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D2.1 Barley lines with improved straw quality

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UNIVERSITY OF DUNDEE (UNIVDUN)

COUNCIL FOR AGRICULTURAL RESEARCH AND ECONOMICS (CREA)

Deliverable description

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0.1	25/June/2024	UNIVDUN	Initial submission for review
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0.5			

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Glossary of terms

FT-IR	Fourier Transformed Infrared Spectroscopy
GWAS	Genome-Wide Association Study

1. Introduction

The objectives of this work package are to (a) identify barley lines with different straw lignin content and/or composition including some with higher levels of lignin, and, (b) identify barley lines with increased straw protein content without penalty on final grain yield. We will furthermore use Genome Wide Association Studies (GWAS) to identify genomic loci and allelic variants for candidate genes influencing straw lignin or protein content.

In this initial year's work, twenty lines with different straw lignin content or higher protein amount were to be identified so that they might be used for trait pyramiding in Task 3.1 and for field trials in Task 4.2. Lines with enriched content of proteins in the straw will also be used to feed BSF larvae in Task 5.1, whereas lines with different lignin content and/or properties will used for the activities planned in Task 5.2 and Task 5.3.

Our first deliverable therefore, as described here, is the identification of twenty barley cultivars with either high or low straw lignin amount, or with high straw protein content.

2. Characterisation of straw lignin content in spring barley

A collection of elite 2-row spring barley cultivars was previously grown in two successive years in a field polytunnel in Dundee (UK) and samples of straw had been taken from the 2nd internode culm (leaves removed) for lignin measurement. After ball milling the internodes to a fine powder, soluble material was extracted and the extract-free cell wall residue was analysed for lignin content using Fourier Transformed Infrared Spectroscopy (FT-IR). This spectroscopic procedure had been previously calibrated using a partial least squares model against the standard gravimetric procedure of Klason lignin determination which is very accurate but not sufficiently high throughput to use on large populations of cultivars. Figure 1 shows the excellent fit between the Klason and FT-IT procedures as applied to our straw samples.

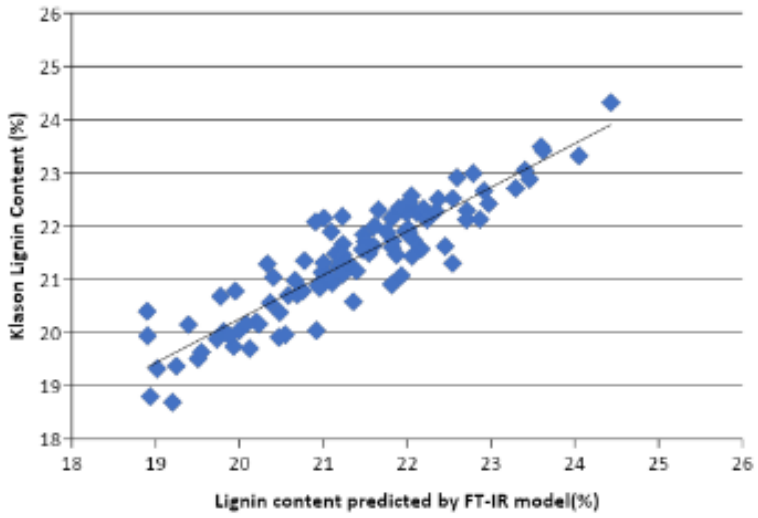


Figure 1: *Klason lignin determinations were used to train a high-throughput FT-IR model to predict straw lignin content.*

For 77 cultivars that were grown in both years trials, the FT-IR evaluated lignin contents in straw are presented in Table 1. These are average values for 3-5 replicates for each cultivar.

