

Supplementary Table S1  
Primers used in this study

Gene	Forward (5'-3')	Reverse (5'-3')	Purpose
<i>CHS1-Glyma.08G109400</i> cds	ATGGTGAGTGTGAAGAGATTC	TCAGAGAGTGACACTGCGGAGC	gene cloning
<i>CHS3-Glyma.08G109300</i> cds	ATGGTCAGTGTGAAGAGATCCGTA	TTAGACAGTGACACTGCGGAGCACA	gene cloning
<i>CHS4-Glyma.08G110700</i> cds	ATGGTGAGTGTGAAGAGATTCTGA	TTAGACAGTGACACTGCGGAGTACA	gene cloning
<i>Glyma.08g122400</i> cds	ATGAATGCTAGAACGCTGGCA	CTAGATGTAATTGCTAGTTGA	gene cloning
<i>Glyma.08g125100</i> cds	ATGGAGGTGACCAACCTGT	CTAGAAATGGGAAGGATGGA	gene cloning
<i>Glyma.08G137600</i> cds	ATGGGAACGTCTAGACATGT	TCATATAGCTCCCAATCAATC	gene cloning
<i>Glyma.08g145300</i> cds	ATGATGAAGAACGATAGAGA	TCAGGCAGAACGAGAGGAGC	gene cloning
<i>Glyma.08G149200</i> cds	ATGGCAGAGTCTCAGAAACAC	TTAGGCATAATGTATATTGAGGGG	gene cloning
qPCR- <i>Glyma.08g125600</i>	CGCATGCAAGAAATAGGTT	TGTAATTGACTGCACACATT	qRT-PCR expression analysis
qPCR- <i>Glyma.08g119500</i>	TGGTGCATTTCAGAACTCA	TCACGTTATCTGATACCCT	qRT-PCR expression analysis
qPCR- <i>Glyma.08g119600</i>	AACTTAACACTCACAGCTCGTA	GGGTATCAAAGTTGAGATT	qRT-PCR expression analysis
qPCR- <i>Glyma.08g122400</i>	ATAATCCGAACACTGTCCGC	GTTGGTGGTGGCTGATTAGT	qRT-PCR expression analysis
qPCR- <i>Glyma.08g125000</i>	TTCCCTGGAACAAGGTTCAT	CATTCGGTCATAAACCTTC	qRT-PCR expression analysis
qPCR- <i>Glyma.08g125100</i>	CTCAGAAACTACTTGCCAAA	AAATTTAAGGAAAGCTTG	qRT-PCR expression analysis
qPCR- <i>Glyma.08g128600</i>	CAGAAAGTTGAGGACTTGT	ATTGGCATGTGGCTGAAATT	qRT-PCR expression analysis
qPCR- <i>Glyma.08g145300</i>	TCATGGTGGCAACTCTGTTCTC	CTCCTCGCTTCTTCTCCTCCT	qRT-PCR expression analysis
qPCR- <i>Glyma.08G137600</i>	TCTAAGACACCACGGAGCCTC	TTGCCACGTCAGACTCGACCT	qRT-PCR expression analysis
qPCR- <i>Glyma.08G149200</i>	TTGGAGAGGATTCAGAACCGA	CCCATTTCTCTGCCTAACACC	qRT-PCR expression analysis
qRT- <i>Actin11</i>	ATGACCCAAATCATGTTGAGACC	TCAGTTAGATCACGACCAGCGA	qRT-PCR expression analysis
<i>GmSFT1(Glyma.08G137600)</i>	AGCCACCATGCTCGAGATGGGAAC TGCTATAGACATGTAC	TGCTCACCATCTCGAGTATAAGCTTC CCAATCAATCTCCAC	vector construction

Table S2

ANOVA of GR, NSR and EC in two RIL populations.

Population	Trait	Source	DF <sup>a</sup>	MS <sup>b</sup>	F <sup>c</sup>	Population	Trait	Source	DF <sup>a</sup>	MS <sup>b</sup>	F <sup>c</sup>
NJRINP	GR	Genotype	280	0.37	2.43***	NJRI4P	GR	Genotype	160	0.43	9.08***
		Env	1	1.87	12.32***			Env	1	0.64	13.34***
		Block	1	0.00	0.00			Block	1	0.00	0.00
		Gen × Env	265	0.17	1.09**			Gen × Env	159	0.10	2.01**
		Error	485	0.15				Error	302	0.05	
	NSR	Genotype	280	0.17	12.05***			Genotype	160	0.44	11.23***
		Env	1	46.23	3300.74***			Env	1	0.29	7.26**
		Block	1	0.07	4.83		NJRI4P	Block	1	0.00	0.01
		Gen × Env	264	0.13	9.29***			Gen × Env	158	0.09	2.37**
		Error	482	0.01				Error	282	0.04	
EC	EC	Genotype	280	131017.63	23.36***			Genotype	160	185786.52	8.69***
		Env	1	139454.82	24.87***			Env	1	137737.31	6.44**
		Block	1	23624.23	4.21			Block	1	15016.54	0.70
		Gen × Env	269	13471.06	2.4**			Gen × Env	159	37284.26	1.74**
		Error	541	5607.53				Error	312	21388.16	

\*\*\*  $p<0.001$ ; \*\*  $p<0.01$ . <sup>a</sup> Degrees of freedom. <sup>b</sup> Mean square. <sup>c</sup> F value is for determining significance.

Table S3

Analysis of nucleotide mutation position of *CHS1*, *CHS3* and *CHS4*.

Gene	Position(bp)	Nucleotide variation	Amino acid variation	
			Variation way	Variation type
<i>CHS1</i>	396	T/C	-	S
	498	C/T		
	531	C/T		
<i>CHS3</i>	219	T/C	-	S
	225	A/G		
	243	G/C		
	255	T/A		
	258	C/T		
<i>CHS4</i>	531	C/T		

x/y = x nucleotide change into y nucleotide, - = No variation, S = Synonymous mutations, N = Non-synonymous mutations.



