginia/USA (Kline, 1972) but susceptible in Scandinavia (Knudsen, 1986), were moderately susceptible at Darzau. Varieties with 'Thibaut'-resistance, reported to carry marker for Rdg2a (Arru *et al.*, 2002), were moderately susceptible under field conditions at Darzau (Table 2). This suggests that there are similarities in the climatic conditions and/or the origin of the pathogen populations between Darzau and Scandinavia but not with all the other listed locations. But as long as the same isolates will not be tested under different conditions it can only be supposed that the differences concern the local races of the pathogen.

Table 1. Number of spring barley accessions in particular from Ethiopia, East -Asia and Scandinavia, and the total of tested accessions; their distribution to logarithmic distinguished groups of susceptibility to stripe disease (*Pyrenophora graminea*) under natural field infection conditions at Darzau/Germany.

Origin	N. tested accessions	Infected plants %								
		0-0.75	0.75-2	2-4	4-7	7-13	13-21	21-36	36-60	>60
Ethiopia	33			2	5	9	3	10	4	
Nepal	27	8	5	2	5	4	3			
Buthan	10	9	1							
India	50	7	8	17	8	6	3	1		
China	49	26	8	7	3	5				
Korea	25	22	1	1	1					
Japan	63	38	9	5	4	4		1	1	1
Finland	65	12	11	17	16	7	2			
Sweden	9	3	1	1	1	3				
Norway	8	1	3	2	1	1				
Denmark	24	9	8	2	4		1			
Total	612	212	107	119	76	57	18	16	6	1

Table 2. Susceptibility to *P. graminea* of some accessions at Darzau related to results at Fiorenzuola and in literature.

Accession	Infected plants %		Elsewhere referred		
	Natural infection (Darzau)	Artificial infection (Fiorenzuola) with strain		resistant	susceptible
		I2	I5		
<u>'Vada'-Resistance</u>					
Vada (<i>Rdg1a</i>)	0	9	10		
Zita (<i>Rdg1a</i>)	0			Europe ^K	Morocco ^K
<u>'Thibaut'-Resistance</u>					
Thibaut (<i>Rdg2a</i>)	1	2	85		
Haruna Nijo (<i>Rdg2a</i>)	3	4	88		
Galleon (<i>Rdg2a</i>)	0	8	81		
<u>'Lion'-Resistance</u>					
Lion	1			India ^M	
Velvet (t:Lion)	3			USA ^L , North Africa ^K	Wisconsin ^{SR} , Europe ^K
Plains (t:Lion)	2				
Glabron (t:Lion)	1				
<u>Bohemian origin</u>					
Betzes	0			Europe ^K	Wales ^K
Erbet (t:Betzes)	0				
Scout (t:Betzes)	0	0	2		
Hanna	0			Denmark ^S	
<u>Landraces</u>					
Olli / FI	1			Denmark ^S	Canada ^T
Trysil / NO	0			Denmark ^S	
Tystofte Kors / DK	0			Denmark ^S	
Archer / UK	0			Denmark ^S	
Chevallier (t:Archer)	0			Denmark ^S	
<u>Other accessions</u>					
Persicum (Clho 2249)	7			USA ^R , Russia ^G	
Scharkin (K 22240)	14			Uzbekistan ^{bp}	
La Plata Capita (K 21960)	7			Uzbekistan ^{bp}	
K 22598	1				
Bulgarskij 250 (K 23690)	0			Uzbekistan ^{bp}	
Nutans 705 (K 26411)	0			Uzbekistan ^b	
Nutans 779 (K 26416)	0			Uzbekistan ^b	
KM 1192 / CZ	3			Czech Republic ^Z	
Modia / UY	0			Denmark ^N	
Ricardo / UY	0			Denmark ^U	
Moshi Mugi / JP	0	0	45	Romania ^O	
Vijaya / IN	4	83	89	India ^{AT}	
Nackta	1	0	0		
Zeiners Immune	0	0	4		
Kodama 13 / JP	0	0	7		
Kobai Sai / JP	0	0	12		
Hsien Hsien / CN	0	0	16		
Dangomugi / JP	0	0	41		
Condor	0	0	90		
Yane Hadaka Selection	0	5	26		
Mitsukiko 1 / JP	0	13	56		
Hiberna	0	15	55		
Fayette	1	26	31		
Yonezawa Mochi / JP	0	19	94		
HOR 144 / CN	0	88	92		
Xizang (PI 328693) / CN	0	93	87		
PI 306469	0	96	100		
PI 328625	3	99	100		
Roemer (PI 194555)	1	100	78		
Clho 10636	1	100	96		

t: type of resistance; A Arru *et al.* (2002); AT Atheya (1974); b Baigulova (1976); bp Baigulova and Pitonya (1979); d Dobrev *et al.* (1986); g Gromyko and Korol'skaya (1976); K Knudsen (1986); L Kline (1972); M Mathur and Bhatnagar (1992); N Nilsson (1975); O Bobes *et al.* (1974); R Army (1945); S Skou and Haahr (1987); SR Shands and Army (1944); T Tekauz (1983); U Skou *et al.* (1994); Z Zenishcheva and Minarzhik (1983).

