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Research article

Identification of auxin response factor in the pan-genome and their expression pattern analysis in cucumber

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Abstract: Auxin response factors (ARFs) are important transcription factors that regulate the expression of early genes in response to auxin. Auxin is a crucial phytohormone that controls plant growth and development. Even though *ARF* genes have been identified in the cucumber (*Cucumis sativus*) genome version 3.0, the features of this gene family are unclear in the cucumber pan-genome and in the newly assembled genome v4.0. We identified a total of 18 CsARF genes, including two CsARF13s in Gy14. All ARF proteins, except ARF5 and 7, showed differences in protein length; all of them showed amino acid variation in the cucumber pan-genome, including 13 accessions. We also analyzed their organ-specific expression and their expression patterns in fruit development and in response to biotic stresses. Importantly, we found that the overexpression of CsARF12 significantly enhanced the resistance of cucumber cotyledons to gray mold infection. Therefore, this study lays a foundation for understanding the biological functions of CsARF genes and provides *CsARF12* as a candidate gene to improve the cucumber resistance to gray mold.
Keywords: cucumber; pan-genome; *ARF* gene; biotic stress response; gray mold

Supplementary

Table S1. The variation of CsARF amino acids in 13 cucumber accessions (See file named "molsci-12-03-019-s002").
Table S2. Primers used in instantaneous infection experiment (See file named "molsci-12-03-019-s002").
Figure S1. The gene developmental tree, conserved protein motifs, and gene structure of *CsARF* genes.
Figure S2. The cis-acting elements of *CsARF* genes in the pan-genome.
