

A pandemic clonal lineage of the wheat blast fungus

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Wheat blast, caused by *Magnaporthe oryzae* (Syn. *Pyricularia oryzae*), was recently reported in Africa for the first time. To determine the relationship between African wheat blast isolates from Zambia with other populations from South America and Bangladesh, we analyzed the genotyping data recently published by [Tembo et al. \(2021\)](#). The dataset consists of 84 single nucleotide polymorphisms (SNPs) obtained by multiplex amplicon sequencing from 186 isolates amended with data from 67 isolates mined from publicly available genome sequences. We observed that the Zambian isolates (N=13, 2018-2020) are identical for the 84 SNPs to isolates from Bangladesh (N=81, 2016-2020) and one genotype from South America (Bolivia, N=1, 2012). We conclude that the "B71" clonal lineage that emerged in South East Asia in 2016 and traces its origins to South America is now established in Zambia. Further analyses using whole genome sequences are needed to validate this conclusion. These findings call for urgent action, including a mitigation plan to prevent further spread.

Methods and Results

Dataset

Tembo et al. (2021) recently reported genotyping data for 84 SNPs for each of 186 *M. oryzae* samples. To complement this dataset and increase the coverage, we extracted the 84 SNPs from each of the publicly available genome sequences of 67 isolates (Table 1) and added to the SNP data set of Tembo et al. (2021). This resulted in a SNP matrix containing 84 SNPs x 253 isolates (Supplemental Dataset 1).

Phylogenetic analysis of SNP data

To estimate the relationships among the fungal isolates, we performed phylogenetic analysis on 253 sets of concatenated SNP sequences using the maximum likelihood algorithm and Tamura-Nei model (Tamura and Nei, 1993) implemented in MEGA X software (Kumar et al., 2018), allowing the sites with gaps to be incorporated. We found that wheat-infecting *M. oryzae* isolates from Bangladesh and Zambia clustered together with B71 isolate from Bolivia differentiating clearly from other lineages (Figure 1). We refer to the isolates belonging to this group as "B71 lineage". In contrast, wheat-infecting isolates from Brazil split into two diverse groups.

To examine the B71 lineage further, we extracted data from 88 samples from this lineage and the neighbouring clade of Brazilian isolates, and performed phylogenetic analysis as described above. Again, we found that isolates from B71 lineage clustered closely together (Figure 2). On closer look at the nucleotide levels, all the isolates within B71 lineage showed identical SNP sequences whenever a SNP was called. We conclude that the B71 lineage is likely to be clonal.

Genotyping data from ‘MonsterPlex’ are robust and accurate

We inspected duplicated samples (for example, BTMP-4-1 and BTJP-41; BTJP4_3 and BTJP-43; BTJP4-5 and BTJP4_5; BTJP-6 and BTJP_6) from MonsterPlex genotyping experiments (Tembo et al. 2021) and found that they all produced identical SNP patterns (Supplemental Dataset 1), indicating that the genotyping method is robust.

We further estimated the accuracy of the genotyping method by comparing SNP data acquired from 40 isolates using MonsterPlex to the SNPs extracted from matching genome sequences. We found that SNPs from both acquisition methods were identical whenever a SNP call was made (100%, 3302/3302 sites). In total, 58 sites had gaps with missing data from MonsterPlex (1.7%, 58/3360) (Supplemental Dataset 2).

Conclusions

- The B71 clonal lineage is now confirmed in three continents (Figure 4).
- We rule out a local origin of the Zambian population.
- The Zambian population was introduced to the country from South America, Bangladesh or an unsampled location.
- All Zambian samples (2018-2020) belong to the B71 lineage indicating that they probably originate from a single introduction.
- All Bangladesh samples (2016-2020) belong to the B71 lineage indicating that there was probably no additional introductions to the country.

Perspectives

These findings highlight the pandemic nature of *M. oryzae* B71 lineage, which has the capacity to spread outside of its likely source of origin in South America. A better understanding of possible dispersal routes is important to help develop a mitigation plan to prevent further spread.

On the upside, the knowledge gained in South America and Bangladesh, e.g. disease management, sources of resistance, fungicides etc., can now be confidently applied to Zambia. Future analyses of genome sequences will provide higher resolution in detecting B71 lineage variants of potential epidemiological importance.