Only a very few of the promising accessions identified under field conditions at Darzau showed less than 4% infected plants in the sandwich-tests with isolates I2 and I5. Most accessions without stripe disease infection at Darzau were more or less susceptible to the monoonidial Italian isolates (Table 2). This suggests that there are differences in aggressivity between the local stripe races present at Darzau and the isolates I2 and I5 used at Fiorenzuola for the sandwich-tests.

'Kodama 13' (OU J362), 'Zeiners Immune' (HOR 333), 'Scout' (HOR 11475) and 'Nackta' (BGRC 5592) were resistant to *P. graminea* under field conditions at Darzau and in sandwich test at Fiorenzuola. 'Kodama 13' (a selection from Kodama, released 1920) originates from a Japanese landrace. The other four accessions can be traced back to Bohemian landraces. Other accessions like 'Kobai Sai 1', 'Dangomugi', 'Mochi Mugi', 'Hsien Hsien' and 'Condor', which were resistant at Darzau, and also resistant to Isolate I2, were more or less susceptible to Isolate I5 (Table 2).

PI 382625 and HOR 139 reacted to natural infections at Darzau becoming dark green during tillering and drying up before the shooting stage. Another kind of reaction, a special type of escape from the disease, was described by Skou and Haahr (1987) for 'Modia', but could not be observed on this neither on other accessions at Darzau. They found typical stripe symptoms during early development (DC 31), but no leaves with symptoms later at DC 61.

Delogu *et al.* (1989) described escaping as a process in which plants with no symptoms, but infected in juvenile growth stages, escaped further fungal colonization during growth, leading to a healthy adult plant. Therefore escaping is a kind of resistance. Different percentages of infection are related to different degrees of resistance. It is suggested to describe the moderate level of resistance of an accession to natural field inoculation (from 0.75 to 7% of infected plants for at least two test cycles, Table 1) as partial resistance. The complete resistance of the land races of European origin to leaf stripe disease was described likely due to an accumulation of partial resistances after genetic analysis of crosses with a susceptible variety (Tekauz, 1983). The land races were also better adapted to European environments, in particular cold and humid weather. The cross breeding which took place over decades has split up these partial resistances. Therefore accumulation of partial resistances in modern varieties seems to be rather accidental. On the other hand one can be sure that cross breeding with a wide range of modern varieties and selection under natural infection conditions will again lead to an accumulation of partial resistances.

The surprisingly high rate of resistances in accessions from Nepal, Bhutan, China, Korea, Japan points to a better adaptation to colder temperatures during the juvenile development of barley. In Nepal and Bhutan barley is cultivated in high areas during summer, in Korea and Japan during the cold, but frost-free winter.

This suggests that the infection of barley by leaf stripe

is less probable in varieties able to tolerate cold temperatures at the juvenile stage. The higher level of disease resistance of cold tolerant genotypes could be a pleiotropic effect ascribable to a generally healthy status of the plant when an infection attempt occurs under cold temperatures. Dark green leaves under cold temperatures are expected to be a marker of such a more healthy status as the chlorophyll concentration shows a correlation to frost tolerance (Teeri *et al.*, 1974; Mueller, 1998).

In some Ethiopian accessions it was observed that the inhibition of ear emergence and the prolongation of tillering caused by the infection is also accompanied by the appearance of dark green leaves instead of light green leaves, which are more common among this group. Therefore they acquire better cold tolerance, but, because of the weak roots, they also become more drought susceptible. The disease appears to prolong a growth stage and induce characteristics (cold tolerance) that these accessions lacked before.

Accessions with resistance to leaf stripe disease were identified from almost all regions where this disease is known to occur. Most resistant accessions observed in this study have their origin in East Asia. Ethiopian accessions were particularly susceptible. Ethiopian landraces have been used as a source of resistance for many other diseases (Jørgensen, 1992). Thus this genetic material found its way into modern varieties. Our data suggests that this practice has led to a genetic erosion of genes in modern varieties conferring resistance to leaf stripe disease in comparison with former European land races.