Figure S3. Single amino acid differences with amino acid properties in the pan-genome.
Figure S4. Single amino acid differences without changes in the amino acid properties in the pan-genome.
Figure S5. The absence of amino acid fragments in the pan-genome.
Figure S6. Insertions of amino acid fragments in the pan-genome.
Figure S7. The phylogenetic tree of ARF proteins from the cucumber variety 9930 and *Arabidopsis* genomes.
Figure S8. The conserved protein motifs and gene structure of CsaV4_2G000004 and CsaV3_2G001050.

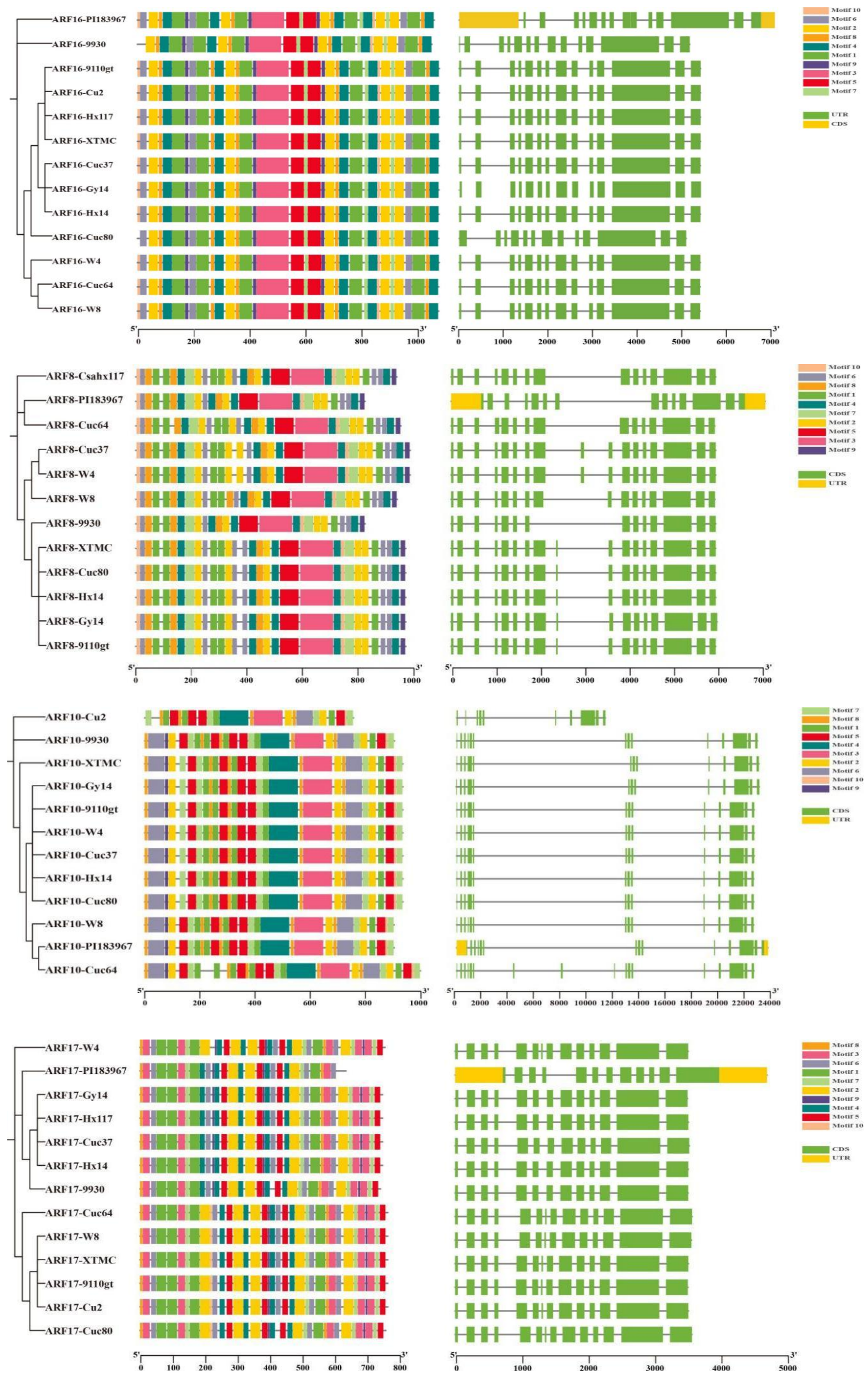
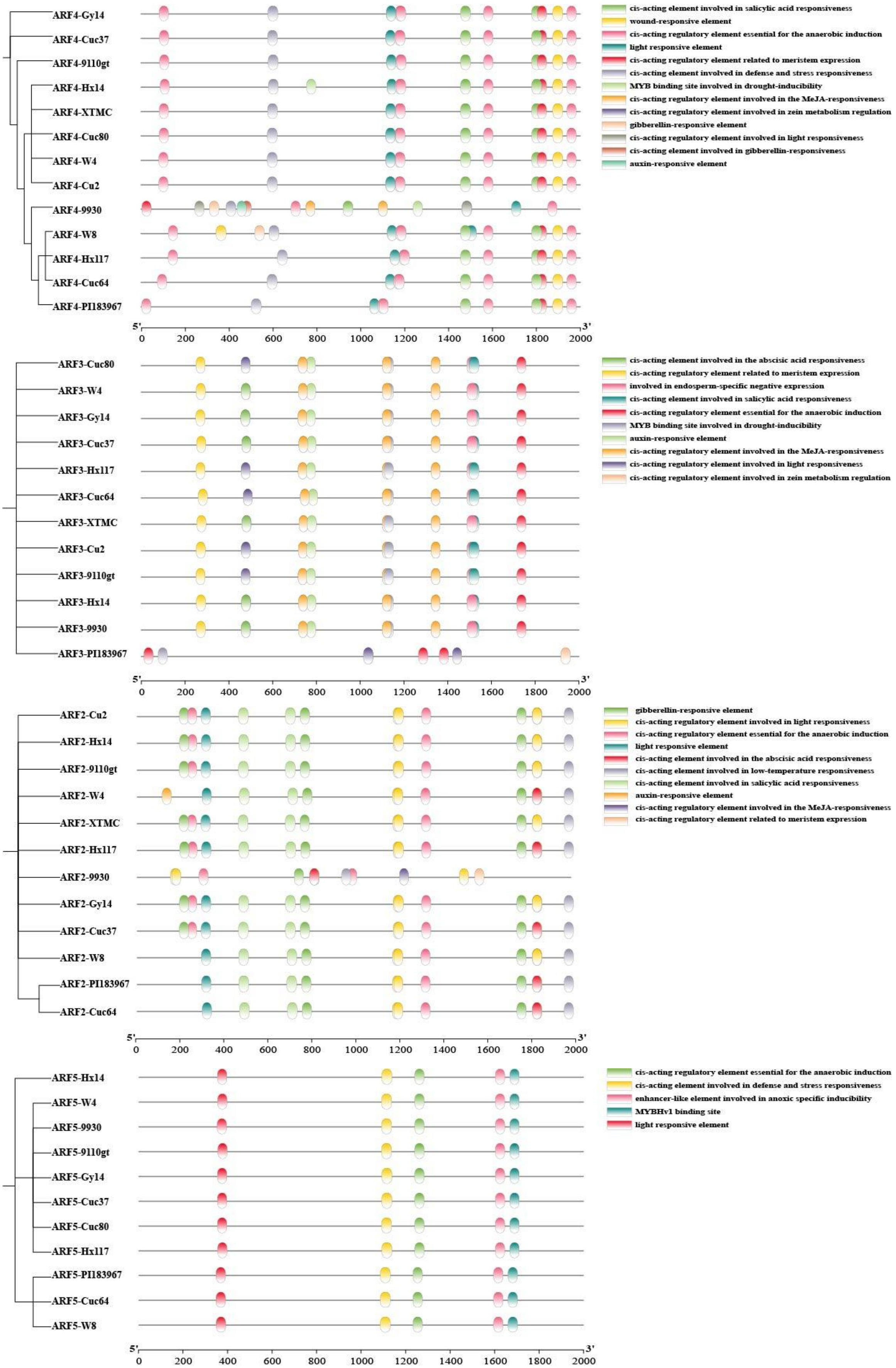
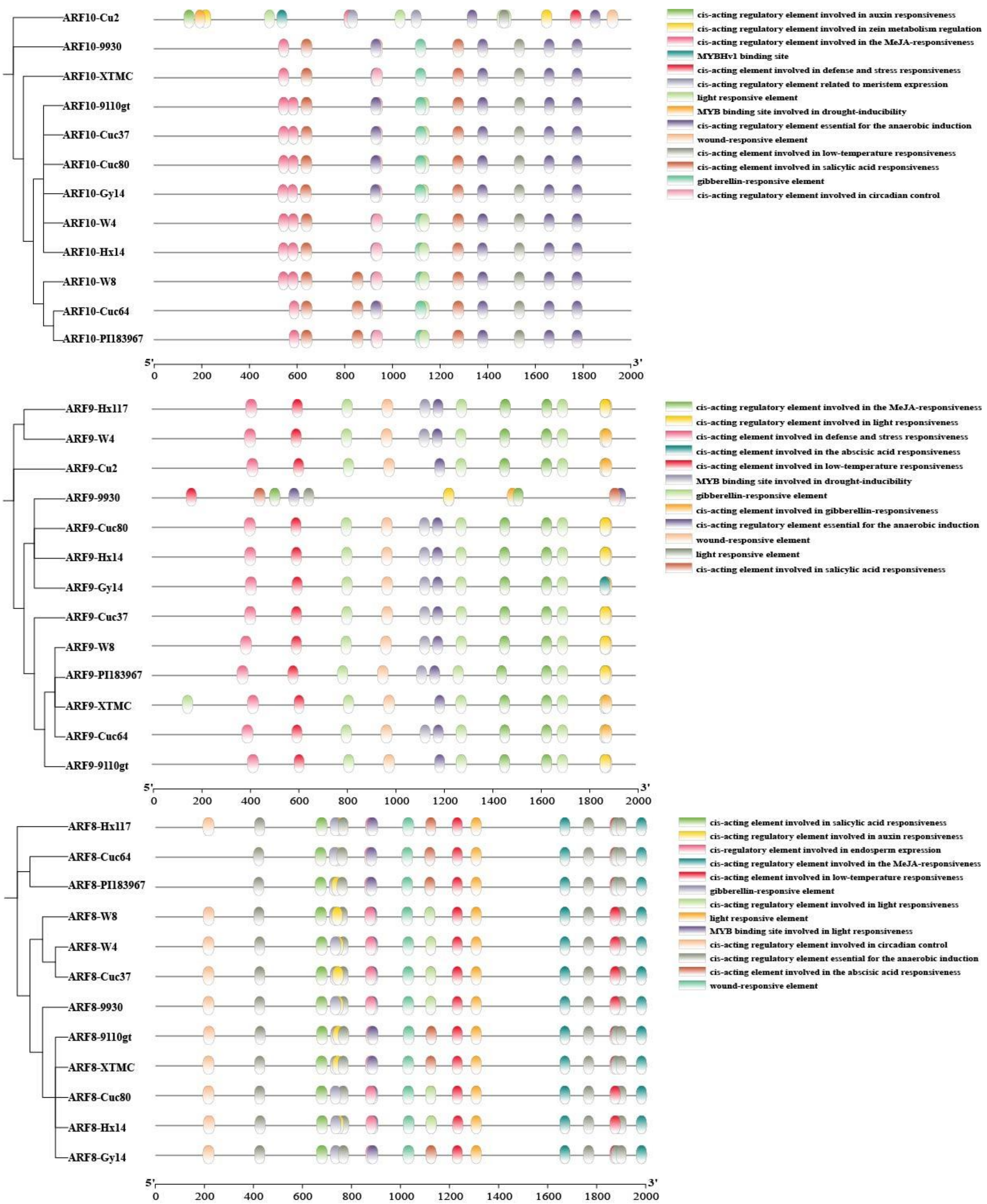
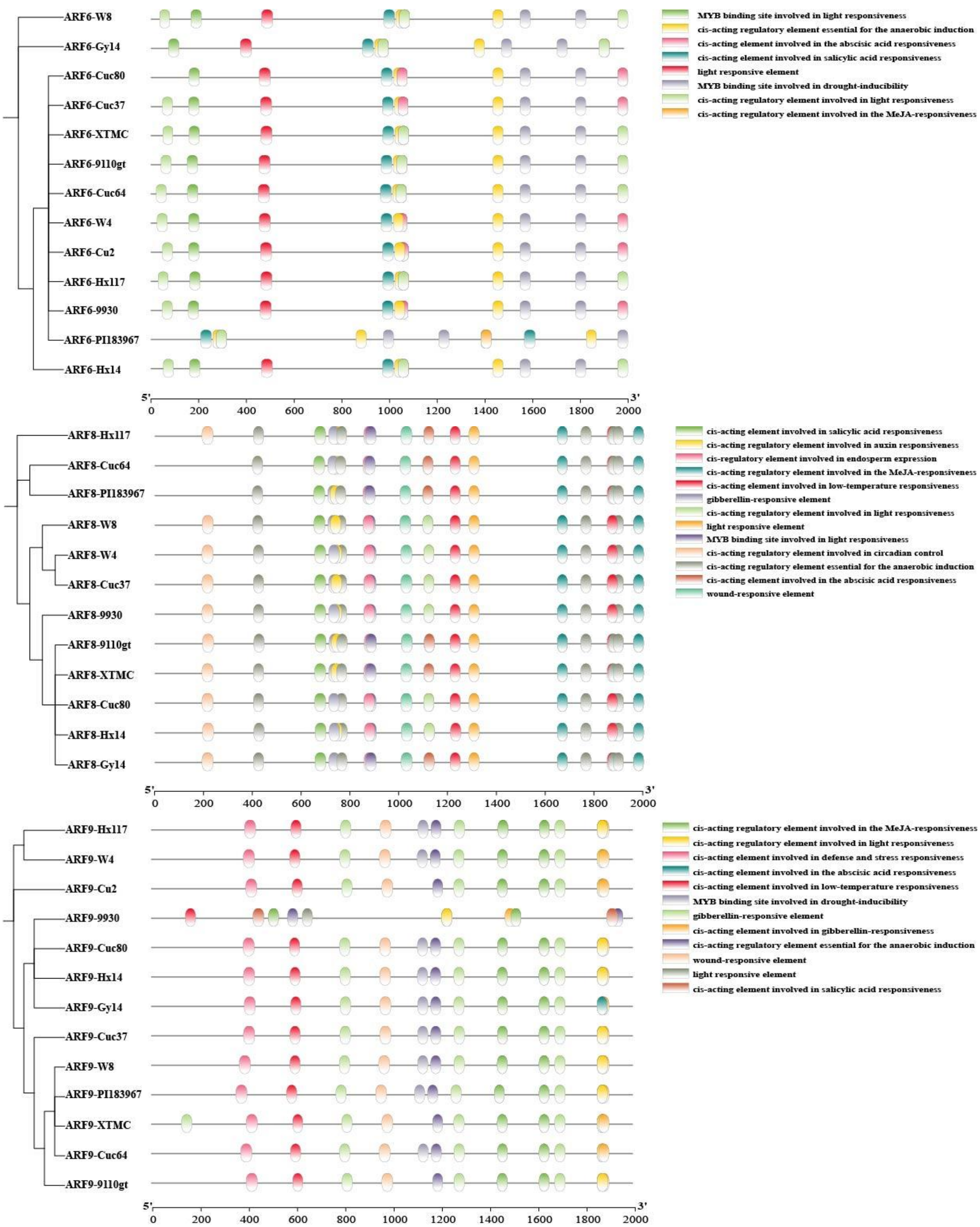


Figure S1. The gene developmental tree, conserved protein motifs, and gene structure of *CsARF* genes.

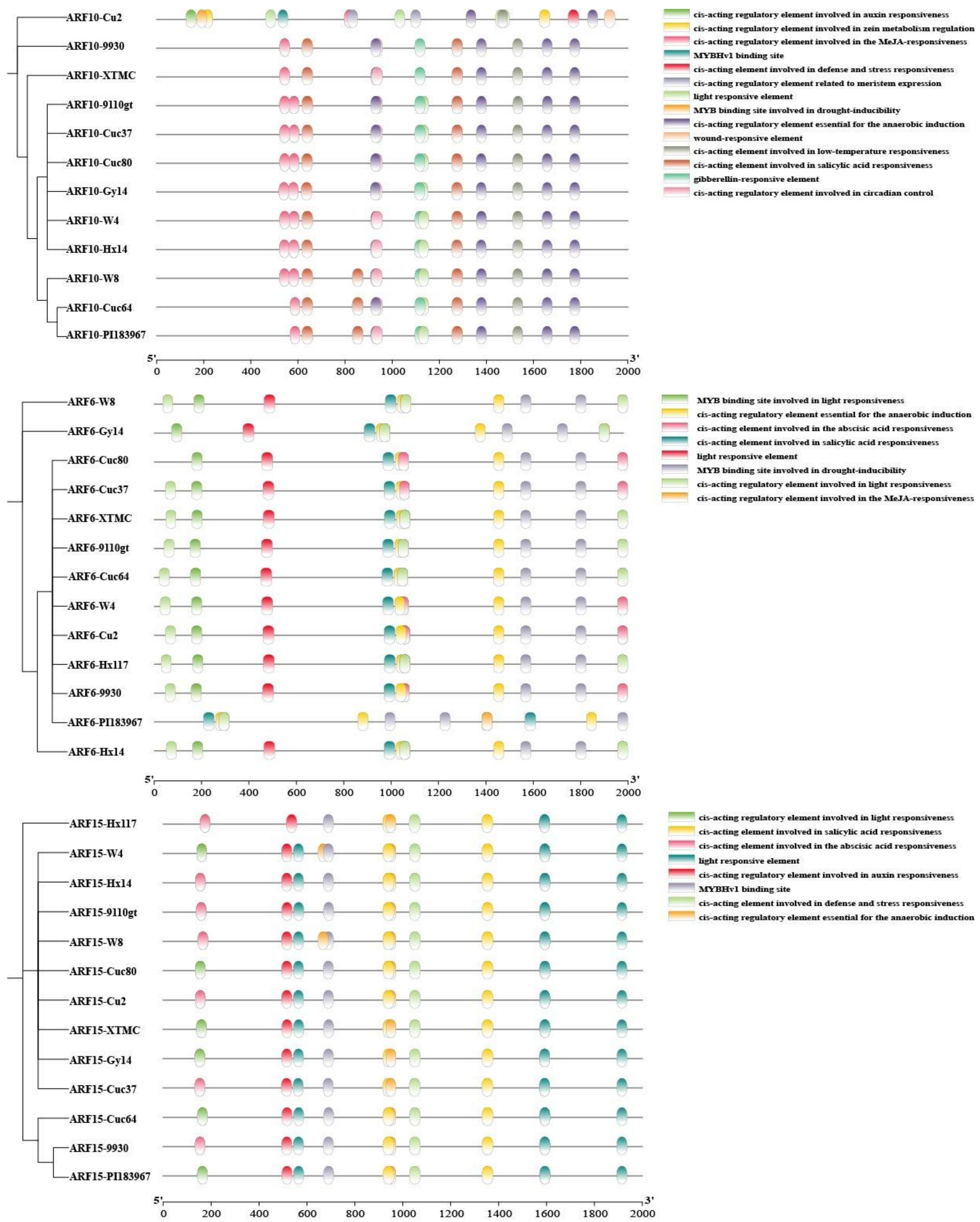


ARF4-Gy14
ARF4-Cuc37
ARF4-9110gt
ARF4-Hx14
ARF4-XTMC
ARF4-Cuc80
ARF4-W4
ARF4-Cu2
ARF4-9930
ARF4-W8
ARF4-Hx117
ARF4-Cuc64
ARF4-PI183967
5'
3'
0 200 400 600 800 1000 1200 1400 1600 1800 2000
cis-acting element involved in salicylic acid responsiveness
wound-responsive element
cis-acting regulatory element essential for the anaerobic induction
light responsive element
cis-acting regulatory element related to meristem expression
cis-acting element involved in defense and stress responsiveness
MYB binding site involved in drought-inducibility
cis-acting regulatory element involved in the MeJA-responsiveness
cis-acting regulatory element involved in zein metabolism regulation
gibberellin-responsive element
cis-acting regulatory element involved in light responsiveness
cis-acting element involved in gibberellin-responsiveness
auxin-responsive element
ARF3-Cuc80
ARF3-W4
ARF3-Gy14
ARF3-Cuc37
ARF3-Hx117
ARF3-Cuc64
ARF3-XTMC
ARF3-Cu2
ARF3-9110gt
ARF3-Hx14
ARF3-9930
ARF3-PI183967
5'
3'
0 200 400 600 800 1000 1200 1400 1600 1800 2000