Table S4

Functional annotation of 10 candidate genes associated with seed-flooding tolerance located in the QTL hotspots on Chr.08.

Gene	Function annotation
<i>Glyma.08g119500</i>	FASCICLIN-like arabinogalactan-protein 12
<i>Glyma.08g119600</i>	FASCICLIN-like arabinogalactan-protein 11
<i>Glyma.08g122400</i>	Carbohydrate-binding X8 domain superfamily protein
<i>Glyma.08g125000</i>	Cytochrome P450, family 716, subfamily A, polypeptide 1
<i>Glyma.08g125100</i>	Cytochrome P450, family 716, subfamily A, polypeptide 1
<i>Glyma.08g125600</i>	UDP-glycosyltransferase 74 F1
<i>Glyma.08g128600</i>	Cytochrome P450, family 716, subfamily A, polypeptide 1
<i>Glyma.08G137600</i>	Ethylene response factor/AP2 domain
<i>Glyma.08g145300</i>	Integrase-type DNA-binding superfamily protein/AP2 domain
<i>Glyma.08G149200</i>	Integrase-type DNA-binding superfamily protein/AP2 domain

**A**

P1-CHS1	ATGGTCAGTTGAAGAGATCTGTAAGCGCAACGTGCCAGAAGGGCCCTGCCACTGTCTATGGCTATTGGCACCGCAACTCTCCAACACTGCGTGGATCAGAGTACCATCTCCGATATTATTCGGCATACCAACAGCGA	140
P2-CHS1	ATGGTCAGTTGAAGAGATCTGTAAGCGCAACGTGCCAGAAGGGCCCTGCCACTGTCTATGGCTATTGGCACCGCAACTCTCCAACACTGCGTGGATCAGAGTACCATCTCCGATATTATTCGGCATACCAACAGCGA	140
P3-CHS1	ATGGTCAGTTGAAGAGATCTGTAAGCGCAACGTGCCAGAAGGGCCCTGCCACTGTCTATGGCTATTGGCACCGCAACTCTCCAACACTGCGTGGATCAGAGTACCATCTCCGATATTATTCGGCATACCAACAGCGA	140
P1-CHS1	GCACATGACCGAGCTAAAGAAAATTCAAGCCATGTCGATAAGTGTGTTAAGAAGGATCATGTACTTAAACGAGATCTGCTTAAAGAAGGATCTGCTTAAAGAAGGAGATCCGAGTTTGCTGTTACATGGCACCTTCGATGCAA	280
P2-CHS1	GCACATGACCGAGCTAAAGAAAATTCAAGCCATGTCGATAAGTGTGTTAAGAAGGATCATGTACTTAAACGAGATCTGCTTAAAGAAGGAGATCCGAGTTTGCTGTTACATGGCACCTTCGATGCAA	280
P3-CHS1	GCACATGACCGAGCTAAAGAAAATTCAAGCCATGTCGATAAGTGTGTTAAGAAGGATCATGTACTTAAACGAGATCCGAGTTTGCTGTTACATGGCACCTTCGATGCAA	280
P1-CHS1	GCACAGACATGGTGGTTGAGGTTACCAAACTTGGGAAAGAGGCTCAACTAAGGAAATCAAGGAATGGGTCACCCAAAGTCAAGATTACCCATCTCATCTTGGCACACAGTGGTCGACATGCCGGTGTGCT	420
P2-CHS1	GCACAGACATGGTGGTTGAGGTTACCAAACTTGGGAAAGAGGCTCAACTAAGGAAATGGGTCACCCAAAGTCAAGGAAATTACCCATCTCATCTTGGCACACAGTGGTCGACATGCCGGTGTGCT	420
P3-CHS1	GCACAGACATGGTGGTTGAGGTTACCAAACTTGGGAAAGAGGCTCAACTAAGGAAATGGGTCACCCAAAGTCAAGGAAATTACCCATCTCATCTTGGCACACAGTGGTCGACATGCCGGTGTGCT	420
P1-CHS1	GATTATCAGCTACTAACTATTAGGCCCTGGCCCTCCGTCAGCGTTACATGTGTTAAGAAGGCTCAACCAAGGCTGTTGGGACGGTCTTCGTTGGCAAGGCTTGGGACGGTCTTCGTTGGCAAGGCTTGGGACGGTCT	560
P2-CHS1	GATTATCAGCTACTAACTATTAGGCCCTGGCCCTCCGTCAGCGTTACATGTGTTAAGAAGGCTCAACCAAGGCTGTTGGGACGGTCTTCGTTGGCAAGGCTTGGGACGGTCTTCGTTGGCAAGGCTTGGGACGGTCT	560
P3-CHS1	GATTATCAGCTACTAACTATTAGGCCCTGGCCCTCCGTCAGCGTTACATGTGTTAAGAAGGCTCAACCAAGGCTGTTGGGACGGTCTTCGTTGGCAAGGCTTGGGACGGTCTTCGTTGGCAAGGCTTGGGACGGTCT	560
P1-CHS1	TGTCGTTTCTCTGAGATCACCGAGTCACATTGCGGCCCAACTGACACCCATCTGATGGCTTGGGCAAGGCTGTTGGGAGATGGTGCAGGCCGCTGTCATGTTGGATCAGACCCCTTACCGTTGAAAAGGCT	700
P2-CHS1	TGTCGTTTCTCTGAGATCACCGAGTCACATTGCGGCCCAACTGACACCCATCTGATGGCTTGGGCAAGGCTGTTGGGAGATGGTGCAGGCCGCTGTCATGTTGGATCAGACCCCTTACCGTTGAAAAGGCT	700
P3-CHS1	TGTCGTTTCTCTGAGATCACCGAGTCACATTGCGGCCCAACTGACACCCATCTGATGGCTTGGGCAAGGCTGTTGGGAGATGGTGCAGGCCGCTGTCATGTTGGATCAGACCCCTTACCGTTGAAAAGGCT	700
P1-CHS1	CTTGTTCAGCTGTCGACTGCCAGAACATCTCCAGACAGTGAAGGGCTATTGATGACACCTTCCGCAAGTGTGTCACCTTCATCTCCAAGGATTTCTGACTCATCCAAAGAATTGAGAAC	840
P2-CHS1	CTTGTTCAGCTGTCGACTGCCAGAACATCTCCGACAGACTGACACCCATCTGATGGCTTGGGCAAGGCTGTTGGGAGATGGTGCAGGCCGCTGTCATGTCAGAAATTGAGAAC	840
P3-CHS1	CTTGTTCAGCTGTCGACTGCCAGAACATCTCCGACAGACTGACACCCATCTGATGGCTTGGGCAAGGCTGTTGGGAGATGGTGCAGGCCGCTGTCATGTCAGAAATTGAGAAC	840
P1-CHS1	GCCTTGTGTAAGGCCCTTCAACCCCTGGGAATCTGGATTACAATCTCTGATGGCTTGGGCAACCCCTGGGACGGCCAATTGGGACCAAGTGGCTGAAGGCTGAAAATGGAGCTACTAG	980
P2-CHS1	GCCTTGTGTAAGGCCCTTCAACCCCTGGGAATCTGGATTACAATCTCTGATGGCTTGGGCAACCCCTGGGACGGCCAATTGGGACCAAGTGGCTGAAGGCTGAAAATGGAGCTACTAG	980
P3-CHS1	GCCTTGTGTAAGGCCCTTCAACCCCTGGGAATCTGGATTACAATCTCTGATGGCTTGGGCAACCCCTGGGACGGCCAATTGGGACCAAGTGGCTGAAGGCTGAAAATGGAGCTACTAG	980
P1-CHS1	ACATGTGTCAGCGAGTATGGTAAACATGTCAGCTGATGGCTTGGGCAACCCCTGGGACGGCCAATTGGGACACCTTCCGAGACTTGGGCTGTCATCTCCAAAGGATTTGGGCTGTCATTTGGGTTTCGCC	1120
P2-CHS1	ACATGTGTCAGCGAGTATGGTAAACATGTCAGCTGATGGCTTGGGCAACCCCTGGGACGGCCAATTGGGACACCTTCCGAGACTTGGGCTGTCATCTCCAAAGGATTTGGGCTGTCATTTGGGTTTCGCC	1120
P3-CHS1	ACATGTGTCAGCGAGTATGGTAAACATGTCAGCTGATGGCTTGGGCAACCCCTGGGACGGCCAATTGGGACACCTTCCGAGACTTGGGCTGTCATCTCCAAAGGATTTGGGCTGTCATTTGGGTTTCGCC	1120
P1-CHS1	CTGGACTCACCGTTGAGACTGTTGCTCCGCACTGTCACTCTCTGA	1167
P2-CHS1	CTGGACTCACCGTTGAGACTGTTGCTCCGCACTGTCACTCTCTGA	1167
P3-CHS1	CTGGACTCACCGTTGAGACTGTTGCTCCGCACTGTCACTCTCTGA	1167