Table 1: *77 cultivars for which straw lignin was measured by FT-IR in two successive years ranked by cultivar average lignin content in year 1.*

	Year 1	Year 2
Cultivar	**Average Lignin (%)**	**Average Lignin (%)**
MELTAN	25.20	21.63
PITCHER	25.15	21.28
TOUCAN	25.12	22.18
TARTAN	25.04	22.99
CRIBBAGE	24.87	21.72
LIVET	24.84	20.79
CHARIOT	24.76	21.13
IMULA	24.72	21.54
AURIGA	24.68	22.50
LYSIBA	24.50	20.47
MALA	24.36	21.85
WELAM	24.36	21.61
GLEN	24.30	21.61
COCKTAIL	24.27	21.68
CAMPALA	24.24	21.19
PENTHOUSE	24.23	22.76
THISTLE	24.12	21.81
MACAW	24.09	22.07
TANKARD	24.05	21.23
LINA	24.03	20.57
HOPPER	24.02	21.91
INGRID	24.02	20.76
ASPEN	23.98	21.48
ANNI	23.97	21.23
BONUS	23.97	22.19
WESTMINSTER	23.97	20.5
VADA	23.96	21.77
VOLLA	23.91	21.87
BERWICK	23.89	21.75
HOST	23.87	22.24
FAMIN	23.84	21.45
SAFIR	23.84	20.6
SEBASTIAN	23.84	20.75
DALLAS	23.82	20.81
ATRIBUT	23.80	21.77
BINDER	23.79	21.66
DOYEN	23.79	21.74
WREN	23.78	20.9
LUD	23.77	21.27
SPEY	23.66	22.92
SMILLA	23.61	20.01
DEW	23.53	21.35
STENDES	23.52	21.79
IMBER	23.50	21.63
MINSTREL	23.49	21.30
SPARTAN	23.49	21.18
RAINBOW	23.43	22.21
RASA	23.42	20.91
ATHOS	23.38	20.77
RIVIERA	23.38	21.59
TAVERN	23.35	21.68
REGGAE	23.34	21.15
SW_SCANIA	23.28	21.53
PRIMERA	23.26	21.79
CORGI	23.22	20.77
TOBY	23.20	21.41
LADIK	23.16	21.78
CANASTA	23.12	21.59
VORTEX	23.10	21.43
AZURE	23.08	20.81
GORM	23.06	20.96
JAREK	23.02	21.30
HENNI	23.00	19.39

GUNILLA	22.95	20.37
KORAL	22.82	22.24
SPIRAL	22.82	21.41
KRYSTAL	22.76	22.03
AKKA	22.74	21.63
KARRI	22.72	19.35
ORZA	22.69	21.95
STEFFI	22.40	21.04
CRUSADER	22.25	20.93
BRITTA	22.23	21.49
MANDOLIN-1418	21.95	19.85
TARM92	21.70	20.89
KEOPS	21.56	20.08
DANUTA	21.13	20.86

The lignin content ranged from 21.13% to 25.20% in culms in year 1, and from 19.35% to 22.99% in year 2. The difference in range and degree of variation between years likely largely reflects the very different weather and environmental conditions that occurred in the two years. Variation will also be significant due to the random placement of replicates of each cultivar in the field. The statistical design used for the field trial and a multi-environment model that was devised to extract the major sources of error will enable us to significantly improve the correlation in lignin content between years (see Oakey et al., Biotechnol Biofuel 2013, 6:185). This statistical analysis will be performed in the coming months but even the unadjusted data already allow us to select cultivars with consistently high (e.g. Tartan), or consistently low (e.g. Keops) straw lignin (Figure 2). Blue shaded quadrants in Figure 2 are our initial identification of 10 high lignin cultivars and 10 low lignin cultivars.