cis-acting element involved in the abscisic acid responsiveness
cis-acting regulatory element related to meristem expression
involved in endosperm-specific negative expression
cis-acting element involved in salicylic acid responsiveness
cis-acting regulatory element essential for the anaerobic induction
MYB binding site involved in drought-inducibility
auxin-responsive element
cis-acting regulatory element involved in the MeJA-responsiveness
cis-acting regulatory element involved in light responsiveness
cis-acting regulatory element involved in zein metabolism regulation
ARF2-Cu2
ARF2-Hx14
ARF2-9110gt
ARF2-W4
ARF2-XTMC
ARF2-Hx117
ARF2-9930
ARF2-Gy14
ARF2-Cuc37
ARF2-W8
ARF2-PI183967
ARF2-Cuc64
5'
3'
0 200 400 600 800 1000 1200 1400 1600 1800 2000
gibberellin-responsive element
cis-acting regulatory element involved in light responsiveness
cis-acting regulatory element essential for the anaerobic induction
light responsive element
cis-acting element involved in the abscisic acid responsiveness
cis-acting element involved in low-temperature responsiveness
cis-acting element involved in salicylic acid responsiveness
auxin-responsive element
cis-acting regulatory element involved in the MeJA-responsiveness
cis-acting regulatory element related to meristem expression
ARF5-Hx14
ARF5-W4
ARF5-9930
ARF5-9110gt
ARF5-Gy14
ARF5-Cuc37
ARF5-Cuc80
ARF5-Hx117
ARF5-PI183967
ARF5-Cuc64
ARF5-W8
5'
3'
0 200 400 600 800 1000 1200 1400 1600 1800 2000
cis-acting regulatory element essential for the anaerobic induction
cis-acting element involved in defense and stress responsiveness
enhancer-like element involved in anoxic specific inducibility
MYBHv1 binding site
light responsive element

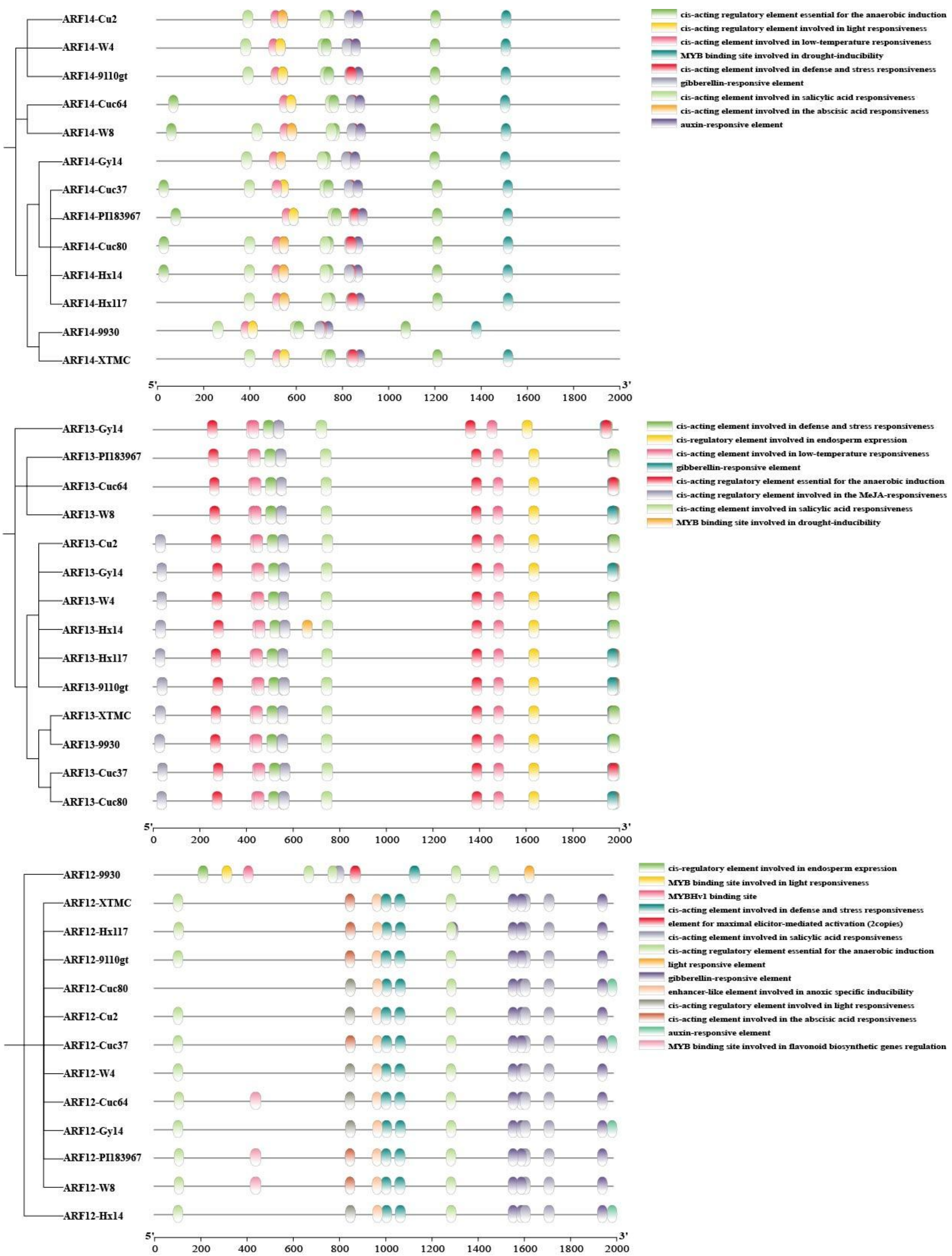

ARF10-Cu2
ARF10-9930
ARF10-XTMC
ARF10-9110gt
ARF10-Cuc37
ARF10-Cuc80
ARF10-Gy14
ARF10-W4
ARF10-Hx14
ARF10-W8
ARF10-Cuc64