**B**

P1-CHS1	MVSVEEIRKAQRAEGPATVMA1GATTPNCVDQSTPDYF1RNTSEHMTLEKEFKRKMCDKSM1KKRMYLNEELKENPNSVCAYMAPSLDARQDMVVVEVPKLGEKAATKA1KEWQPQSK1TH1FCTTSVGDMPGA	140
P2-CHS1	MVSVEEIRKAQRAEGPATVMA1GATTPNCVDQSTPDYF1RNTSEHMTLEKEFKRKMCDKSM1KKRMYLNEELKENPNSVCAYMAPSLDARQDMVVVEVPKLGEKAATKA1KEWQPQSK1TH1FCTTSVGDMPGA	140
P3-CHS1	MVSVEEIRKAQRAEGPATVMA1GATTPNCVDQSTPDYF1RNTSEHMTLEKEFKRKMCDKSM1KKRMYLNEELKENPNSVCAYMAPSLDARQDMVVVEVPKLGEKAATKA1KEWQPQSK1TH1FCTTSVGDMPGA	140
P1-CHS1	DYQLTKLLGLRPSVKRYMMYQQQCFAGTVRLAKDLAENNGARVLVVCSE1TAVTFRGPDTLDLSLVQALFGDAAVIVGSDPPLPVKPLFLQWTAQTILPDSSEGAI1DGH1REVGLTFHLLKDVGTLISKNIK	280
P2-CHS1	DYQLTKLLGLRPSVKRYMMYQQQCFAGTVRLAKDLAENNGARVLVVCSE1TAVTFRGPDTLDLSLVQALFGDAAVIVGSDPPLPVKPLFLQWTAQTILPDSSEGAI1DGH1REVGLTFHLLKDVGTLISKNIK	280
P3-CHS1	DYQLTKLLGLRPSVKRYMMYQQQCFAGTVRLAKDLAENNGARVLVVCSE1TAVTFRGPDTLDLSLVQALFGDAAVIVGSDPPLPVKPLFLQWTAQTILPDSSEGAI1DGH1REVGLTFHLLKDVGTLISKNIK	280
P1-CHS1	ALVEAFQPLG1SDYNS1FWIAPGPPA1LDQVEAKLGLPEKMEATHRVLSEYGMSSACVLFLDQMRKKS1ENGLTGTCLDWVFLFGFPGLTVETVLRSTVL	388
P2-CHS1	ALVEAFQPLG1SDYNS1FWIAPGPPA1LDQVEAKLGLPEKMEATHRVLSEYGMSSACVLFLDQMRKKS1ENGLTGTCLDWVFLFGFPGLTVETVLRSTVL	388
P3-CHS1	ALVEAFQPLG1SDYNS1FWIAPGPPA1LDQVEAKLGLPEKMEATHRVLSEYGMSSACVLFLDQMRKKS1ENGLTGTCLDWVFLFGFPGLTVETVLRSTVL	388