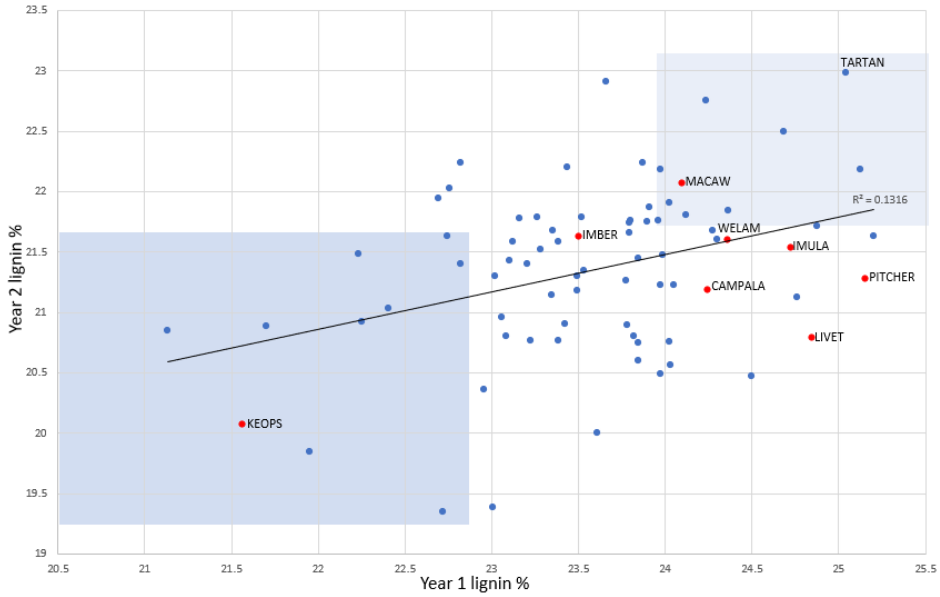


Figure 2: *Correlation of cultivar culm lignin in year 1 and year 2 (unadjusted raw data). Some cultivars that are present in current new field trials are named. Shaded blue quadrants indicate 10 high lignin and 10 low lignin cultivars.*

This data greatly exceeds the 20 cultivar dataset (10 high lignin cultivars and 10 lower lignin cultivars) we initially intended to present and gives a much broader perspective on lignin variation across a larger number of cultivars. Using this data, it will be possible to improve the selection of barley cultivars for industrial purposes where high or low straw lignin is desired.

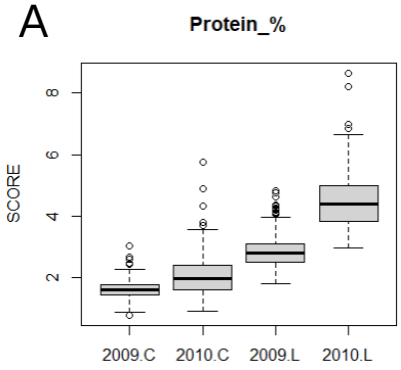
3. Characterisation of protein content in barley straw

Straw samples from ca. 120 winter barley cultivars of European origin, grown in the field at CREA-Genomics and Bioinformatics in Fiorenzuola d'Arda in 2009 and 2010, have been analysed for protein content by near-infrared spectroscopy (NIRS). For each genotype, both leaf and culm samples were collected from 2 replicated plots over the two years and milled to a 1-mm size. Each sample was read twice at NIRS, for a total of >2000 spectra generated.

Since a calibration curve for straw was not available, the samples were read with a curve developed for hay, whose protein content is higher with respect to straw. For this reason, based on a Principal Component Analysis, 100 spectra were selected that represent the whole diversity of the collection, and the protein content of the correspondent samples was determined through a CHN Elemental analyser. Based on these results, a new calibration curve specific for straw was calculated, for a more precise estimation of the protein content of the available sample.

As shown in Figure 3, protein content was significantly higher in leaves with respect to culms, and in 2010 with respect to 2009. Still, the genotype factor was the second most significant one in the three-way ANOVA.

A



B

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
GENOTYPE	122	528.02	4.33	32.9143	< 2.2e-16	***
YEAR	1	483.89	483.89	3679.9799	< 2.2e-16	***
TISSUE	1	1741.84	1741.84	13246.5635	< 2.2e-16	***
GENOTYPE:YEAR	115	122.39	1.06	8.0938	< 2.2e-16	***
GENOTYPE:TISSUE	119	89.52	0.75	5.7212	< 2.2e-16	***
YEAR:TISSUE	1	165.32	165.32	1257.2423	< 2.2e-16	***
GENOTYPE:YEAR:TISSUE	111	37.89	0.34	2.5959	9.977e-16	***
Residuals	1568	206.18	0.13			

