

1 **Harnessing genetic potential of wheat germplasm banks through impact-oriented-**
2 **prebreeding for future food and nutritional security**

3
4 Sukhwinder Singh^{1*†}, Prashant Vikram^{1†}, Deepmala Sehgal^{1†}, Juan Burgueño¹, Achla Sharma²,
5 Sanjay K. Singh³, Carolina P. Sansaloni¹, Ryan Joynson⁴, Thomas Brabbs⁴, Cynthia Ortiz¹,
6 Ernesto Solis-Moya⁵, Velu Govindan¹, Naveen Gupta⁶, Harminder S. Sidhu⁶, Ashwani K.
7 Basandrai⁷, Daisy Basandrai⁷, Lourdes Ledesma-Ramires⁵, Maria P. Suaste-Franco⁵, Guillermo
8 Fuentes-Dávila⁸, Javier I. Moreno⁹, Kai Sonder¹, Vaibhav K. Singh¹⁰, Sanjay Singh¹¹, Sajid
9 Shokat^{12,13}, Mian A. R. Arif¹², Khalil A. Laghari¹³, Puja Srivastava², Sridhar Bhavani¹⁴, Satish
10 Kumar³, Dharam Pal¹⁵, Jai P. Jaiswal¹⁶, Uttam Kumar⁶, Harinder K. Chaudhary⁷, Jose Crossa¹,
11 Thomas S. Payne¹, Muhammad Imtiaz¹⁸, Virinder S. Sohu², Gyanendra P. Singh³, Navtej S.
12 Bains², Anthony Hall^{4,19}, Kevin V. Pixley^{1*}

13
14 ¹International Maize and Wheat Improvement Center (CIMMYT), Carretera México-Veracruz Km. 45, El Batán,
15 Texcoco, México, C.P. 56237

16 ²Department Plant Breeding & Genetics, Punjab Agriculture University, Ludhiana-141004, India

17 ³ICAR-Indian Institute of Wheat and Barley Research, Karnal-132001, India

18 ⁴Earlham Institute, Norwich, Norfolk, NR4 7UG, UK

19 ⁵Carretera Celaya-San Miguel de Allende, Km .6.5, C.P. 38110, Celaya, Guanajuato, México

20 ⁶Borlaug Institute for South Asia (BISA), CIMMYT, Ladhawal, Punjab, 141004, India

21 ⁷CSK Himachal Pradesh Agricultural University Palampur, H.P. India, 176062

22 ⁸INIFAP-CIRNO, Campo Experimental Norman E. Borlaug, Apdo. Postal 155, Km 12 Norman E. Borlaug, Cd.
23 Obregon, Sonora, Mexico CP 85000

24 ⁹INIFAP, Interior Parque Los Colomos S/N, Colonia Providencia, CP. 44660, Guadalajara, Jalisco

25 ¹⁰ICAR-Indian Agricultural Research Institute (IARI), New Delhi - 110 012, INDIA

26 ¹¹National Research Center for Plant Biotechnology, New Delhi - 110 012, INDIA

27 ¹²Nuclear Institute for Agriculture and Biology, Faisalabad -38000, Pakistan

28 ¹³Department of Plant and Environmental Sciences, Crop Science, University of Copenhagen, Højbakkegård Allé 13,
29 DK-2630, Taastrup, DENMARK

30 ¹⁴Nuclear Institute of Agriculture, Tando Jam, Sindh, - 70050, Pakistan,

31 ¹⁵CIMMYT - World Agroforestry Centre (ICRAF), United Nations Avenue, Gigiri. P.O. Box 1041–00621, Nairobi,
32 Kenya

33 ¹⁶ICAR-Indian Agricultural Research Institute, Regional Station, Shimla-171004, India

34 ¹⁷Department of Genetics & Plant Breeding, G.B. Pant University of Agriculture & Technology, Pantnagar- 263145
35 Uttarakhand, India

36 ¹⁸CIMMYT – Pakistan, NARC CSI Complex, Park Road, Islamabad- 44000, Pakistan

37 ¹⁹School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ, UK

38
39 [†]Equal contributions

40 ^{*}Corresponding authors:

41 Sukhwinder Singh | Wheat Lead- Seeds of Discovery

42 International Maize and Wheat Improvement Center (CIMMYT)

43 Tel: +52 (595) 952 1900 (2104) Email: suk.singh@cgiar.org

44 Kevin Pixley | Director, Genetic Resources Program

45 International Maize and Wheat Improvement Center (CIMMYT)

46 Carretera México-Veracruz Km. 45, El Batán, Texcoco, México, C.P. 56237

47 Tel: +52-595-9521900 (1397) Email: k.pixley@cgiar.org

48

49

50

1 **Supplementary Table 1. Haplotype variation among three sets of germplasm; exotic, pre breeding,**
 2 **and elite**
 3

Chromosome	Germplasm set	Total number of haplotypes	Length of haplotypes (number of SNPs in a haplotype): min to max	Genomic regions in PBLs where large HBs in elites were broken (genetic position in cM based on 64K map)
1A	Elite	10	2-36	
	Exotic	33	2-7	
	PBLs	32	2-10	190.3-194.7, 195.8- 196.6, 204.3-204.7, 206.7-207.0, 208.1-218.3, 225.2-226.0, 226.4-228.4, 313.4-343.3
1B	Elite	4	2-3	
	Exotic	23	2-8	
	PBLs	14	2-5	
1D	Elite	1	4	
	Exotic	14	2-6	
	PBLs	11	2-3	75-78
2A	Elite	7	2-6	
	Exotic	19	2-3	
	PBLs	18	2-6	
2B	Elite	9	2-10	
	Exotic	27	2-5	
	PBLs	30	2-6	68-69
2D	Elite	3	3-8	
	Exotic	20	2-8	