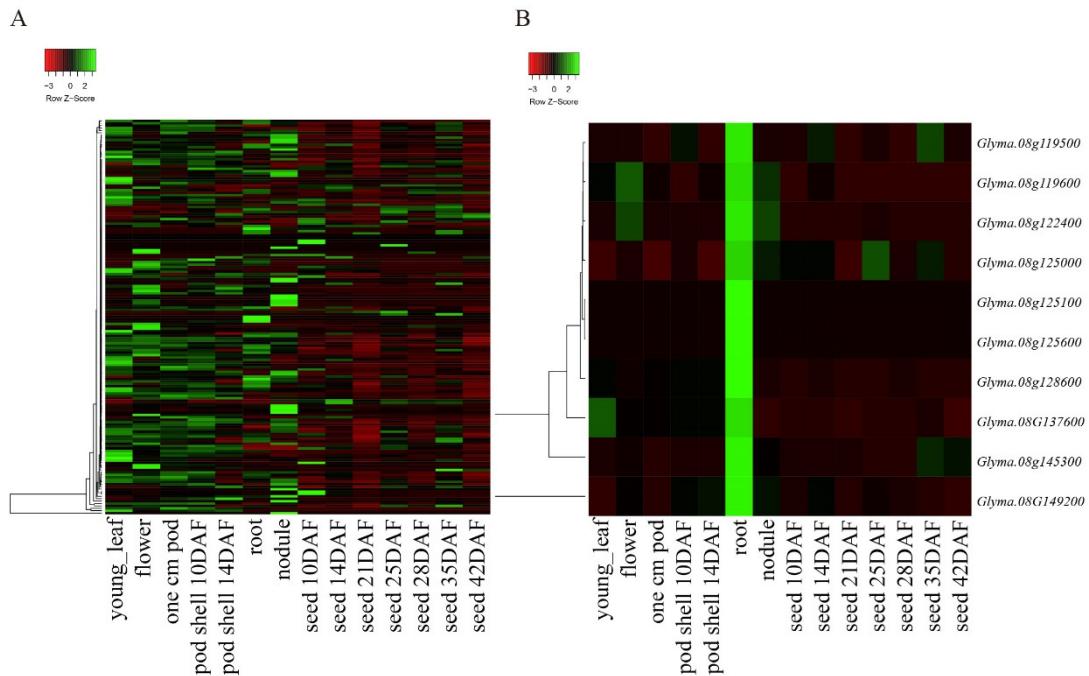
**C**

P1-CHS3	ATGGTCAGTTGAAGAGATCTGTAATGCCACAGTCAGAGGGCCCTGCCACTGTCTATGGCTATTGGCACCGCAACTCTCCAACACTGCGTGGATCAGAGTACCATCTCCGATACCAACAGCGA	140
P2-CHS3	ATGGTCAGTTGAAGAGATCTGTAATGCCACAGTCAGAGGGCCCTGCCACTGTCTATGGCTATTGGCACCGCAACTCTCCAACACTGCGTGGATCAGAGTACCATCTCCGATACCAACAGCGA	140
P3-CHS3	ATGGTCAGTTGAAGAGATCTGTAATGCCACAGTCAGAGGGCCCTGCCACTGTCTATGGCTATTGGCACCGCAACTCTCCAACACTGCGTGGATCAGAGTACCATCTCCGATACCAACAGCGA	140
P1-CHS3	GCACATGACCGAGCTAAAGAAAATTCAAGCCATGTCGATAAGTGTGTTAAGAAGGATCATGTACTTAAACGAGATCTGCTTAAAGAAGGAGATCCGAGTTTGCTGTTACATGGCACCTTCGATGCAA	280
P2-CHS3	GCACATGACCGAGCTAAAGAAAATTCAAGCCATGTCGATAAGTGTGTTAAGAAGGATCATGTACTTAAACGAGATCTGCTTAAAGAAGGAGATCCGAGTTTGCTGTTACATGGCACCTTCGATGCAA	280
P3-CHS3	GCACATGACCGAGCTAAAGAAAATTCAAGCCATGTCGATAAGTGTGTTAAGAAGGATCATGTACTTAAACGAGATCTGCTTAAAGAAGGAGATCCGAGTTTGCTGTTACATGGCACCTTCGATGCAA	280
P1-CHS3	GCACAGACATGGTGGTTGAGGTTACCAAACTTGGGAAAGAGGCTCAACTAAGGAAATGGGTCACCCAAAGTCAAGGAAATTACCCATCTCATCTTGGCACAGAGTACCATCTCCGATATTATTCGGCATACCAACAGCGA	420
P2-CHS3	GCACAGACATGGTGGTTGAGGTTACCAAACTTGGGAAAGAGGCTCAACTAAGGAAATGGGTCACCCAAAGTCAAGGAAATTACCCATCTCATCTTGGCACAGAGTACCATCTCCGATATTATTCGGCATACCAACAGCGA	420
P3-CHS3	GCACAGACATGGTGGTTGAGGTTACCAAACTTGGGAAAGAGGCTCAACTAAGGAAATGGGTCACCCAAAGTCAAGGAAATTACCCATCTCATCTTGGCACAGAGTACCATCTCCGATATTATTCGGCATACCAACAGCGA	420
P1-CHS3	GATTATCAGCTACTAACTATTAGGCCCTGGCCCTCCGTCAGCGTTACATGTGTTAAGAAGGCTCAACCGCTTGGGACGGCTTGGCACCGCTTGGGCAAGGCTGTCATTTGGCACCTTCGATGCAA	560
P2-CHS3	GATTATCAGCTACTAACTATTAGGCCCTGGCCCTCCGTCAGCGTTACATGTGTTAAGAAGGCTCAACCGCTTGGGACGGCTTGGCACCTTCGATGCAA	560