Figure 3A: *Boxplots reporting the distribution of protein content in leaf (L) and culm (C) samples collected in 2009 and 2010 at CREA, Fiorenzuola d'Arda.* ***Figure 3B:*** *Result of the three-way ANOVA based on >2000 protein content data.*

Figure 4 describes the variation observed for protein content in the collection of winter barley cultivars. The full set of data are reported in Annex 1. The protein content ranges from 1.03% to 5.33% in culms (average 1.85%) and from 2.44% to 6.93% in leaves (average 3.67%). Two-row and six-row barleys are equally represented among the cultivars with highest and lowest straw protein content (Table 2).

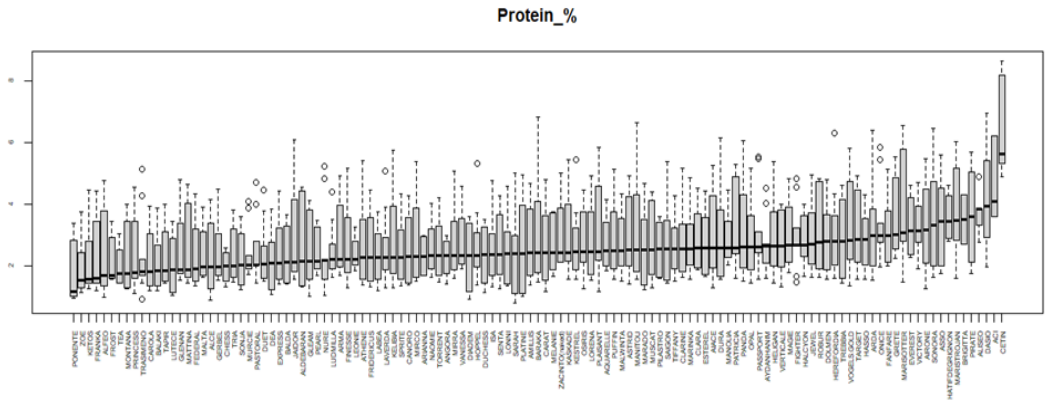


***Figure 4**:Boxplots reporting the distribution of protein content in ca. 120 winter barley cultivars of European origin.*

***Table 2**: List of cultivars with highest and lowest straw protein content.*

Genotype	RowType	Prot_C_2009	Prot_C_2010	Prot_C_Mean	Prot_L_2009	Prot_L_2010	Prot_L_Mean	Prot_GrandMean
CETIN	6	NA	5.33	5.33	NA	6.93	6.93	5.86
ACI	2	NA	NA	NA	4.10	4.92	4.51	4.51
DASIO	2	2.04	3.75	2.89	4.14	6.80	5.47	3.92
ALISEO	6	NA	NA	NA	3.07	4.27	3.67	3.67
HATIFDEGRIGNON	6	NA	NA	NA	2.88	4.28	3.58	3.58
MARISTROJAN	2	2.83	2.35	2.59	3.76	5.42	4.59	3.39
ONICE	6	2.10	3.25	2.68	2.83	5.65	4.24	3.30
SONORA	6	1.68	2.85	2.27	3.38	6.21	4.79	3.28
BRIGITTA	2	NA	2.70	2.70	NA	4.31	4.31	3.24
MARISOTTER	2	1.58	3.03	2.30	2.90	6.06	4.48	3.17
ARDA	2	1.80	2.63	2.21	3.52	5.68	4.60	3.17
BALDA	6	1.42	1.55	1.49	2.37	3.42	2.90	2.05
SONJA	2	1.52	1.27	1.39	2.56	3.51	3.03	2.05
ALCE	2	0.90	1.54	1.22	2.38	4.11	3.24	2.03
CAROLA	6	1.35	1.41	1.38	2.08	3.92	3.00	2.03
ZOE	6	1.41	1.26	1.34	2.43	3.61	3.02	2.01
DEA	6	1.23	1.37	1.30	2.51	3.44	2.98	1.97
TEA	2	1.55	NA	1.55	2.78	NA	2.78	1.96
LUTECE	6	1.26	1.13	1.19	2.53	3.23	2.88	1.87
DIADEM	2	1.09	1.08	1.09	2.59	3.37	2.98	1.84
PONENTE	6	1.00	1.07	1.03	2.83	3.21	3.02	1.83
CHESS	2	1.51	NA	1.51	2.44	NA	2.44	1.82