	PBLs	10	2-12	114-118
3A	Elite	5	2-8	
	Exotic	16	2-5	
	PBLs	20	2-5	
3B	Elite	10	2-8	
	Exotic	23	2-5	
	PBLs	33	2-6	130.8-131.5, 185.4 – 185.6, 285.5 – 286.1
3D	Elite	1	5	
	Exotic	11	2-22	
	PBLs	9	2-5	
4A	Elite	4	3-7	
	Exotic	11	2-5	
	PBLs	14	2-4	181.7 – 190.0, 211.0-215.4
4B	Elite	4	2-9	
	Exotic	9	2-4	
	PBLs	6	2-3	
4D	Elite	1	3	
	Exotic	8	2	
	PBLs	6	2	
5A	Elite	10	2-5	
	Exotic	20	2-6	
	PBLs	20	2-5	
5B	Elite	11	2-15	
	Exotic	28	2-6	

	PBLs	37	2-4	23.3-23.8, 23.9-25.5, 63.7-69.1, 100.4-106.1, 107.2-115.7
5D	Elite	1	7	
	Exotic	6	2-3	
	PBLs	3	2-5	217.9-228.8
6A	Elite	5	3-8	
	Exotic	13	2-4	
	PBLs	19	2-6	110.8-112.3
6B	Elite	3	2-6	
	Exotic	14	2-4	
	PBLs	15	2-6	
6D	Elite	4	2-3	
	Exotic	10	2-39	
	PBLs	6	2-3	
7A	Elite	11	2-7	
	Exotic	25	2-6	
	PBLs	28	2-6	
7B	Elite	10	2-8	
	Exotic	16	2-3	
	PBLs	23	2-5	61.6-75.1, 76.7-79.0
7D	Elite	1	2	
	Exotic	21	2-23	
	PBLs	6	2-4	

1
2
3
4

1 **Supplementary Table 2. Contribution of exotic and elite alleles in ten crosses with equal to or**
2 **more than 16 pre-breeding lines selected after 7 cycles of selection (separate excel sheet attached)**

3
4 **Supplementary Table 3. Haplotype associations with grain yield and related traits (separate excel**
5 **sheet attached)**

6
7 **Supplementary Table 4. Haplotype blocks associated with grain yield under heat stress and**
8 **irrigated situation**

9

Haplotype	Chromosome	Irrigated		Heat-2016		Heat-2017	
		<i>P</i> -value	R ² (%)	<i>P</i> -value	R ² (%)	<i>P</i> -value	R ² (%)
HB18.1	6D	9.88E-05	2.8	3.30E-12	6.1	5.3076E-07	3.7
HB8.22	3B	8.33E-04	3.4	6.29E-04	3.6	0.00204	3.0
HB16.10	6A	3.42E-05	4.2	9.90E-04	3.4	0.00045675	3.4
HB18.2	6D	1.38E-06	3.7	3.30E-12	6.2	2.44E-04	3.0

10

1
2
3
4

Supplementary Table 5A. Haplotype associations for yellow rust resistance in 984 PBLs

Haplotype	Chromosome	<i>P</i> -value	Location-PAU	Location-Palampur
HB1.21	1A	0.00103		
HB1.31	1A	0.000113		**
HB1.32	1A	8.16E-05		**
HB2.1	1B	0.000924		***
HB2.7	1B	3.76E-06	**	**
HB3.4	1D	0.000426	***	
HB3.7	1D	0.000151		**
HB5.2	2B	0.00155		***
HB5.23	2B	0.00138	**	**
HB19.4	7A	1.74E-05		**
HB20.22	7B	0.00309	**	**

5 P-Value significance level: *: < 0.01; **: < 0.01; *** :<0.0001

6
7
8

Supplementary Table 5B. Haplotype associations for powdery mildew resistance in the pre-breeding germplasm set

Haplotype	Chromosome	<i>P</i> -value	R ²
HB14.36	5B	3.17E-05	0.0308
HB17.11	6B	1.09E-04	0.0281

9
10
11
12
13
14
15
16
17
18

1 **Supplementary Table 6. Genes found within trait-associated intervals along with their molecular function and genome annotation**
 2 **(separate excel sheet attached)**

3
 4 **Supplementary Table 7. Eight allelic variants of gene Traes_6DS_84A4D85F.1 with SIFT score <0.05**
 5

Variant ID	Chromosome: bp	Alleles	Class	Source	Conseq.Type	SIFT	Transcript
EPITAEV07251078	6D:3099529	G/C	SNP	Inter-homoeologous	Missense variant	0	<u>Traes_6DS_84A4FD85F1</u>
EPITAEV01106843	6D:3099718	T/G	SNP	Inter-homoeologous	Missense variant	0	<u>Traes_6DS_84A4FD85F1</u>
EPITAEV01106544	6D:3099856	T/C	SNP	Inter-homoeologous	Missense variant	0	<u>Traes_6DS_84A4FD85F1</u>
EPITAEV01106553	6D:3099833	G/A	SNP	Inter-homoeologous	Missense variant	0.01	<u>Traes_6DS_84A4FD85F1</u>
EPITAEV01106611	6D:3099439	C/T	SNP	Inter-homoeologous	Missense variant	0.02	<u>Traes_6DS_84A4FD85F1</u>
EPITAEV01106815	6D:3099471	G/T	SNP	Inter-homoeologous	Missense variant	0.02	<u>Traes_6DS_84A4FD85F1</u>
EPITAEV01106694	6D:3099160	T/C	SNP	Inter-homoeologous	Missense variant	0.03	<u>Traes_6DS_84A4FD85F1</u>
EPITAEV01106840	6D:3099708-3099709	GG/CA	substitution	Inter-homoeologous	Missense variant	0.04	<u>Traes_6DS_84A4FD85F2</u>