ARF10-PI183967
5'
3'
0 200 400 600 800 1000 1200 1400 1600 1800 2000
cis-acting regulatory element involved in auxin responsiveness
cis-acting regulatory element involved in zein metabolism regulation
cis-acting regulatory element involved in the MeJA-responsiveness
MYBHv1 binding site
cis-acting element involved in defense and stress responsiveness
cis-acting regulatory element related to meristem expression
light responsive element
MYB binding site involved in drought-inducibility
cis-acting regulatory element essential for the anaerobic induction
wound-responsive element
cis-acting element involved in low-temperature responsiveness
cis-acting element involved in salicylic acid responsiveness
gibberellin-responsive element
cis-acting regulatory element involved in circadian control
ARF9-Hx117
ARF9-W4
ARF9-Cu2
ARF9-9930
ARF9-Cuc80
ARF9-Hx14
ARF9-Gy14
ARF9-Cuc37
ARF9-W8
ARF9-PI183967
ARF9-XTMC
ARF9-Cuc64
ARF9-9110gt
5'
3'
0 200 400 600 800 1000 1200 1400 1600 1800 2000
cis-acting regulatory element involved in the MeJA-responsiveness
cis-acting regulatory element involved in light responsiveness
cis-acting element involved in defense and stress responsiveness
cis-acting element involved in the abscisic acid responsiveness
cis-acting element involved in low-temperature responsiveness
MYB binding site involved in drought-inducibility
gibberellin-responsive element
cis-acting element involved in gibberellin-responsiveness
cis-acting regulatory element essential for the anaerobic induction
wound-responsive element
light responsive element
cis-acting element involved in salicylic acid responsiveness
ARF8-Hx117
ARF8-Cuc64
ARF8-PI183967
ARF8-W8
ARF8-W4
ARF8-Cuc37
ARF8-9930
ARF8-9110gt
ARF8-XTMC
ARF8-Cuc80
ARF8-Hx14
ARF8-Gy14
5'
3'
0 200 400 600 800 1000 1200 1400 1600 1800 2000
cis-acting element involved in salicylic acid responsiveness