These cultivars were sown again in autumn 2023 at CREA - Fiorenzuola d'Arda under 2 different nitrogen fertilization levels for confirmation of the observed phenotype. Straw samples were collected on June 20, 2024 but not yet processed. Similarly, >500 ICARDA accessions kindly provided to BEST-CROP were sown in autumn 2023 and will be eventually considered for evaluation of straw protein content, to confirm previous observations.

Annex 1 – Variation for protein content in the collection of winter barley cultivars

Genotype	RowType	Prot_C_2009	Prot_C_2010	Prot_C_Mean	Prot_L_2009	Prot_L_2010	Prot_L_Mean	Prot_GrandMean
CETIN	6	NA	5.33	5.33	NA	6.93	6.93	5.86
ACI	2	NA	NA	NA	4.10	4.92	4.51	4.51
DASIO	2	2.04	3.75	2.89	4.14	6.80	5.47	3.92
ALISEO	6	NA	NA	NA	3.07	4.27	3.67	3.67
HATIFDEGRIGNON	6	NA	NA	NA	2.88	4.28	3.58	3.58
MARISTROJAN	2	2.83	2.35	2.59	3.76	5.42	4.59	3.39
ONICE	6	2.10	3.25	2.68	2.83	5.65	4.24	3.30
SONORA	6	1.68	2.85	2.27	3.38	6.21	4.79	3.28
BRIGITTA	2	NA	2.70	2.70	NA	4.31	4.31	3.24
MARISOTTER	2	1.58	3.03	2.30	2.90	6.06	4.48	3.17
ARDA	2	1.80	2.63	2.21	3.52	5.68	4.60	3.17
ASSO	2	1.86	2.57	2.21	4.19	4.97	4.58	3.16
GRETE	6	2.24	2.86	2.55	2.78	5.22	4.00	3.13
VOGELS.GOLD	6	1.91	2.22	2.07	3.53	5.73	4.63	3.09
PANDA	2	2.11	1.88	2.00	3.71	5.77	4.74	3.09
HELIGAN	2	1.82	2.56	2.19	3.47	5.36	4.42	3.08
AIRONE	2	1.40	2.70	2.05	3.77	5.24	4.51	3.03
MANITOU	6	1.56	2.25	1.91	3.20	6.10	4.65	3.00
EVEREST		2.38	NA	2.38	4.21	NA	4.21	2.99
VICTORY	2	1.94	2.84	2.39	3.29	4.45	3.87	2.98
PIRATE	6	1.75	2.16	1.95	3.64	5.37	4.50	2.97
ZACINTO	2	2.11	2.29	2.20	3.74	4.45	4.09	2.96
PASSPORT	6	1.60	2.52	2.06	3.06	5.51	4.28	2.95
BARAKA	2	1.54	2.77	2.16	2.42	5.74	4.08	2.93
ROBUR	6	1.67	2.10	1.88	4.12	4.74	4.43	2.90
HERFORDIA	6	1.63	2.58	2.10	2.87	5.33	4.10	2.90
MALWINTA	2	1.73	3.41	2.57	2.73	4.02	3.37	2.89
FANFARE	2	1.99	2.41	2.20	3.18	4.67	3.93	2.89
ARMA	6	1.99	1.87	1.93	3.63	4.91	4.27	2.87
MAGIE	2	1.81	2.66	2.24	2.76	4.78	3.77	2.85
PUFFIN	2	1.74	2.11	1.93	3.96	4.44	4.20	2.84
CLARINE	2	1.73	2.37	2.05	2.95	5.07	4.01	2.84
FIGHTER	2	1.56	2.66	2.11	3.11	4.69	3.90	2.83
PATRICIA	6	1.69	2.49	2.09	2.64	5.14	3.89	2.81
JEWEL	2	1.63	2.58	2.10	2.84	4.76	3.80	2.78
AIACE	2	1.59	2.10	1.84	3.67	4.71	4.19	2.78
MANOLIA	2	2.45	2.05	2.25	2.85	4.21	3.53	2.76
TARGET	2	1.77	2.30	2.03	2.91	4.69	3.80	2.74
JAIDOR	6	1.60	1.98	1.79	2.54	5.79	4.16	2.74
DURA	6	1.58	1.89	1.74	3.14	5.28	4.21	2.73
OPAL	2	1.49	2.76	2.13	2.61	4.56	3.58	2.71