6

1 **Supplementary Table 8A. Pre-breeding lines identified as potential donors for grain yield under**
 2 **heat stress**

GID	Exotic parent type	2015-16	2016-17
		----- kg ha ⁻¹ -----	
7641495	Synthetic	2261	2346
7644075	Synthetic	2325*	2418*
7645422	Synthetic	2338*	2488*
7645970	Synthetic	2214	2407*
7689940	Landrace	2415*	2362
BAJ #1	Check	2144	2216
VOROBAY	Check	1769	1985
SOKOLL	Check	NA	2023
LSD(0.05)		526	386

3 In crop season 2015-16 and 2016-17, 984 and 230 PBLs respectively, were evaluated under heat stress
 4 environment at Ciudad Obregon, Mexico. The number of PBLs with grain yield equal/comparable to Baj#1
 5 and Vorobey (checks) in 2015-16 were **610** and **908** respectively, and, in 2016-17 they were **165** and **193**
 6 respectively. *: PBLs yielding significantly higher than Vorobey.
 7

8 **Supplementary Table 8B. Pre-breeding lines performing comparable or better than checks for**
 9 **grain yield under drought stress**

GID	Exotic parent type	2015-16	2016-17
		----- kg ha ⁻¹ -----	
7643084	Synthetic	3587	4510
7642492	Synthetic	3480	4574
7688508	Landrace	3360	4787*
7687479	Synthetic	3167	5198*
7642491	Synthetic	2766	5151* [#]
VOROBAY	Check	3346	4613
BAJ #1	Check	3111	4858
SOKOLL	Check	NA	3968
LSD(0.05)		868	457

10 A total of **984** and **230** PBLs were evaluated in crop season of 2015-16 and 2016-17 respectively, under
 11 drought stress environment at Ciudad Obregon, Mexico. Of these, grain yield of **940** and **799** PBLs were
 12 equal/comparable to Baj#1 and Vorobey, in the crop season 2015-16 respectively. In the 2016-17 season,
 13 PBLs with grain yield equal/ comparable to Baj#1 and Vorobey were **43** and **89**, respectively. *: **Three**
 14 PBLs yielded significantly higher than Vorobey in the crop season 2016-17; #: **One** PBL yielded
 15 significantly higher than Baj#1 in the crop season 2016-17.
 16

17 **Supplementary Table 9. List of exotics with minor haplotypes associated with agronomic traits**

18 **Supplementary Table 10. List of elites, exotics and pre-breeding lines used in this study. Separate**
 19 **excel sheet attached.**

20 **10-A: Salient features of the 25 elites that were either released varieties or better performing lines**
 21 **in CIMMYT's multi-location trials. These elites were used for developing pre-breeding germplasm**
 22

1 **populations; 10-B: Details of 165 exotics involved in 183 crosses representing 984 pre-breeding**
 2 **lines; 10-C: Details of 183 three-way crosses representing 984 pre-breeding lines**

3
 4 **Supplementary Table 11. (A) Details of 10,111 SNP makers used for analysis in this study; (B)**
 5 **Details of 7,180 SNP markers used for haplotype analysis in this study. Separate excel sheet**
 6 **attached**

7
 8 **Supplementary Table 12. Location and size of QTL intervals and number of annotated genes**

Interval	Chromosome	Interval start	Interval end	Interval size (Mbp)	Number of Genes	Number of GY associated genes* (NB-LRR Resistance Associated genes)10
HB5.23	2B	6579864 41	6902774 42	32.29	279	
HB10.5	4A	1237265 09	1376101 08	13.88	61	(GY related genes) 4
HB16.1 0	6A	4180390 34	4465922 70	28.55	138	(GY related genes) 14
HB18.1	6D	4555134	6843670	2.29	48	(GY related genes) 6

10 *Flagged as previously associated with grain yield by KnetMiner

11
 12
 13 **SUPPLEMENTARY FIGURE LEGENDS**

14
 15 **Fig. S1.** Pictorial presentation of haplotype blocks comparison between of elite and PBL haplotype
 16 block map. Figure shows the distribution of same 36 SNPs in elite and PBL haplotype map

17
 18 **Fig. S2.** SNP allele profiles on all 21 chromosomes (columns) for pre-breeding lines (PBLs) of 10
 19 crosses (rows). Black lines delimit chromosomes and crosses. Black dots inside the black lines are
 20 missing values, while blue and orange dots represent elite- and exotic-specific alleles, respectively.
 21 Green color corresponds to unidentified, or alleles originating from recombination of elite and
 22 exotics. Chromosomal regions where elite, exotic or recombinant alleles prevailed in the PBLs
 23 appear as vertical patterns of the same color.

24
 25 **Fig. S3:** Phylogenetic tree of the *Traes_6DS_84A4D85F.1* candidate gene, from EnsemblPlants
 26 database, (<http://plants.ensembl.org/index.html>) showing closest similarity with the gene
 27 *F775_22033* in *Aegilops tauschii*, coding for isoflavone reductase IRL-like protein.