cis-acting regulatory element involved in auxin responsiveness
cis-regulatory element involved in endosperm expression
cis-acting regulatory element involved in the MeJA-responsiveness
cis-acting element involved in low-temperature responsiveness
gibberellin-responsive element
cis-acting regulatory element involved in light responsiveness
light responsive element
MYB binding site involved in light responsiveness
cis-acting regulatory element involved in circadian control
cis-acting regulatory element essential for the anaerobic induction
cis-acting element involved in the abscisic acid responsiveness
wound-responsive element



ARF6-W8
ARF6-Gy14
ARF6-Cuc80
ARF6-Cuc37
ARF6-XTMC
ARF6-9110gt
ARF6-Cuc64
ARF6-W4
ARF6-Cu2
ARF6-Hx117
ARF6-9930
ARF6-PI183967
ARF6-Hx14
5' 3'
0 200 400 600 800 1000 1200 1400 1600 1800 2000
MYB binding site involved in light responsiveness
cis-acting regulatory element essential for the anaerobic induction
cis-acting element involved in the abscisic acid responsiveness
cis-acting element involved in salicylic acid responsiveness
light responsive element
MYB binding site involved in drought-inducibility
cis-acting regulatory element involved in light responsiveness
cis-acting regulatory element involved in the MeJA-responsiveness
ARF8-Hx117
ARF8-Cuc64
ARF8-PI183967
ARF8-W8
ARF8-W4
ARF8-Cuc37
ARF8-9930
ARF8-9110gt
ARF8-XTMC
ARF8-Cuc80
ARF8-Hx14
ARF8-Gy14
5' 3'
0 200 400 600 800 1000 1200 1400 1600 1800 2000
cis-acting element involved in salicylic acid responsiveness
cis-acting regulatory element involved in auxin responsiveness
cis-regulatory element involved in endosperm expression
cis-acting regulatory element involved in the MeJA-responsiveness
cis-acting element involved in low-temperature responsiveness
gibberellin-responsive element
cis-acting regulatory element involved in light responsiveness