Under organic farming conditions in Northern Germany subject to natural infection by leaf stripe disease, the 'Betzes', 'Vada', and the 'East-Asian' resistances can be used for breeding. The results at Darzau suggest that selection of plants with stripe disease resistance under enhanced natural infection conditions is sufficient for the development of resistant lines, because the expected disease pressure is lower than in other Northern European countries. We consider that lines selected with less than 3 diseased plants per m² after natural infection are sufficiently resistant for these purposes. At this level neither a loss in yield nor in quality needs to be expected (Skou *et al.*, 1992).

Selection under enhanced natural infection can lead to sustainable varieties based on an accumulation of partial resistances conferring diminished susceptibility instead of a genetically fragile monogenic resistance.

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Table 3. 148 Gene bank accessions without any symptoms of stripe disease under natural infection conditions at Darzau/Germany during all - at least two - seasons. C/N=Covered or Naked, C=Covered, N=Naked; Acc No = Accession number; AT: Austria; AU: Australia; BG: Bulgaria; BO: Bolivia; BT: Buthan; CA: Canada; CZ: Czeck Republic; CN: China; DE: Germany; DK: Denmark; EG: Egypt; FI: Finland; FR: France; IN: India; JP: Japan; KR: Korea; MN: Mongolia; NL: Netherlands; NO: Norway; NP: Nepal; RU: Romania; SE: Sweden; UK: United Kingdom; US: United States; UY: Uruguay.