CLARA	2	1.94	2.02	1.98	3.01	4.43	3.72	2.67
AYDANHANIM	2	1.88	2.39	2.14	2.68	4.27	3.48	2.67
VANESSA	2	2.00	2.08	2.04	2.65	4.47	3.56	2.65
MARINKA	2	1.67	2.48	2.08	2.57	4.41	3.49	2.64
TREBBIA	6	1.66	1.59	1.63	3.75	4.58	4.16	2.64
KELIBIA	2	1.57	1.82	1.70	2.79	5.32	4.05	2.64
LAVERDA	6	1.55	2.60	2.07	2.46	4.49	3.48	2.63
LORENA	6	1.62	1.96	1.79	3.35	4.35	3.85	2.62
HASSO	6	1.97	1.91	1.94	3.13	4.12	3.63	2.62
KASKADE	2	1.66	2.32	1.99	2.53	4.57	3.55	2.61
KESTREL	2	1.68	2.17	1.93	2.62	4.59	3.61	2.60
PLAISANT	6	1.21	2.45	1.83	2.34	5.17	3.75	2.60
GLENAN	6	1.57	1.80	1.68	3.24	4.64	3.94	2.59
HOPPEL	6	1.83	1.96	1.89	2.71	4.52	3.62	2.58
MIRRA	6	1.89	1.85	1.87	3.00	4.30	3.65	2.58
MIRCO	6	1.61	1.63	1.62	2.93	5.06	3.99	2.57
ESTEREL	6	1.68	1.91	1.80	3.16	4.16	3.66	2.54
AMILLIS	2	1.46	1.94	1.70	2.90	4.66	3.78	2.53
HALCYON	2	1.66	2.69	2.18	2.29	3.81	3.05	2.53
FINESSE	2	1.54	2.01	1.78	2.51	4.76	3.64	2.52
PASTORAL	2	1.59	2.04	1.81	2.76	4.36	3.56	2.51
TIFFANY	2	1.90	1.99	1.94	2.72	3.98	3.35	2.51
SAIGON	2	1.44	1.93	1.68	2.62	4.80	3.71	2.49
ASTRID	2	1.40	2.05	1.72	2.71	4.53	3.62	2.48
AQUARELLE	2	1.85	2.24	2.04	2.44	3.79	3.11	2.47
DOLMEN	2	1.57	NA	1.57	2.49	4.23	3.36	2.47
ATHENE	6	1.54	1.67	1.60	2.83	4.69	3.76	2.46
GERBEL	6	1.62	1.92	1.77	2.87	4.08	3.47	2.45
SENTA	6	1.75	1.71	1.73	2.82	4.25	3.53	2.45
OSIRIS	6	1.35	2.29	1.82	2.59	4.18	3.38	2.45
ISA	6	1.39	1.96	1.68	2.72	4.36	3.54	2.42
FEDERAL	6	1.43	1.80	1.62	3.05	4.18	3.61	2.42
LUDMILLA	6	1.65	2.02	1.83	2.62	3.95	3.29	2.41
PILASTRO	6	1.60	1.71	1.66	3.40	3.61	3.50	2.39
MELANIE	2	1.66	2.14	1.90	2.51	3.74	3.12	2.39
MURCIE	2	1.79	1.98	1.89	2.26	4.00	3.13	2.38
MALTA	2	1.72	1.76	1.74	2.91	3.71	3.31	2.37
MATTINA	6	1.52	1.75	1.64	2.58	4.34	3.46	2.37
FRIDERICUS	6	1.35	1.82	1.59	2.68	4.38	3.53	2.36
FRANKA	6	1.59	1.33	1.46	3.18	4.25	3.71	2.36
MUSCAT	6	1.52	1.68	1.60	2.69	4.29	3.49	2.36
VERTICALE	2	1.41	1.97	1.69	2.75	3.90	3.33	2.35
ALFEO	2	1.20	1.54	1.37	3.15	4.48	3.81	2.35
CARAT	2	1.17	2.08	1.63	2.61	4.21	3.41	2.34
NAOMIE	6	1.77	1.97	1.87	2.44	3.65	3.05	2.34
NURE	2	1.06	1.93	1.50	2.14	5.03	3.59	2.33
CANORO	6	1.62	1.41	1.51	2.93	4.18	3.56	2.33