**Supplementary Fig. S1. Multiple sequence alignment describing the base sequence and amino acids sequence of *CHS1*, *CHS3* and *CHS4* in P1(PI342618B), P2(NN86-4) and P3(NN493-1).** (A),(C),(E) represent multiple base sequence alignment of *CHS1*, *CHS3* and *CHS4*. (B),(D),(F) represent multiple amino acids sequence alignment of *CHS1*, *CHS3* and *CHS4*.

D		
P1-CHS3	MVSVEEIRNAQRAEGPATVMA GTATPPNCVDQSTYPDYFRTINSEHMETELKERFKRMCDSMIKKRYMLNEEILKENPSVCAYMAPSLDARQDMVVVEVPKLGEKEATAKIKEWGQPKSKITHLIFCTTSGVDMPGAA	140
P2-CHS3	MVSVEEIRNAQRAEGPATVMA GTATPPNCVDQSTYPDYFRTINSEHMETELKEFKRMCDSMIKKRYMLNEEILKENPSVCAYMAPSLDARQDMVVVEVPKLGEKEATAKIKEWGQPKSKITHLIFCTTSGVDMPGAA	140
P3-CHS3	MVSVEEIRNAQRAEGPATVMA GTATPPNCVDQSTYPDYFRTINSEHMETELKEFKRMCDSMIKKRYMLNEEILKENPSVCAYMAPSLDARQDMVVVEVPKLGEKEATAKIKEWGQPKSKITHLIFCTTSGVDMPGAA	140
P1-CHS3	DYQLTKLLGLRPSVKRYMMYQQGCFAGGTVLRLAKDLAENNNKGARVLYVVCSEITAVTFRGPDTDHLDSLVGQALFGDAAAIVGSDPLPVKEPLFQLWVTAQTILPDSEGAIDGHLREVGLTFHLLKDVPGLISKNIK	280
P2-CHS3	DYQLTKLLGLRPSVKRYMMYQQGCFAGGTVLRLAKDLAENNNKGARVLYVVCSEITAVTFRGPDTDHLDSLVGQALFGDAAAIVGSDPLPVKEPLFQLWVTAQTILPDSEGAIDGHLREVGLTFHLLKDVPGLISKNIK	280
P3-CHS3	DYQLTKLLGLRPSVKRYMMYQQGCFAGGTVLRLAKDLAENNNKGARVLYVVCSEITAVTFRGPDTDHLDSLVGQALFGDAAAIVGSDPLPVKEPLFQLWVTAQTILPDSEGAIDGHLREVGLTFHLLKDVPGLISKNIK	280
P1-CHS3	ALVEAFQPLG1SDYNSIP#IAHPGPAILDQVEAKGLKPEKMEATHVLSYGNMSSACVLFILDQMRRKSIEENGLTTGEGLDWGVLFGFPGPLTVETVLRSLRVTV	388
P2-CHS3	ALVEAFQPLG1SDYNSIP#IAHPGPAILDQVEAKGLKPEKMEATHVLSYGNMSSACVLFILDQMRRKSIEENGLTTGEGLDWGVLFGFPGPLTVETVLRSLRVTV	388
P3-CHS3	ALVEAFQPLG1SDYNSIP#IAHPGPAILDQVEAKGLKPEKMEATHVLSYGNMSSACVLFILDQMRRKSIEENGLTTGEGLDWGVLFGFPGPLTVETVLRSLRVTV	388
E		
P1-CHS4	ATGGTGAGTTGAAGAGATTCTGAAGGCCAACGTCAGAGGCTTCAACTGTCATGGCTATGGCACCGCCACTCTCCAACTCGCTGGATCAGAGTACCTATCTGACTATTATTCGGCATACCAACAGCGAA	140
P2-CHS4	ATGGTGAGTTGAAGAGATTCTGAAGGCCAACGTCAGAGGCTTCAACTGTCATGGCTATGGCACCGCCACTCTCCAACTCGCTGGATCAGAGTACCTATCTGACTATTATTCGGCATACCAACAGCGAA	140
P3-CHS4	ATGGTGAGTTGAAGAGATTCTGAAGGCCAACGTCAGAGGCTTCAACTGTCATGGCTATGGCACCGCCACTCTCCAACTCGCTGGATCAGAGTACCTATCTGACTATTATTCGGCATACCAACAGCGAA	140
P1-CHS4	GCACATGACGGAGCTCAAAGAAAATCAAACCGATGTGATAAGTCGATGATTAAGAAGCGATACATGACTTAAACCAAGAGATCTGAGAGAATCCAGTGTGTCATAATGGCACCTCTGGATGCAA	280
P2-CHS4	GCACATGACGGAGCTCAAAGAAAATCAAACCGATGTGATAAGTCGATGATTAAGAAGCGATACATGACTTAAACCAAGAGATCTGAGAGAATCCAGTGTGTCATAATGGCACCTCTGGATGCAA	280
P3-CHS4	GCACATGACGGAGCTCAAAGAAAATCAAACCGATGTGATAAGTCGATGATTAAGAAGCGATACATGACTTAAACCAAGAGATCTGAGAGAATCCAGTGTGTCATAATGGCACCTCTGGATGCAA	280
P1-CHS4	GCGAACAGCATGGGGTTAGGGGTTACCAAGGCTGCAACTAAGGCATCAAGGATGGGTCAACCCAGTCAAGGATTTGACCATCTTTCGACCAACTAGTTGTTGCGACATGGCTGGTGT	420
P2-CHS4	GCGAACAGCATGGGGTTAGGGGTTACCAAGGCTGCAACTAAGGCATCAAGGATGGGTCAACCCAGTCAAGGATTTGACCATCTTTCGACCAACTAGTTGTTGCGACATGGCTGGTGT	420
P3-CHS4	GCGAACAGCATGGGGTTAGGGGTTACCAAGGCTGCAACTAAGGCATCAAGGATGGGTCAACCCAGTCAAGGATGGGTCAACCCAGTCAAGGATTTGACCATCTTTCGACCAACTAGTTGTTGCGACATGGCTGGTGT	420
P1-CHS4	GATTATCAGCTACTAAACTATTAGGCTTCTGCTCTCGTCAAGGTTACATGATGTTACCMACAAGGCTGCTTGGCCGCTGCAGCGTCTTGGCCAAAGACCTTGTGAAACAACAAAGGGTGTCTGCTG	560
P2-CHS4	GATTATCAGCTACTAAACTATTAGGCTTCTGCTCTCGTCAAGGTTACATGATGTTACCMACAAGGCTGCTTGGCCAAAGACCTTGTGAAACAACAAAGGGTGTCTGCTG	560
