Protocol

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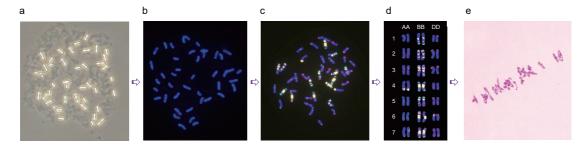
A platform for whole-genome speed introgression from *Aegilops tauschii* to wheat for breeding future crops

In the format provided by the authors and unedited

| 1 | A platform for whole genome speed introgression from <i>Aegilops</i> | | | | | |
|-----|--|--|--|--|--|--|
| 2 | tauschii to wheat for breeding future crops | | | | | |
| 3 | | | | | | |
| 4 | Hao Li ^{1,2,4} , Lele Zhu ^{1,4} , Ruixiao Fan ^{1,4} , Zheng Li ¹ , Yifan Liu ¹ , Aaqib Shaheen ¹ , Fang Nie ¹ , Can Li ¹ , | | | | | |
| 5 | Xuqin Liu ¹ , Yuanyuan Li ¹ , Wenjuan Liu ¹ , Yingying Yang ¹ , Tutu Guo ¹ , Yu Zhu ¹ , Mengchen Bu ¹ , | | | | | |
| 6 | Chenglin Li, Huihui Liang ¹ , Shenglong Bai ¹ , Feifei Ma ¹ , Guanghui Guo ² , Zhen Zhang ² , Jinling | | | | | |
| 7 | Huang ^{1,3} , Yun Zhou ¹ *, Chun-Peng Song ¹ * | | | | | |
| 8 | | | | | | |
| 9 | ¹ State Key Laboratory of Crop Stress Adaptation and Improvement, School of Life Sciences, | | | | | |
| 10 | Henan University, Kaifeng, China | | | | | |
| 11 | ² State Key Laboratory of Crop Stress Adaptation and Improvement, College of Agriculture, | | | | | |
| 12 | Henan University, Kaifeng, China | | | | | |
| 13 | ³ Department of Biology, East Carolina University, Greenville, NC, USA | | | | | |
| 14 | | | | | | |
| 15 | ⁴ These authors contributed equally to this work. | | | | | |
| 16 | *Emails: CP.S. (songcp@henu.edu.cn) or Y.Z. (zhoumouyun@vip.henu.edu.cn) | | | | | |
| 17 | | | | | | |
| 18 | Secondaria Flamma 1.2 | | | | | |
| 19 | Supplementary Figures 1-2 | | | | | |
| 20 | Supplementary Table 1-3 | | | | | |
| 21 | Supplementary Table 1 and Table 3 in separate Excel file | | | | | |
| 22 | Supplementary Video file | | | | | |
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25 Supplementary Figures

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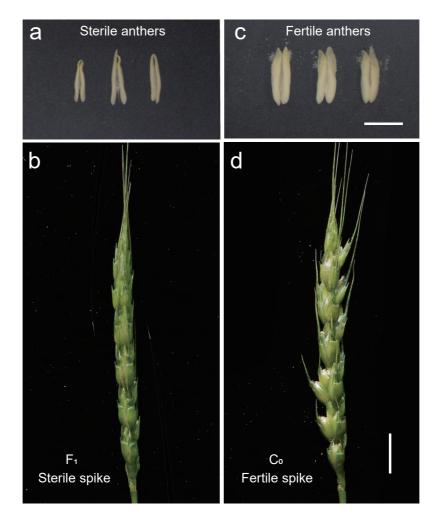


28 Supplementary Fig. 1. Mitotic chromosome analysis during the developmet of AWIs.

a, a good mitotic cell showing clear chromosome shape under the phase contrast channel. b, a
captured image of a cell showing chromosome shape with a 100× oil lens under the DAPI channel
using a fluorescence microscope. c, a merged image of four channels (DAPI, FITC, TXRED and
CY5) into a single picture after capturing. d, label and process the karyotype analysis on each
image according to the standard karyotype of wheat. Blue: DAPI, green: oligo-pSc119.2-1, red:
oligo-pTa535-1, yellow: oligo-(GAA)₁₀ and pTa71-2. e, a good meiotic cell showing clear
chromosome pairing.

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39 Supplementary Fig. 2. Spikes and anthers of a F_1/C_0 chimeric hybrid after colchicine

40 treatment.

41 a and b, a sterile spike showing sterile anthers and no seedset. c and d, a fertile spike showing fertile

42 anthers and seedset. Bar = 2 mm(a, c); Bar = 1 cm(b, d).

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47 Supplementary Table 1. The inheritance frequencies of *Ae. tauschii* T093 alleles evaluated by 55K
48 SNP array in derived BC₁F₁, BC₂F₁ and BC₃F₁ generation.
49 See Excel file
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52 Supplementary Table 2. Summary of the genes with variations between *Ae. tauschii* T093 and the
53 D subgenome of bread wheat AK58. A total of 16055 genes showed variations in coding sequence

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54 regions.
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| | Category – | SNP | | Indel | |
|----------------|-------------------|------------|----------------|---------|----------------|
| Region | | Numbers | Affected genes | Numbers | Affected genes |
| Intron | - | 326,111 | 10,352 | 10,163 | 4,391 |
| Splicing sites | - | 236 | 250 | 61 | 63 |
| | stop gain | 621 | 567 | 101 | 99 |
| | stop loss | 226 | 221 | 5 | 5 |
| F | synonymous SNV | 35,541 | 10,382 | - | - |
| Exon | nonsynonymous SNV | 40,005 | 11,167 | - | - |
| | nonframeshift | - | - | 2,053 | 1,744 |
| | frameshift | - | - | 9,990 | 3,346 |
| Total | - | 18,609,736 | 13,701 | 521,436 | 3,869 |

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58 Supplementary Table 3. Statistics of introgressed fragment sizes in derived BC<sub>4</sub>F<sub>1</sub>, BC<sub>4</sub>F<sub>2</sub> and
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59 BC<sub>4</sub>F<sub>4</sub> generations.
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60 See Excel file
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63 Supplementary Video 1. A video file showing how to emasculate the spikes of *Ae. tauschii*.