Supplemental Datasets

Supplemental Dataset 1. SNP data for 253 samples.

Supplemental Dataset 2. Samples used to compare SNP data from MonsterPlex genotyping method with genome sequence.

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Figure 1

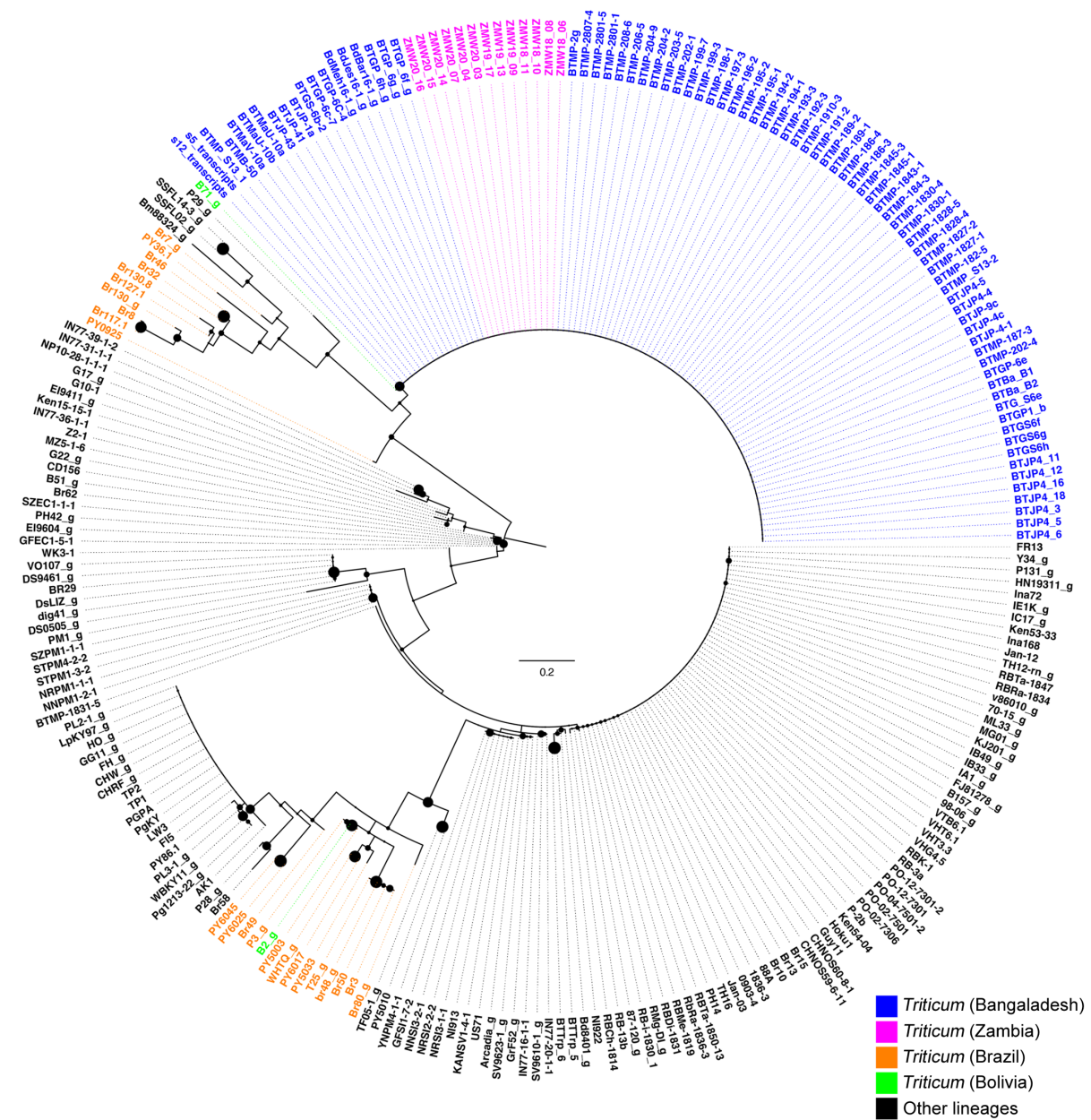


Figure 1. Genotyping with 84 SNPs differentiates wheat-infecting *M. oryzae* isolates from Bangladesh, Zambia, and Bolivia (B71) from other lineages. Phylogenetic relationship of 253 isolates were computed from the SNP sites by Maximum Likelihood method and Tamura-Nei model. The tree with the highest log likelihood (-1730.25) is shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 253 nucleotide sequences. There was a total of 84 positions in the final dataset. Relative bootstrap values are shown with filled black circles on nodes.

