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**Modification of a novel x-type high-molecular-weight glutenin subunit gene from *Aegilops markgrafii* to improve dough strength of wheat flour**

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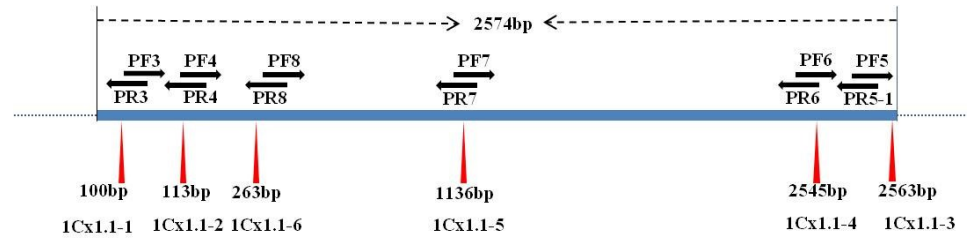
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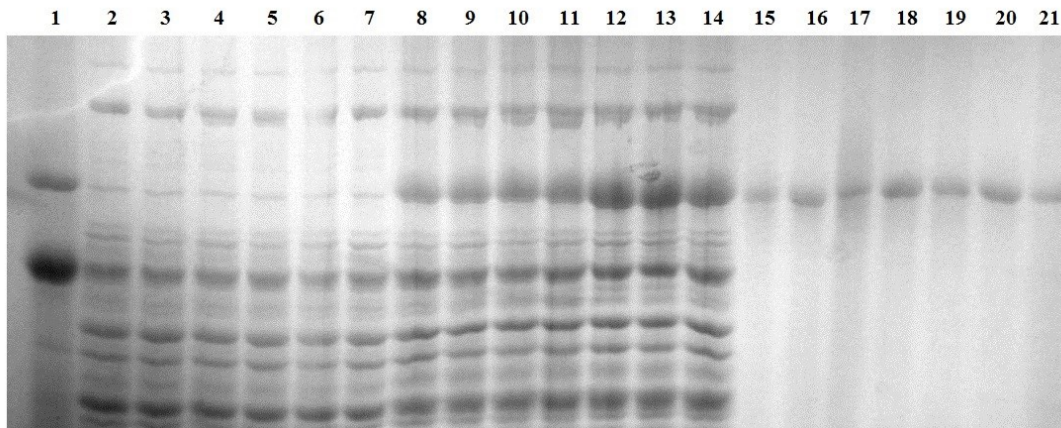
## Supplementary materials

**Fig. S1**



**Fig. S1.** The diagram of site-directed mutagenesis of *ICx1.1*. black arrows indicate the primers used for site-directed mutagenesis, red triangle points at the mutation site, dotted line shows the pMD18-T Vector.

**Fig. S2**



**Fig. S2.** SDS-PAGE analysis of *E. coli* expressed and purified proteins of 1Cx1.1 and Mut 1Cx1.1-1 to Mut 1Cx1.1-6. Lane 1: HMW-GSs in the seed of *Ae. markgrafii* Y46; lane 2: proteins expressed from BL21(DE3); lane 3: proteins expressed under IPTG induced BL21(DE3); lane 4: proteins expressed without IPTG induced BL21 (DE3) transformed with pEASY-E1; lane 5: proteins expressed under IPTG induced BL21 (DE3) transformed with pEASY-E1; lane 6: proteins expressed without IPTG induced BL21 (DE3) transformed with 1Cx1.1; lane 7: proteins expressed without IPTG induced BL21 (DE3) transformed with Mut 1Cx1.1-1; lane 8-lane 14: proteins expressed under IPTG induced BL21 (DE3) transformed with 1Cx1.1, Mut 1Cx1.1-1, Mut 1Cx1.1-2, Mut 1Cx1.1-3, Mut 1Cx1.1-4, Mut 1Cx1.1-5 and Mut 1Cx1.1-6; lane 15-lane 21: purified protein of 1Cx1.1, Mut 1Cx1.1-1, Mut 1Cx1.1-2, Mut 1Cx1.1-3, Mut 1Cx1.1-4, Mut 1Cx1.1-5 and Mut 1Cx1.1-6.

**Table S1. The primers used in this study. The positions of single-base substitution mutation were are shown in bold italics**

Primer	Sequences (5'-3')
PF1	ATGGCTAAGCGGC/TTA/GGTCCTCTTTG
PR1	CTATCACTGGCTA/GGCCGACAATGCG
PF2	GAAGGTGAGGCCTCTGGGCAACTACA
PR2	CTATCACTGGCTGGCCGACAATGCGTCG
PF3	ACCAGCAGCTCCGAGACATT <b>T</b> GCCCCAAGT
PR3	<b>A</b> AATGTCTCGGAGCTGCTGGTCCATGACCT
PF4	GACATTAGCCCCAAGTGCT <b>G</b> CCCCGTTGTC
PR4	<b>C</b> AGCACTTGGGGCTAATGTCTCGGAGCTGC
PF5	GCGGCGACGCATTGTCGGCC <b>T</b> GCCAGTGAT
PR5-1	<b>A</b> GGCCGACAATGCGTCGCCGCCCTCCAGCC
PR5-2	CTATCACTGGCAGGCCGACAATGCGTCG
PF6	AATGTGCCGGCTGGAGGGC <b>T</b> GCGACGCATT
PR6	<b>A</b> GCCCTCCAGCCGGCACATTGCCGGCAGCT
PF7	CAAGTGTACTIONACCCAACTT <b>G</b> TCCGCAACAG
PR7	<b>C</b> AAGTTGGGTAGTACACTTGTTGCCCTTGT
PF8	ACCTGCACTGCTAAAAAGGT <b>G</b> TTACCCAAG
PR8	<b>C</b> ACCTTTTTAGCAGTGCAGGTATTCCCAA