light responsive element
MYB binding site involved in light responsiveness
cis-acting regulatory element involved in circadian control
cis-acting regulatory element essential for the anaerobic induction
cis-acting element involved in the abscisic acid responsiveness
wound-responsive element
ARF9-Hx117
ARF9-W4
ARF9-Cu2
ARF9-9930
ARF9-Cuc80
ARF9-Hx14
ARF9-Gy14
ARF9-Cuc37
ARF9-W8
ARF9-PI183967
ARF9-XTMC
ARF9-Cuc64
ARF9-9110gt
5' 3'
0 200 400 600 800 1000 1200 1400 1600 1800 2000
cis-acting regulatory element involved in the MeJA-responsiveness
cis-acting regulatory element involved in light responsiveness
cis-acting element involved in defense and stress responsiveness
cis-acting element involved in the abscisic acid responsiveness
cis-acting element involved in low-temperature responsiveness
MYB binding site involved in drought-inducibility
gibberellin-responsive element
cis-acting element involved in gibberellin-responsiveness
cis-acting regulatory element essential for the anaerobic induction
wound-responsive element
light responsive element
cis-acting element involved in salicylic acid responsiveness



ARF10-Cu2
ARF10-9930
ARF10-XTMC
ARF10-9110gt
ARF10-Cuc37
ARF10-Cuc80
ARF10-Gy14
ARF10-W4
ARF10-Hx14
ARF10-W8
ARF10-Cuc64
ARF10-PI183967
5'
3'
0 200 400 600 800 1000 1200 1400 1600 1800 2000
cis-acting regulatory element involved in auxin responsiveness
cis-acting regulatory element involved in zein metabolism regulation
cis-acting regulatory element involved in the MeJA-responsiveness
MYBHv1 binding site
cis-acting element involved in defense and stress responsiveness
cis-acting regulatory element related to meristem expression
light responsive element
MYB binding site involved in drought-inducibility
cis-acting regulatory element essential for the anaerobic induction
wound-responsive element
cis-acting element involved in low-temperature responsiveness