Figure 2

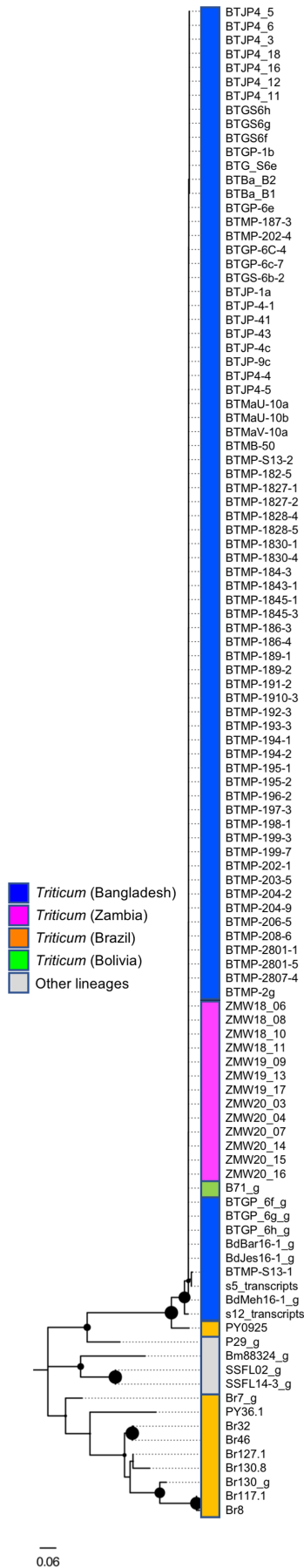


Figure 2. Wheat-infecting *Magnaporthe oryzae* lineages from Zambia and Bangladesh are clustered together with B71 isolate from Bolivia. Phylogenetic relationship of *Triticum* isolates were computed from the SNP sites by Maximum Likelihood method and Tamura-Nei model. The tree with the highest log likelihood (-666.22) is shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 108 nucleotide sequences. There was a total of 84 positions in the final dataset. Relative bootstrap values are shown with filled black circles on nodes.

Figure 3

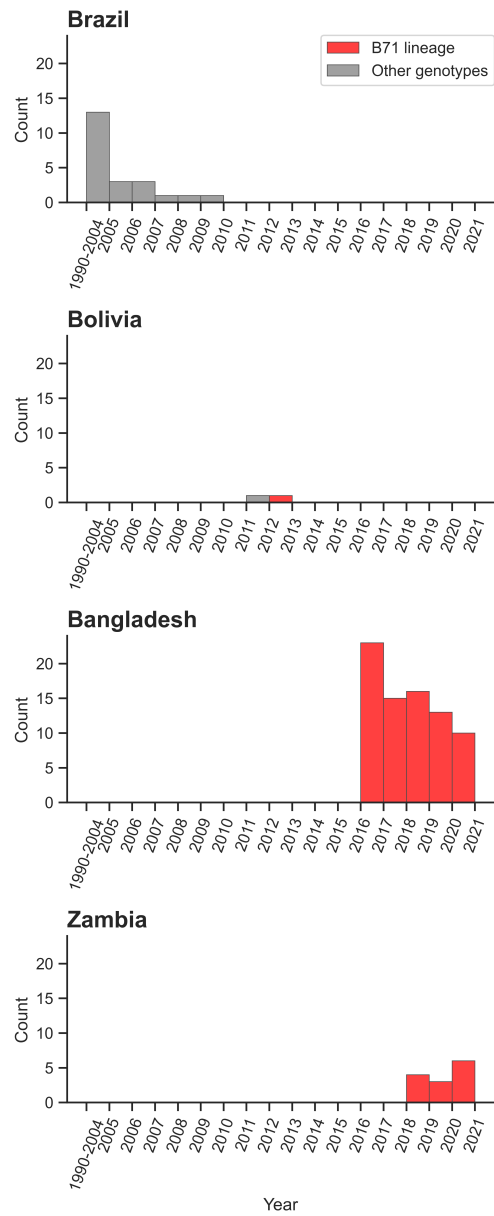


Figure 3. Summary of sample count and collection year for wheat-infecting *Magnaporthe oryzae* samples used in this study, categorized by geography. Isolates belonging to the B71 lineage are highlighted in red.