P3-CHS4	GATTATCAGCTACTAAACTATTAGGCTTCTGCTCTCGTCAAGGTTACATGATGTTACCMACAAGGCTGCTTGGCCAAAGACCTTGTGAAACAACAAAGGGTGTCTGCTG	560
P1-CHS4	TGTGTTTGTCTGAGATCACCGCAGTCACATTCGGCCCAACTGACACCCATCTTGATAGCCTTGTGGTCAAGCCTTGTGGAGATGTTGCAAGCCGCTGTCTTGGCCAAAGACCTTGTGAAACAACAAAGGGTGTCTGCTG	700
P2-CHS4	TGTGTTTGTCTGAGATCACCGCAGTCACATTCGGCCCAACTGACACCCATCTTGATAGCCTTGTGGTCAAGCCTTGTGGAGATGTTGCAAGCCGCTGTCTTGGCCAAAGACCTTGTGAAACAACAAAGGGTGTCTGCTG	700
P3-CHS4	TGTGTTTGTCTGAGATCACCGCAGTCACATTCGGCCCAACTGACACCCATCTTGATAGCCTTGTGGTCAAGCCTTGTGGAGATGTTGCAAGCCGCTGTCTTGGCCAAAGACCTTGTGAAACAACAAAGGGTGTCTGCTG	700
P1-CHS4	CTTGTTTCACTGTTCTGGACTGCCAGAACATCTTCCGAGACAGTGAAGGGCTTATGTGATGGCTGCAACTTCTCCATCTTGGCTGCAAGGATCTCTGGCTGCTTGGCTGCAAGGATCTCTGGCTG	840
P2-CHS4	CTTGTTTCACTGTTCTGGACTGCCAGAACATCTTCCGAGACAGTGAAGGGCTTATGTGATGGCTGCAACTTCTCCATCTTGGCTGCAAGGATCTCTGGCTGACTCTTCAGAAATTATGGAGAG	840
P3-CHS4	CTTGTTTCACTGTTCTGGACTGCCAGAACATCTTCCGAGACAGTGAAGGGCTTATGTGATGGCTGCAACTTCTCCATCTTGGCTGCAAGGATCTCTGGCTGACTCTTCAGAAATTATGGAGAG	840
P1-CHS4	GCCTTGGTTGAAGCCTCCAACCCCTGGAAATCTCGGATTACAACTCTTCTGGATTGACACCCCTGGTGGCCGGAATTTGGACCAAGTGGAGCTAAGTGGCTGAAAGCTGAAACAAATGGAGACTAG	980
P2-CHS4	GCCTTGGTTGAAGCCTCCAACCCCTGGAAATCTCGGATTACAACTCTTCTGGATTGACACCCCTGGTGGCCGGAATTTGGACCAAGTGGAGCTAAGTGGCTGAAAGCTGAAACAAATGGAGACTAG	980
P3-CHS4	GCCTTGGTTGAAGCCTCCAACCCCTGGAAATCTCGGATTACAACTCTTCTGGATTGACACCCCTGGTGGCCGGAATTTGGACCAAGTGGAGCTAAGTGGCTGAAAGCTGAAACAAATGGAGACTAG	980
P1-CHS4	GCATGTGCTCAGGGAGTATGTAACATGTCAGTGTGCTTATTCTGGATCAAATCGGAAGAAATCAATAGAAAATGGACTTGGCACAACCGGCGAAGGCTTGTACTGGGTGTCTTGGGTTTCGGT	1120
P2-CHS4	GCATGTGCTCAGGGAGTATGTAACATGTCAGTGTGCTTATTCTGGATCAAATCGGAAGAAATCAATAGAAAATGGACTTGGCACAACCGGCGAAGGCTTGTACTGGGTGTCTTGGGTTTCGGT	1120
P3-CHS4	GCATGTGCTCAGGGAGTATGTAACATGTCAGTGTGCTTATTCTGGATCAAATCGGAAGAAATCAATAGAAAATGGACTTGGCACAACCGGCGAAGGCTTGTACTGGGTGTCTTGGGTTTCGGT	1120
P1-CHS4	CTGGACTCACTGTTGAGACTGTTGACTCCGCAGTGTCAGTCTAA	1167
P2-CHS4	CTGGACTCACTGTTGAGACTGTTGACTCCGCAGTGTCAGTCTAA	1167
P3-CHS4	CTGGACTCACTGTTGAGACTGTTGACTCCGCAGTGTCAGTCTAA	1167
F		
P1-CHS4	MVSVEEIRKAQRAEGPATVMA GTATPPNCVDQSTYPDYFRTINSEHMETELKERFKRMCDSMIKKRYMLNEEILKENPSVCAYMAPSLDARQDMVVMEVPKLGEKEATAKIKEWGQPKSKITHLIFCTTSGVDMPGAA	140
P2-CHS4	MVSVEEIRKAQRAEGPATVMA GTATPPNCVDQSTYPDYFRTINSEHMETELKEFKRMCDSMIKKRYMLNEEILKENPSVCAYMAPSLDARQDMVVMEVPKLGEKEATAKIKEWGQPKSKITHLIFCTTSGVDMPGAA	140
P3-CHS4	MVSVEEIRKAQRAEGPATVMA GTATPPNCVDQSTYPDYFRTINSEHMETELKEFKRMCDSMIKKRYMLNEEILKENPSVCAYMAPSLDARQDMVVMEVPKLGEKEATAKIKEWGQPKSKITHLIFCTTSGVDMPGAA	140
P1-CHS4	DYQLTKLLGLRPSVKRYMMYQQGCFAGGTVLRLAKDLAENNNKGARVLYVVCSEITAVTFRGPDTDHLDSLVGQALFGDAAAIVGSDPLPVKEPLFQLWVTAQTILPDSEGAIDGHLREVGLTFHLLKDVPGLISKNIK	280
P2-CHS4	DYQLTKLLGLRPSVKRYMMYQQGCFAGGTVLRLAKDLAENNNKGARVLYVVCSEITAVTFRGPDTDHLDSLVGQALFGDAAAIVGSDPLPVKEPLFQLWVTAQTILPDSEGAIDGHLREVGLTFHLLKDVPGLISKNIK	280
P3-CHS4	DYQLTKLLGLRPSVKRYMMYQQGCFAGGTVLRLAKDLAENNNKGARVLYVVCSEITAVTFRGPDTDHLDSLVGQALFGDAAAIVGSDPLPVKEPLFQLWVTAQTILPDSEGAIDGHLREVGLTFHLLKDVPGLISKNIK	280
P1-CHS4	ALVEAFQPLG1SDYNSIP#IAHPGPAILDQVEAKGLKPEKMEATHVLSYGNMSSACVLFILDQMRRKSIEENGLTTGEGLDWGVLFGFPGPLTVETVLRSLRVTV	388
P2-CHS4	ALVEAFQPLG1SDYNSIP#IAHPGPAILDQVEAKGLKPEKMEATHVLSYGNMSSACVLFILDQMRRKSIEENGLTTGEGLDWGVLFGFPGPLTVETVLRSLRVTV	388
P3-CHS4	ALVEAFQPLG1SDYNSIP#IAHPGPAILDQVEAKGLKPEKMEATHVLSYGNMSSACVLFILDQMRRKSIEENGLTTGEGLDWGVLFGFPGPLTVETVLRSLRVTV	388