cis-acting element involved in salicylic acid responsiveness
gibberellin-responsive element
cis-acting regulatory element involved in circadian control
ARF6-W8
ARF6-Gy14
ARF6-Cuc80
ARF6-Cuc37
ARF6-XTMC
ARF6-9110gt
ARF6-Cuc64
ARF6-W4
ARF6-Cu2
ARF6-Hx117
ARF6-9930
ARF6-PI183967
ARF6-Hx14
5'
3'
0 200 400 600 800 1000 1200 1400 1600 1800 2000
MYB binding site involved in light responsiveness
cis-acting regulatory element essential for the anaerobic induction
cis-acting element involved in the abscisic acid responsiveness
cis-acting element involved in salicylic acid responsiveness
light responsive element
MYB binding site involved in drought-inducibility
cis-acting regulatory element involved in light responsiveness
cis-acting regulatory element involved in the MeJA-responsiveness
ARF15-Hx117
ARF15-W4
ARF15-Hx14
ARF15-9110gt
ARF15-W8
ARF15-Cuc80
ARF15-Cu2
ARF15-XTMC
ARF15-Gy14
ARF15-Cuc37
ARF15-Cuc64
ARF15-9930
ARF15-PI183967
5'
3'
0 200 400 600 800 1000 1200 1400 1600 1800 2000
cis-acting regulatory element involved in light responsiveness
cis-acting element involved in salicylic acid responsiveness
cis-acting element involved in the abscisic acid responsiveness
light responsive element
cis-acting regulatory element involved in auxin responsiveness
MYBHv1 binding site
cis-acting element involved in defense and stress responsiveness
cis-acting regulatory element essential for the anaerobic induction


ARF14-Cu2
ARF14-W4
ARF14-9110gt
ARF14-Cuc64
ARF14-W8
ARF14-Gy14
ARF14-Cuc37
ARF14-PI183967
ARF14-Cuc80
ARF14-Hx14
ARF14-Hx117
ARF14-9930
ARF14-XTMC
5'
3'
0
200
400
600
800
1000
1200
1400
1600
1800
2000
cis-acting regulatory element essential for the anaerobic induction
cis-acting regulatory element involved in light responsiveness
cis-acting element involved in low-temperature responsiveness
MYB binding site involved in drought-inducibility