Figure 4

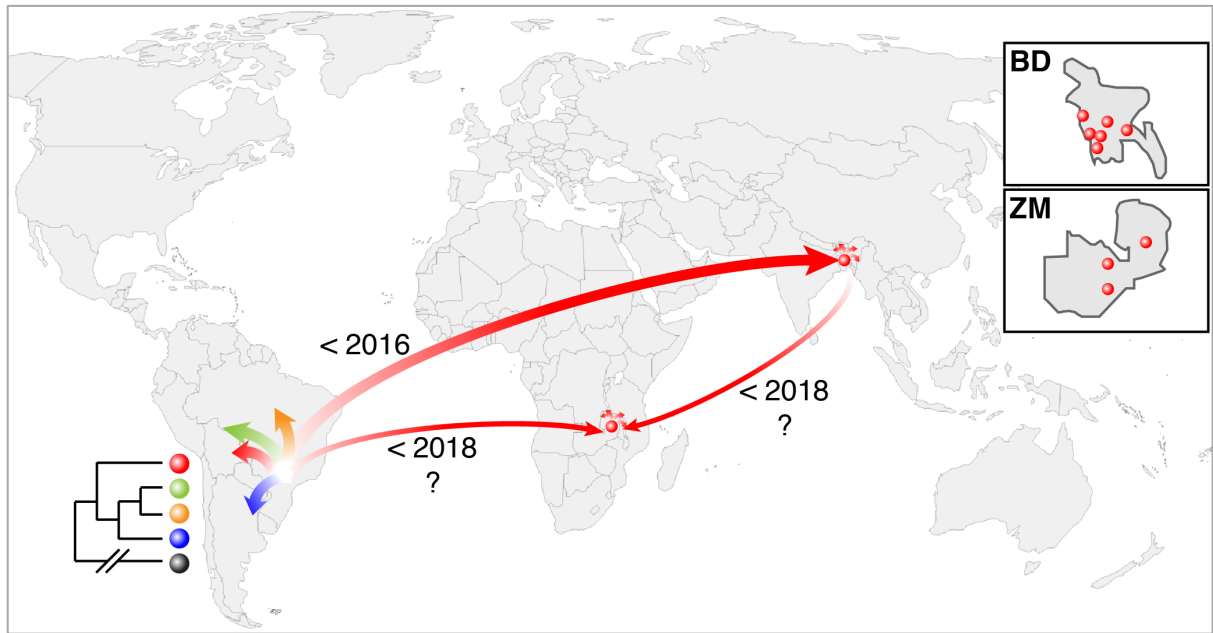


Figure 4. Dispersal of the *Magnaporthe oryzae* B71 clonal lineage of across three continents. Colours of spheres and arrows indicate lineages of the wheat blast fungus. Arrows indicate possible routes and years of dispersal. Inserts on right side: Spheres indicate sampling locations in Bangladesh (BD) and Zambia (ZM).

Table 1. *Magnaporthe oryzae* isolates from which 84 SNPs were extracted from their genomic sequences.¹ BUSCO completeness scores were based on Sordariomycota database (Seppy et al., 2019)