28
 29 **Fig. S4:** BLAST analysis of two SNPs in HB18.1 revealed a significant hit for the SNP 2 (clone
 30 ID 1067078), with a candidate gene, *Traes_6DS_84A4D85F.1*. This gene is homologous to the
 31 rice gene *LOC_Os06g27770.1*, coding for isoflavone reductase. Allelic variants are shown in
 32 UTR, coding and intron regions of the gene (<http://plants.ensembl.org/index.html>). The dark
 33 yellow colored rectangular boxes represent missense mutations (causing deleterious amino acid
 34 changes) in the coding region.

35
 36 **Fig. S5.** Average TKW of PBLs with alleles AC, AT and TC (X-axis) for HB18.2. PBLs with
 37 haplotype AC had highest TKW. The haplotype AC was present in 6% of PBLs.

38

1 **Fig. S6.** Different regions in the world map (i) to which the exotic parents belong and (ii) where
2 pre-breeding germplasms have been shared

3

4 **Fig. S7.** Presenting number of crosses made in the last 5 years for wheat pre-breeding and
5 development stages of germplasm and anticipated product delivery timeline has been illustrated

6

7 **Fig. S8.** Graph showing a three dimensional principal component analysis graph of 1711 gene
8 bank accessions out of which 244 exotics in different genetic spaces. The selected and unselected
9 genotypes are presented with red and yellow dots respectively