**Supplementary Fig. S1. Multiple sequence alignment describing the base sequence and amino acids sequence of *CHS1*, *CHS3* and *CHS4* in P1(PI342618B), P2(NN86-4) and P3(NN493-1).** (A),(C),(E) represent multiple base sequence alignment of *CHS1*, *CHS3* and *CHS4*. (B),(D),(F) represent multiple amino acids sequence alignment of *CHS1*, *CHS3* and *CHS4*.



**Supplementary Fig. S2. Expression analysis of candidate genes in the QTL hotspot(8-2) region related to seed-flooding tolerance.** (A) Heat maps of expression levels for 164 candidate genes in different soybean tissues. (B) Heat maps of expression levels for 10 screened candidate genes associated with seed-flooding tolerance in different tissues. The RNA-seq data were obtained from Soybase (<https://www.soybase.org/soyseq/>).

**A**

P1-Glyma. 08g122400	ATGAATGCTAGAACCTGGACCTCGTATTATTCCTTA	TGCTTTCTTGGTTCAGGTAGGAACAACCAAAATAAATAAATGGAAAAAGGAATCTCTCATCGGAATTGGGTACTCAATTAGATG	139
P2-Glyma. 08g122400	ATGAATGCTAGAACCTGGACCTCGTATTATTCCTTA	TGCTTTCTTGGTTCAGGTAGGAACAACCAAAATAAATAAATGGAAAAAGGAATCTCTCATCGGAATTGGGTACTCAATTAGATG	140
P3-Glyma. 08g122400	ATGAATGCTAGAACCTGGACCTCGTATTATTCCTTA	TGCTTTCTTGGTTCAGGTAGGAACAACCAAAATAAATAAATGGAAAAAGGAATCTCTCATCGGAATTGGGTACTCAATTAGATG	140

P1-Glyma. 08g122400	TGTTCTATCTGTAATCCCAAACCTTGACACAGAAA	TGCTATATGGAAACCTAACATCTCTAACACCTGACATCAGGCCAACCCAAACACCACACAAATCCAACAACCCAAACCAACGCCA	279
P2-Glyma. 08g122400	TGTTCTATCTGTCATCCCAAACCTTGACACAGAAA	TGCTATATGGAAACCTAACATCTCTAACACCTGACATCAGGCCAACCCAAACCCACACAAATCCAACAACCCAAACGCCA	280
P3-Glyma. 08g122400	TGTTCTATCTGTCATCCCAAACCTTGACACAGAAA	TGCTATATGGAAACCTAACATCTCTAACACCTGACATCAGGCCAACCCAAACCCACACAAATCCAACAACCCAAACGCCA	280