Isolate ID	Host	Year	Location	Accession no.	Reference	BUSCO completeness ¹
70-15	Laboratory strain	NA	NA	SAMN02953596	Dean et al., 2005;	98.2
87-120	<i>Oryza sativa</i>	NA	NA	SAMN08377452	Gladioux et al., 2018	97.5
98-06	<i>Oryza sativa</i>	NA	Jiangsu, China	SAMN03010511	Dong et al., 2015	98.0
Arcadia	<i>Setaria viridis</i>	1998	Lexington, KY, USA	SAMN08009540	Farman et al., 2017; Pieck et al., 2016	96.4
B157	<i>Oryza sativa</i>	1989	India	SAMN03329169	Gowda et al., 2015; Kachroo et al., 1994	96.2
B2	<i>Triticum aestivum</i>	2011	Bolivia	SAMN05580113	Pieck et al., 2016	97.3
B51	<i>Eleusine indica</i>	2012	Quirusillas, Bolivia	SAMN08009542	Farman et al., 2017; Pieck et al., 2016	97.5
B71	<i>Triticum aestivum</i>	2012	Bolivia	SAMN06076154	Peng et al., 2019	98.0
Bd8401	<i>Brachiaria distachya</i>	1984	Philippines	SAMN08009543	Gladioux et al., 2018	98.0
BdBar16-1	<i>Triticum aestivum</i>	2016	Barisal, Bangladesh	SAMN04940126	Malaker et al., 2016	90.9
BdJes16-1	<i>Triticum aestivum</i>	2016	Jessore, Bangladesh	SAMN04942531	Malaker et al., 2016	94.0
BdMeh16-1	<i>Triticum aestivum</i>	2016	Mehepur, Bangladesh	SAMN04942534	Malaker et al., 2016	97.7
Bm88324	<i>Brachiaria mutica</i>	1988	Philippines	SAMN08009544	Gladioux et al., 2018	98.2
Br130	<i>Triticum aestivum</i>	1990	Mato Grosso do Sul, Brazil	SAMN08009547	Farman et al., 2017	94.6
br48	<i>Triticum aestivum</i>	1990	Brazil	NA	Yoshida et al., 2016	96.5
Br7	<i>Triticum aestivum</i>	1990	Parana, Brazil	SAMN08009545	Farman et al., 2017; Pieck et al., 2016	97.6
Br80	<i>Triticum aestivum</i>	1991	Brazil	SAMN08009546	Farman et al., 2017; Pieck et al., 2016	97.9
BTGP_6f	<i>Triticum aestivum</i>	2017	Meherpur, Bangladesh	SAMEA104190810	Soanes et al., 2017b	97.9
BTGP_6g	<i>Triticum aestivum</i>	2017	Meherpur, Bangladesh	SAMEA104190811	Soanes et al., 2017b	97.9
BTGP_6h	<i>Triticum aestivum</i>	2017	Meherpur, Bangladesh	SAMEA104190812	Soanes et al., 2017b	97.8
CHRF	<i>Lolium perenne</i>	1996	Silver Spring, MD, USA	SAMN08009548	Pieck et al., 2016	97.8
CHW	<i>Lolium perenne</i>	1996	Annapolis, MD, USA	SAMN08009549	Pieck et al., 2016	97.7
Dig41	<i>Digitaria sanguinalis</i>	1990	Hyogo, Japan	NA	Yoshida et al., 2016	88.7
DS0505	<i>Digitaria sanguinalis</i>	2005	Zhejiang, China	SAMN04299554	Zhong et al., 2016	97.9