10

11

12

13

14

15

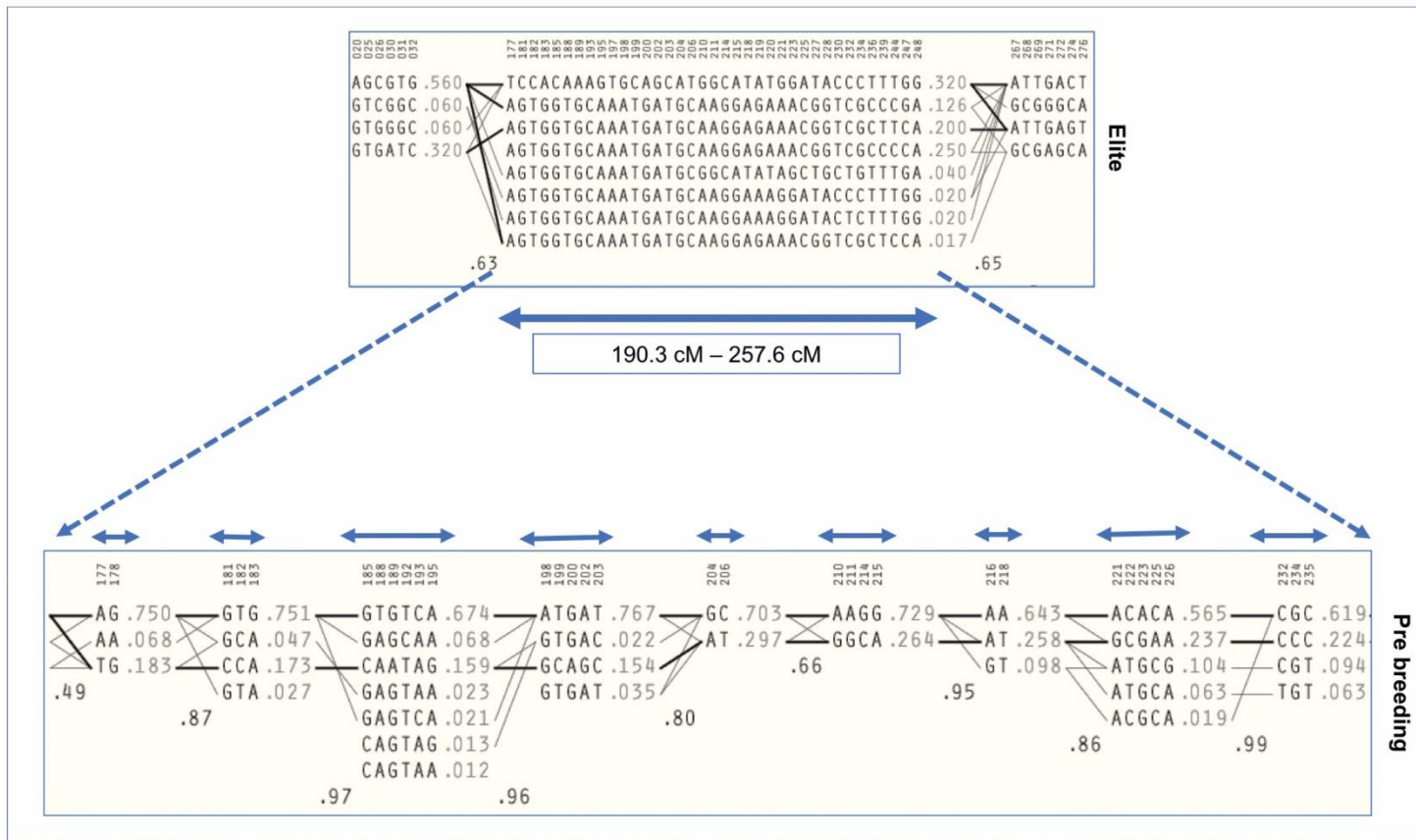
16

17

18

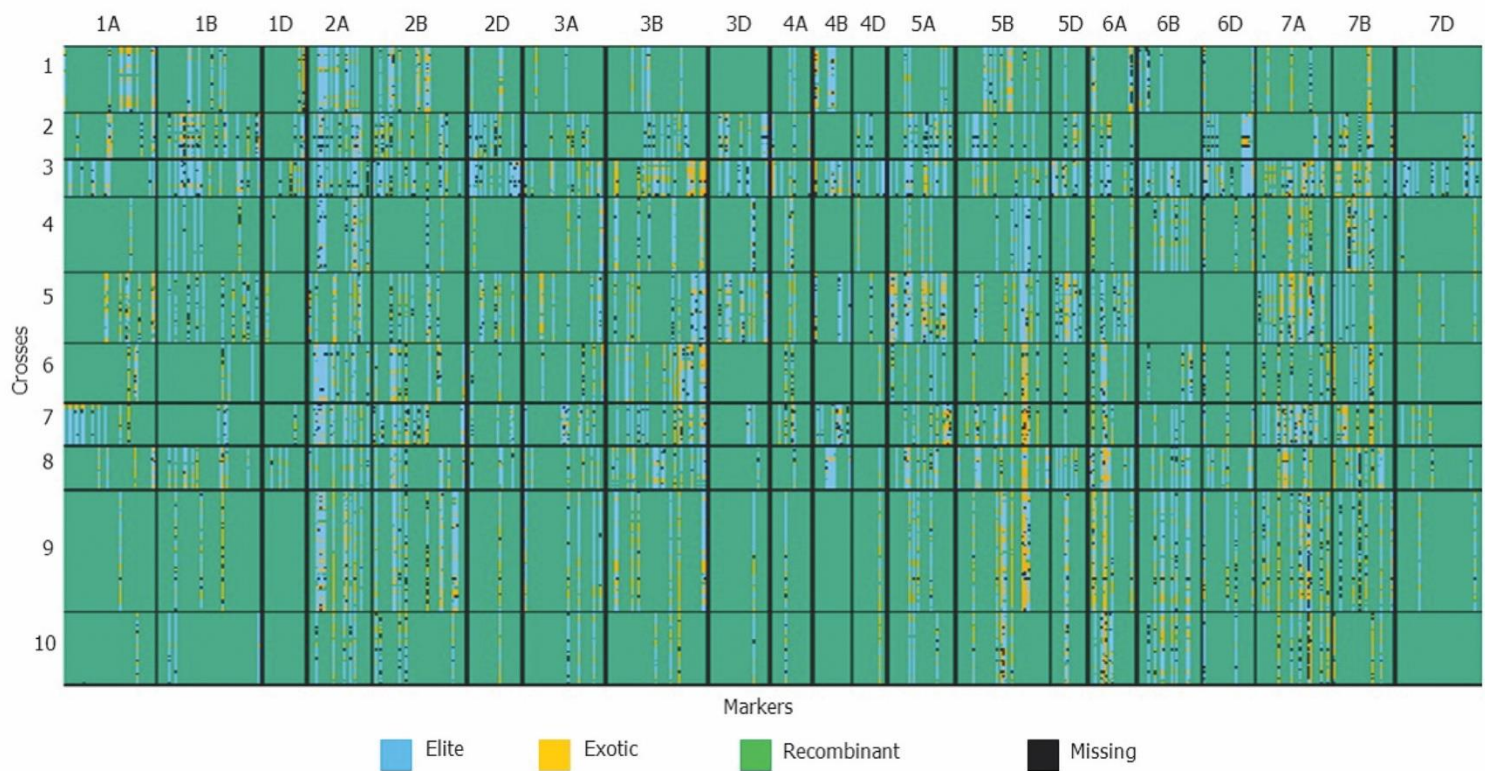
19

20



1
2
3
4
5
6

Fig.S1



1

2 Fig.S2

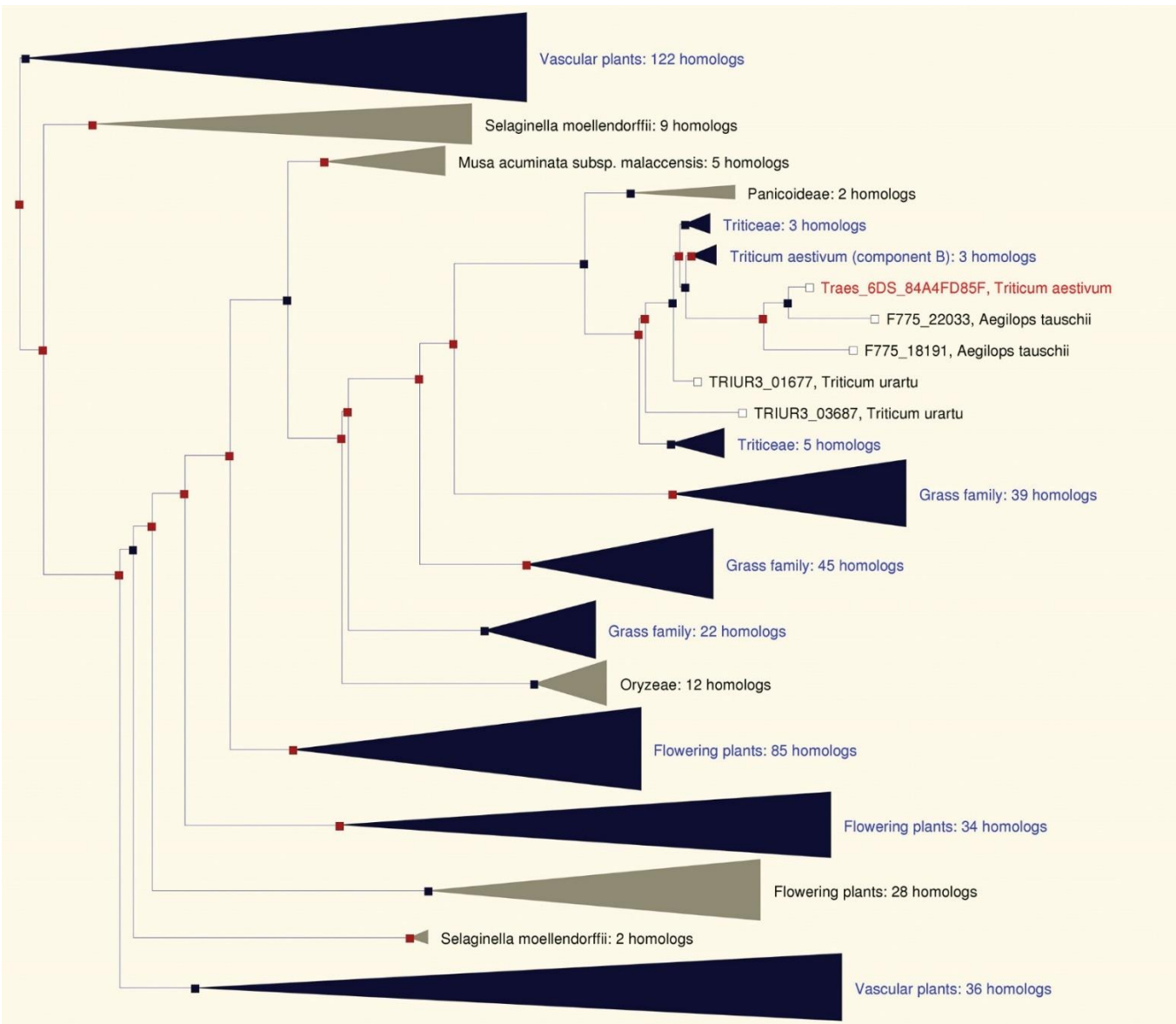