Acc No.	Name	Country	C/N	Row	Acc No.	Name	Country	C/N	Row
BCC 402	TKB69b Thinleygang	BT	N	6	HB 335	McGwire	CA	N	2
BCC 403	TKB73a Khelakha	BT	N	6	HOR 144	Flora	IN	N	6
BCC 405	TKB75a Tongsa	BT	N	6	HOR 166	Mochi Mugi I	JP	N	6
BCC 406	TKB75c Tongsa	BT	N	6	HOR 333	Zeiners Immune	DE	C	2
BCC 407	TKB79a Jakar	BT	N	6	HOR 356	Paradiesgerste	DE	N	2
BCC 410	TKB81a Changka	BT	N	6	HOR 1489	Xizang	CN	N	2
BCC 413	TKB82d Serpuchen	BT	N	6	HOR 4769	Blaue Nackte	UK	N	6
BCC 414	TKB82f Serpuchen	BT	N	6	HOR 7304	Zita	DK	C	2
BCC 419	La Lin Lua 1 Hao	CN	N	6	HOR 11011	Hiberna	DE	N	2
BCC 420	Ning Cheng Da Mai (1)	CN	N	6	HOR 11378	Ricardo	UY	C	6
BCC 423	Gang Tuo Quing Ke 1 Hao	CN	N	6	HOR 11475	Scout	CA	N	2
BCC 426	Jing Pi 198	CN	C	6	HOR 12097	Chevallier Tyst	UK	C	2
BCC 436	Vladivostok	CN	C	6	K 23690	Bolgarskij 250	BG	C	6
BCC 439	Fengtien Black	CN	C	6	K 26411	Nutans 705	RU	C	2
BCC 442	Chientao Lungching	CN	C	6	K 26416	Nutans 779	RU	C	2
BCC 452	Hsin Hsien	CN	N	6	NGB 265	Rauto	FI	C	6
BCC 489	Himalayense Type 5	CN	N	6	NGB 277	Lähde	FI	C	2
BCC 492	Violaceum Sh Type 10	CN	N	6	NGB 309	U.Tammi-Narva	FI	C	2
BCC 497	Tibet White 9	CN	N	6	NGB 317	Kivistö	FI	C	6
BCC 498	Tibet White 16	CN	N	6	NGB 322	Rita-Luopa	FI	C	2
BCC 499	Tibet White 25	CN	N	6	NGB 326	Rehakka-59	FI	C	6
BCC 500	Tibet Violet 1	CN	N	6	NGB 468	Trysil	NO	C	6
BCC 503	Guang Da Mai	CN	N	6	NGB 2565	Kääs, Local Öland	SE	C	2
BCC 504	Ying Chun Da Mai	CN	N	6	NGB 2784	Långstrand 0102; Paavo Mix	FI	C	6
BCC 509	Ke Shan Xi Cheng Da Mai	CN	C	6	NGB 4431	Pääskylä AP0201, Västälä	FI	C	2
BCC 519	Yuan Mai	CN	N	6	NGB 6938	Oslo, KVL 338, Trif.	DK	N	2
BCC 550	Phokar 2	IN	N	6	NGB 7453	Wing	SE	C	2
BCC 558	Martselang 2	IN	N	6	NGB 9346	Trysil	FI	C	6
BCC 574	Dhaneti 2	IN	N	6	NGB 9394	Oslo, KVL 173	DK	N	6
BCC 584	Gauriat 1	IN	C	6	NGB 9436	Nue Grosse	DK	N	2
BCC 589	Satsuki Nijo	JP	C	2	NGB 9472	Östgöta Flaettring	FI	C	2
BCC 612	Mitsukiko 1	JP	N	6	NGB 9503	KVL 388	FI	C	2
BCC 624	Hozoro	JP	C	6	NGB 9590	Modia	UY	C	6
BCC 625	Dairokkaku	JP	C	6	NGB 9622	Rostov	FI	C	6
BCC 627	Akashinriki	JP	N	6	NGB 10700	Betzes	US	C	2
BCC 628	Kinai Nita Hadaka	JP	N	6	NGB 10754	Horn	AT	C	2
BCC 637	Datano	JP	C	6	NGB 11213	Egypt 4	EG	C	6
BCC 638	Kairyo Ogara	JP	C	6	OUC 26	Mushinchiang 1	CN	N	6
BCC 644	Niho	JP	C	6	OIJ 007	Marumi 16	JP	N	6
BCC 645	Yanagiho	JP	C	6	OIJ 036	Kobai Sai 1	JP	N	6
BCC 652	Kome Hadaka	JP	N	6	OIJ 046	Shiro Chinko	JP	N	6
BCC 663	Saeolbori	KR	C	6	OIJ 050	Aichi Kobinkatagi 1	JP	N	6
BCC 664	Kangbori	KR	C	6	OIJ 070	Okayama Hadaka 1	JP	N	6
BCC 668	Alchanbori	KR	C	6	OIJ 084	Oni Hadaka 1	JP	N	6
BCC 674	Doosan #8	KR	C	2	OIJ 104	Kuroshio Hadaka	JP	N	6
BCC 692	Buyong Naked 3	KR	N	6	OIJ 106	Shiro Shinriki	JP	N	6
BCC 695	Tongyeong Covered 1	KR	C	6	OIJ 147	Bijin	JP	N	6
BCC 698	Masan Naked 1	KR	N	6	OIJ 156	Shiro Seto	JP	N	6
BCC 700	Namji Milyang Native	KR	C	6	OIJ 158	Shinonome	JP	N	6
BCC 712	Shirodo	KR	N	6	OIJ 161	Nagajiro	JP	N	6
BCC 721	Hwacheon Native	KR	C	6	OIJ 177	Onomichi	JP	N	6
BCC 726	Anbyeon Native	KR	C	6	OIJ 236	Akane Hadaka	JP	N	6
BCC 727	Harumaki Domugi	KP	C	6	OIJ 362	Kodama 13	JP	N	6
BCC 731	Gho 1	NP	N	6	OIJ 483	Dangomugi	JP	N	6
BCC 735	Annapurna B.C.1	NP	N	6	OIJ 521	Mochi Mugi	JP	N	6
BCC 739	Bimtakothi 9	NP	N	6	OIJ 601	Sumire Mochi	RU	N	6
BCC 749	Ngyak 1	NP	N	6	OIJ 606	Mitsukiko 1	JP	N	6
BCC 756	Tukucha	NP	N	6	OIJ 783	Tokushima Mochimugi 1	JP	N	6
BCC 849	Condor	CA	N	2	OIJ 784	Yonezawa Mochi	JP	N	6
BCC 928	San Benito	BO	N	2	OJK 010	Haggyo Naked 4	KR	N	6
BCC 931	Shonkin	US	N	2	OJK 075	Daecheon Shirodo Hadaka 1	KR	N	6
BCC 1420	Vada	NL	C	2	OJK 115	Zairai Shiro Hadaka	KR	N	6
BCC 1702	Galleon	AU	C	2	OJK 119	Goseshikoku 2	KR	N	6
CIho 521	Bulgarian	BG	C	6	OJK 435	Chukan Komemugi	KR	N	6
CIho 531	Hannchen	SE	C	2	OJN 344	Prok 1	NP	N	6
CIho 6503	Tystofte Kors	DK	C	6	OJU 026	France Naked 1	FR	N	6
CIho 7557	Otis	US	C	2	PI 37707	Tystofte Korsbyg	DK	C	6
CIho 10584	Laevigatum	NL	C	2	PI 65201	Sichuan Chengu	CN	N	6
CIho 13826	Erbet	US	C	2	PI 268183	Weihenstephaner	DE	N	6
CIho 14177	Mongolia	MN	N	6	PI 306469	Romania	RO	N	6
CIho 14726	Big Culm	US	N	6	PI 365587	Lofa Abed	DK	C	2
CIho 16559	Nubet	UK	N	2	PI 467655	Archer	UK	C	2
HB 332	HB332	CA	N	2	PI 538760	Wanubet	US	N	2
HB 334	HB334	CA	N	2	PI 584807	Salka	DK	C	2

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