DUET	2	1.60	1.77	1.68	2.45	4.14	3.29	2.33
LEONIE	2	2.13	1.92	2.02	2.42	3.11	2.76	2.32
PRINCESS	6	1.45	1.51	1.48	2.89	4.23	3.56	2.31
GLEAM	2	1.23	1.60	1.42	3.39	3.81	3.60	2.29
ALDEBARAN	6	1.33	1.87	1.60	2.15	4.49	3.32	2.29
PLATINE	2	1.04	1.76	1.40	2.88	4.37	3.62	2.29
SPRITE	2	1.58	1.58	1.58	2.76	3.95	3.36	2.29
PEARL	2	1.85	1.89	1.87	2.39	3.37	2.88	2.28
EXPRESS	6	1.54	1.58	1.56	2.62	4.07	3.35	2.27
TAPIR	6	1.49	1.61	1.55	3.00	3.64	3.32	2.26
TRASIMENO	2	1.18	1.90	1.54	1.97	4.70	3.34	2.26
MARADO	6	1.38	1.36	1.37	2.61	4.52	3.57	2.25
LONNI	6	1.42	1.60	1.51	2.51	4.07	3.29	2.22
TORRENT	2	1.76	1.58	1.67	2.50	3.60	3.05	2.22
TRIA	2	1.56	1.64	1.60	2.68	3.60	3.14	2.22
MONTANA	2	1.62	1.29	1.45	2.99	3.72	3.35	2.21
LABEA	2	1.40	1.92	1.66	2.35	3.68	3.02	2.20
SARAH	2	0.95	1.88	1.41	2.28	4.35	3.32	2.17
DUCHESS	2	1.32	1.54	1.43	3.10	3.44	3.27	2.17
KETOS	6	1.35	1.48	1.42	2.60	3.80	3.20	2.13
FROST	6	1.56	1.66	1.61	2.43	3.38	2.91	2.13
ARIANNA	6	1.70	NA	1.70	2.95	NA	2.95	2.12
BALAKI	6	1.38	1.45	1.41	2.59	3.68	3.14	2.10
ANGORA	2	1.75	NA	1.75	2.79	NA	2.79	2.10
BALDA	6	1.42	1.55	1.49	2.37	3.42	2.90	2.05
SONJA	2	1.52	1.27	1.39	2.56	3.51	3.03	2.05
ALCE	2	0.90	1.54	1.22	2.38	4.11	3.24	2.03
CAROLA	6	1.35	1.41	1.38	2.08	3.92	3.00	2.03
ZOE	6	1.41	1.26	1.34	2.43	3.61	3.02	2.01
DEA	6	1.23	1.37	1.30	2.51	3.44	2.98	1.97
TEA	2	1.55	NA	1.55	2.78	NA	2.78	1.96
LUTECE	6	1.26	1.13	1.19	2.53	3.23	2.88	1.87
DIADEM	2	1.09	1.08	1.09	2.59	3.37	2.98	1.84
PONENTE	6	1.00	1.07	1.03	2.83	3.21	3.02	1.83
CHESS	2	1.51	NA	1.51	2.44	NA	2.44	1.82