3

4

5

6

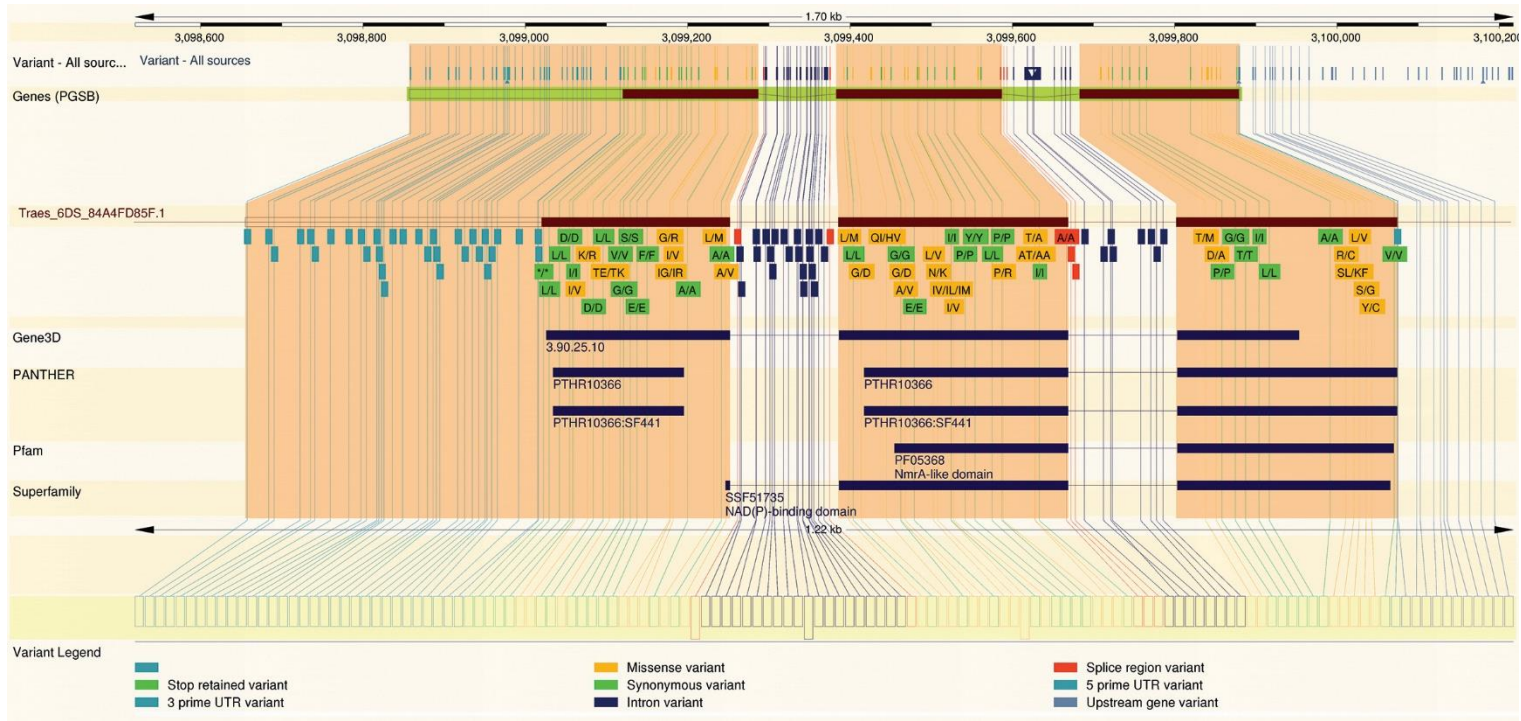
7



1

2 Fig.S3

1



2

3 Fig.S4

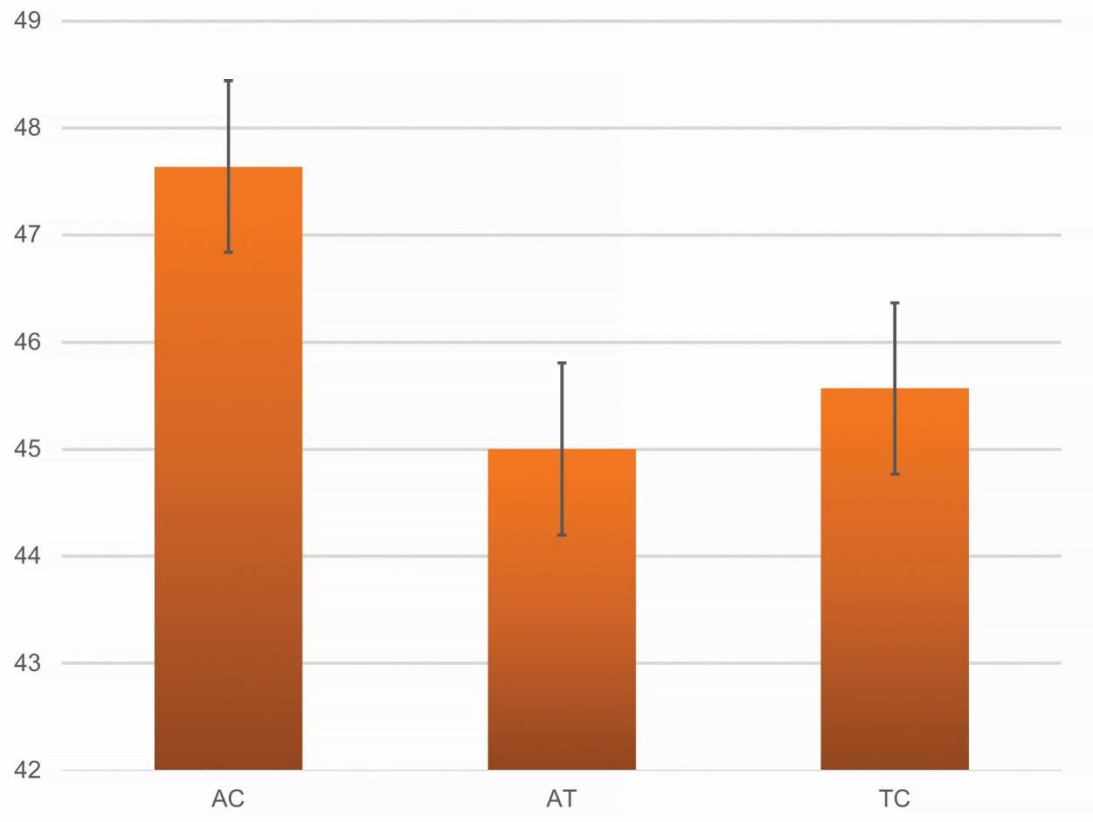
4

5

6

7

8



1

2 Fig.S5