P1-Glyma. 08g122400	CAACCCAACTACAACACCCACAACCCGACTACTACATCTCAGTGGTCAATGGTGTGCTAACAGGGAGCTTCAGACACTGCT	CTACAGGTTGCTTGGGTTGGAGGTGCAGACTGCTCA	419
P2-Glyma. 08g122400	CAACCCAACTACAACACCCACAACCCGACTACTACATCTCAGTGGTCAATGGTGTGCTAACAGGGAGCTTCAGACACTGCT	CTACAGGTTGCTTGGGTTGGAGGTGCAGACTGCTCA	420
P3-Glyma. 08g122400	CAACCCAACTACAACACCCACAACCCGACTACTACATCTCAGTGGTCAATGGTGTGCTAACAGGGAGCTTCAGACACTGCT	CTACAGGTTGCTTGGGTTGGAGGTGCAGACTGCTCA	420

P1-Glyma. 08g122400	GCAATACAACCAAGGAGCAAGCTGTTATAATC	AAACACTGTCGGGATCATGCTTATGCAATTCAAGACTAACAGAGAAATCCAGCACCTACAGCTGTATTTGGAGAACAGCATCTTACAAGCAATG	559
P2-Glyma. 08g122400	GCAATACAACCAAGGAGCAAGCTGTTATAATC	AAACACTGTCGGGATCATGCTTATGCAATTCAAGACTAACAGAGAAATCCAGCACCTACAGCTGTATTTGGAGAACAGCATCTTACAAGCAATG	560
P3-Glyma. 08g122400	GCAATACAACCAAGGAGCAAGCTGTTATAATC	AAACACTGTCGGGATCATGCTTATGCAATTCAAGACTAACAGAGAAATCCAGCACCTACAGCTGTATTTGGAGAACAGCATCTTACAAGCAATG	560

P1-Glyma. 08g122400	TCCAAGTAGTGGGAGTTGCAAATAGCATACCCAA	CTACAGGACACTAACATCCAGGACACCACAACTTGTGCGCCACCAAGTCCAGCTACAGGCTGATGACCCAACTCCAGCAGATGCTAAATCTG	699
P2-Glyma. 08g122400	TCCAAGTAGTGGGAGTTGCAAATAGCATACCCAA	CTACAGGACACTAACATCCAGGACACCACAACTTGTGCGCCACCAAGTCCAGCTACAGGCTGATGACCCAACTCCAGCAGATGCTAAATCTG	700
P3-Glyma. 08g122400	TCCAAGTAGTGGGAGTTGCAAATAGCATACCCAA	CTACAGGACACTAACATCCAGGACACCACAACTTGTGCGCCACCAAGTCCAGCTACAGGCTGATGACCCAACTCCAGCAGATGCTAAATCTG	700

P1-Glyma. 08g122400	GTGGCGGATCAACTGCTTGGTTCAGAACACAGGA	GGCCCAACCCACACAGGACATTGCAATTCTGCTGATGTTGTCACATGTTGCTTGGCTTCCCTCAAGTCAAATTACATCTAG	830
P2-Glyma. 08g122400	GTGGCGGATCAACTGCTTGGTTCAGAACACAGGA	GGCCCAACCCACACAGGACATTGCAATTCTGCTGATGTTGTCACATGTTGCTTGGCTTCCCTCAAGTCAAATTACATCTAG	831
P3-Glyma. 08g122400	GTGGCGGATCAACTGCTTGGTTCAGAACACAGGA	GGCCCAACCCACACAGGACATTGCAATTCTGCTGATGTTGTCACATGTTGCTTGGCTTCCCTCAAGTCAAATTACATCTAG	831

**B**

P1-Glyma. 08G122400	MNARSWHLVLFSLCLFFPSGLCTKILINGKQE	IFSSSELGTQLDDWPVNPTTDGTGPNPVPQTPDTSGPNPTPTNPTPTPTPTPTPTTSSGGQWCVANQGASDTALQVALDYACFGGGADCS	140
P2-Glyma. 08G122400	MNARSWHLVLFSLCLFFPSGLCTKILINGKQE	IFSSSELGTQLDDWPVNPTTDGTGPNPVPQTPDTSGPNPTPTNPTPTPTPTPTPTTSSGGQWCVANQGASDTALQVALDYACFGGGADCS	140
P3-Glyma. 08G122400	MNARSWHLVLFSLCLFFPSGLCTKILINGKQE	IFSSSELGTQLDDWPVNPTTDGTGPNPVPQTPDTSGPNPTPTNPTPTPTPTPTPTTSSGGQWCVANQGASDTALQVALDYACFGGGADCS	140

P1-Glyma. 08G122400	A1QPGASCYNPTVROMASYAFNDYYQKNPAPTSV	IPTFVSPSPPTPMIPPTMLNPGGSTVPGSEPTGSPNTATFAPNSLLMFTGLLASLQLANYI	276
P2-Glyma. 08G122400	A1QPGASCYNPTVROMASYAFNDYYQKNPAPTSV	IPTFVSPSPPTPMIPPTMLNPGGSTVPGSEPTGSPNTATFAPNSLLMFTGLLASLQLANYI	276
P3-Glyma. 08G122400	A1QPGASCYNPTVROMASYAFNDYYQKNPAPTSV	IPTFVSPSPPTPMIPPTMLNPGGSTVPGSEPTGSPNTATFAPNSLLMFTGLLASLQLANYI	276

**Supplementary Fig. S3. Multiple sequence alignment describing the base sequence and amino acids sequence of *Glyma.08G122400* in P1(PI342618B), P2(NN86-4) and P3(NN493-1).** (A) represent multiple base sequence alignment of *Glyma.08G124400*. (B) represent multiple amino acids sequence alignment of *Glyma.08G124400*.