DS9461	<i>Digitaria sanguinalis</i>	1994	Fujian, China	SAMN04318446	Zhong et al., 2016	97.9
DsLIZ	<i>Digitaria sanguinalis</i>	2000	Lexington, KY, USA	SAMN08009550	Pieck et al., 2016	96.8
EI9411	<i>Eleusine indica</i>	1994	Fujian, China	SAMN04318447	Zhong et al., 2016	97.8
EI9604	<i>Eleusine indica</i>	1996	Zhejiang, China	SAMN04318448	Zhong et al., 2016	97.8
FH	<i>Lolium perenne</i>	1997	Hagerstown, MD, USA	SAMN08009551	Farman et al., 2017; Pieck et al., 2016	96.5
FJ81278	<i>Oryza sativa</i>	NA	China	SAMN06050152	Bao et al., 2017	98.0
G17	<i>Eragrostis curvula</i>	1976	Japan	SAMN08009553	Soanes et al., 2017a; Islam et al., 2016	97.4
G22	<i>Eleusine coracana</i>	1976	Japan	SAMN08009554	Gladieux et al., 2018	97.7
GG11	<i>Lolium perenne</i>	1997	Lexington, KY, USA	SAMN08009555	Farman et al., 2017	97.9
GrF52	<i>Setaria viridis</i>	2001	Lexington, KY, USA	SAMN08009556	Gladieux et al., 2018	97.9
HN19311	<i>Oryza sativa</i>	NA	Hunan, China	SAMN02216973	Chen et al., 2013	97.9
HO	<i>Lolium perenne</i>	1996	Richmond, PA, USA	SAMN08009558	Farman et al., 2017	98.0
IA1	<i>Oryza sativa</i>	2009	Arkansas, USA	SAMN08009559	Pieck et al., 2016	98.0
IB33	<i>Oryza sativa</i>	NA	Texas, USA	SAMN08009560	Gladieux et al., 2018	97.8
IB49	<i>Oryza sativa</i>	1992	Arkansas, USA	SAMN08009561	Pieck et al., 2016	98.0
IC17	<i>Oryza sativa</i>	1992	Arkansas, USA	SAMN08009562	Pieck et al., 2016	97.8
IE1K	<i>Oryza sativa</i>	2003	Arkansas, USA	SAMN08009563	Pieck et al., 2016	98.0
KJ201	<i>Oryza sativa</i>		Korea	SAMN02730190		96.4
LpKY97	<i>Lolium perenne</i>	1997	Lexington, KY, USA	SAMN08009564	Farman et al., 2017; Pieck et al., 2016	97.7
MG01	<i>Oryza sativa</i>	2012	India	SAMN02397294	Gowda et al., 2015; Kachroo et al., 1994	96.0
ML33	<i>Oryza sativa</i>	1995	Mali	SAMN08009565	Gladieux et al., 2018	98.0
P131	<i>Oryza sativa</i>	2012	India	SAMN02981399	Xue et al., 2012	97.8
P28	<i>Bomus tectorum</i>	2014	Paraguay	SAMN05864041	Pieck et al., 2016	97.9
P29	<i>Bomus tectorum</i>	2014	Paraguay	SAMN05898532	Pieck et al., 2016	97.1
P3	<i>Triticum durum</i>	2012	Canindeyu, Paraguay	SAMN08009568	Pieck et al., 2016	98.0
Pg1213-22	<i>Festuca arundinaceum</i>	2000	Georgia, USA	SAMN08009569	Pieck et al., 2016	97.8
PH42	<i>Eleusine coracana</i>	1983	Philippines	SAMN08009570	Pieck et al., 2016	95.8
PL2-1	<i>Lolium multiflorum</i>	2002	Pulaski Co., KY, USA	SAMN08009571	Farman et al., 2017	97.7
PL3-1	<i>Lolium multiflorum</i>	2002	Pulaski Co., KY, USA	SAMN08009572	Farman et al., 2017; Pieck et al., 2016	97.5
PM1	<i>Pennisetum americanum</i>	1990	Georgia, USA	SAMN08377453	Gladieux et al., 2018	96.9

RMg-DI	<i>Oryza sativa</i> <i>Stenotaphrum</i>	NA	Madhubani,Bihar,India	SAMN05425585	Kumar et al., 2017	94.3
SSFL02	<i>secundatum</i> <i>Stenotaphrum</i>	2002	Disney World, FL, USA	SAMN08009573	Pieck et al., 2016	97.5
SSFL14-3	<i>secundatum</i>	2014	New Smyrna, FL, USA	SAMN08009574	Gladioux et al., 2018	97.8
SV9610-1	<i>Setaria viridis</i>	1996	Zhejiang, China	SAMN04318449	Zhong et al., 2016	98.2
SV9623-1	<i>Setaria viridis</i>	1996	Zhejiang, China	SAMN04318450	Zhong et al., 2016	98.0
T25	<i>Triticum aestivum</i>	1988	Parana, Brazil	SAMN08009575	Farman et al., 2017; Pieck et al., 2016	97.6
TF05-1	<i>Festuca arundinaceum</i>	2005	Lexington, KY, USA	SAMN08009576	Gladioux et al., 2018	97.9
TH12	<i>Hordeum vulgare</i>	NA	Thailand	NA; available on GEMO	Chiapello et al., 2015, Islam et al., 2016	97.7
v86010	<i>Oryza sativa</i>	NA	Philippines	SAMN06392751	Zhu et al., 2017	96.4
VO107	<i>Digitaria sanguinalis</i>	1981	Texas, USA	SAMN08009577	Gladioux et al., 2018	84.4
WBKY11	<i>Triticum aestivum</i>	2011	Lexington, KY	SAMN08009578	Farman et al., 2017; Pieck et al., 2016	97.9
WHTQ	<i>Triticum aestivum</i>	NA	Brazil	SAMN08009580	Farman et al., 2017	92.9
Y34	<i>Oryza sativa</i>	1982	Yunnan, China	SAMN02981398	Xue et al., 2012	97.9