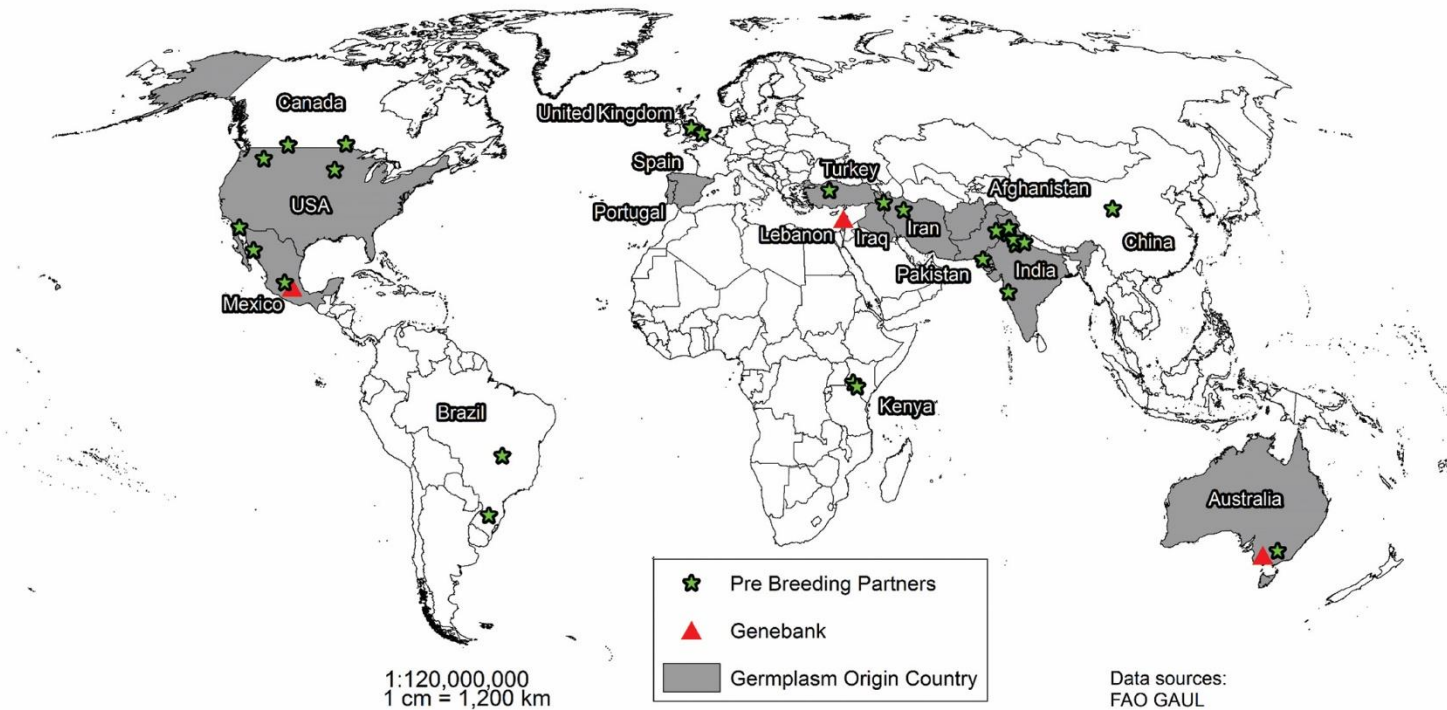
3

4

5

6

1



2

3 Fig.S6





4

5

6

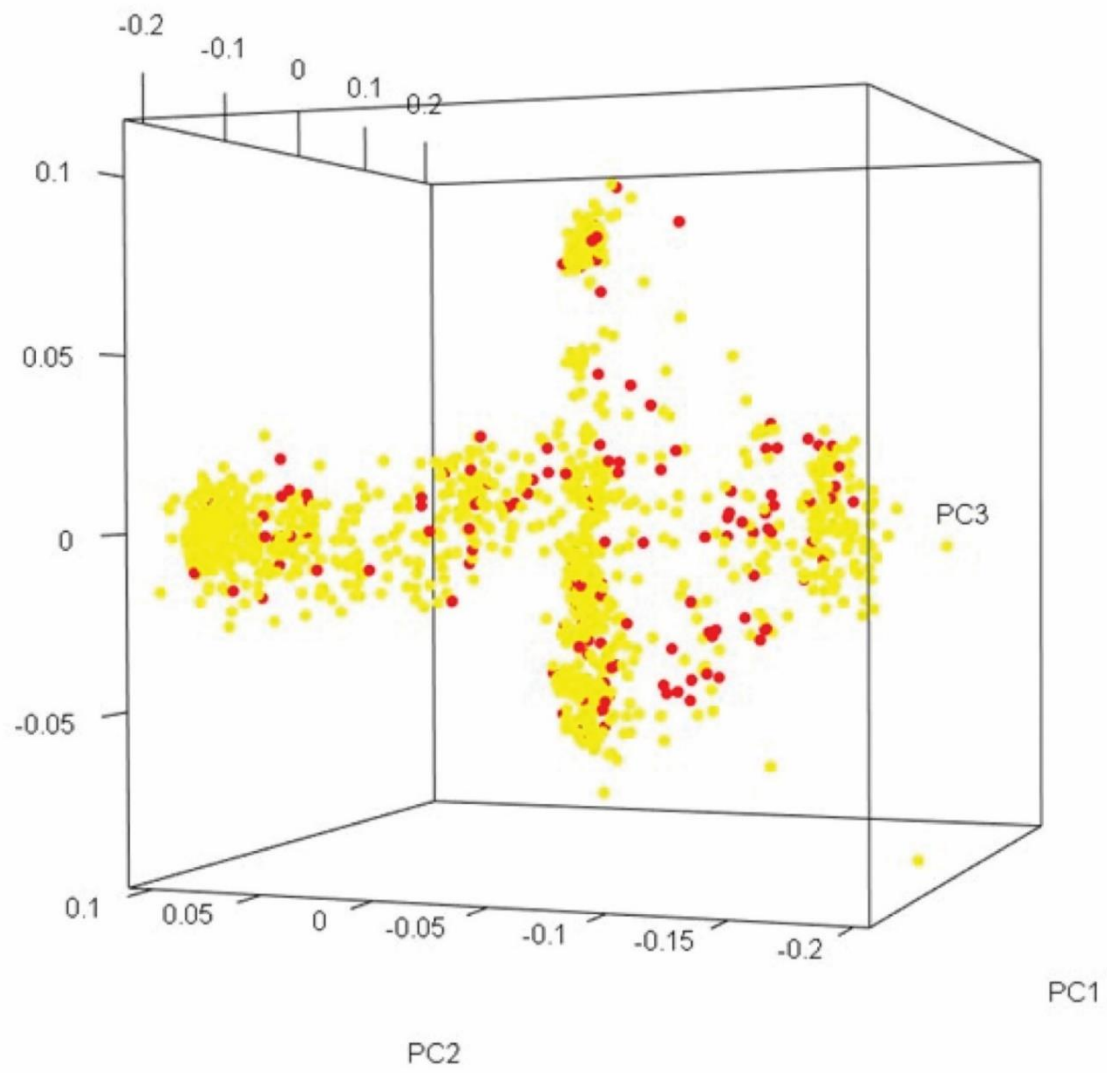
7

8

2016	200	2021	TC ₁ F ₃
			
2015	500	2020	TC ₁ F ₄
			
2014	200	2019	TC ₁ F ₅
			
2013	126	2018	TC ₁ F ₆
			
2012	244	2017	TC₁F₇
Crossing Initiated	Number of crosses	Mobilization to breeding pipelines	Filial stage of pre breeding product

1

2 Fig.S7



1

2 Fig.S8