cis-acting element involved in defense and stress responsiveness
gibberellin-responsive element
cis-acting element involved in salicylic acid responsiveness
cis-acting element involved in the abscisic acid responsiveness
auxin-responsive element
ARF13-Gy14
ARF13-PI183967
ARF13-Cuc64
ARF13-W8
ARF13-Cu2
ARF13-Gy14
ARF13-W4
ARF13-Hx14
ARF13-Hx117
ARF13-9110gt
ARF13-XTMC
ARF13-9930
ARF13-Cuc37
ARF13-Cuc80
5'
3'
0
200
400
600
800
1000
1200
1400
1600
1800
2000
cis-acting element involved in defense and stress responsiveness
cis-regulatory element involved in endosperm expression
cis-acting element involved in low-temperature responsiveness
gibberellin-responsive element
cis-acting regulatory element essential for the anaerobic induction
cis-acting regulatory element involved in the MeJA-responsiveness
cis-acting element involved in salicylic acid responsiveness
MYB binding site involved in drought-inducibility
ARF12-9930
ARF12-XTMC
ARF12-Hx117
ARF12-9110gt
ARF12-Cuc80
ARF12-Cu2
ARF12-Cuc37
ARF12-W4
ARF12-Cuc64
ARF12-Gy14
ARF12-PI183967
ARF12-W8
ARF12-Hx14
5'
3'
0
200
400
600
800
1000
1200
1400
1600
1800
2000
cis-regulatory element involved in endosperm expression
MYB binding site involved in light responsiveness
MYBHv1 binding site
cis-acting element involved in defense and stress responsiveness
element for maximal elicitor-mediated activation (2copies)
cis-acting element involved in salicylic acid responsiveness
cis-acting regulatory element essential for the anaerobic induction
light responsive element
gibberellin-responsive element
enhancer-like element involved in anoxic specific inducibility
cis-acting regulatory element involved in light responsiveness
cis-acting element involved in the abscisic acid responsiveness
auxin-responsive element
MYB binding site involved in flavonoid biosynthetic genes regulation

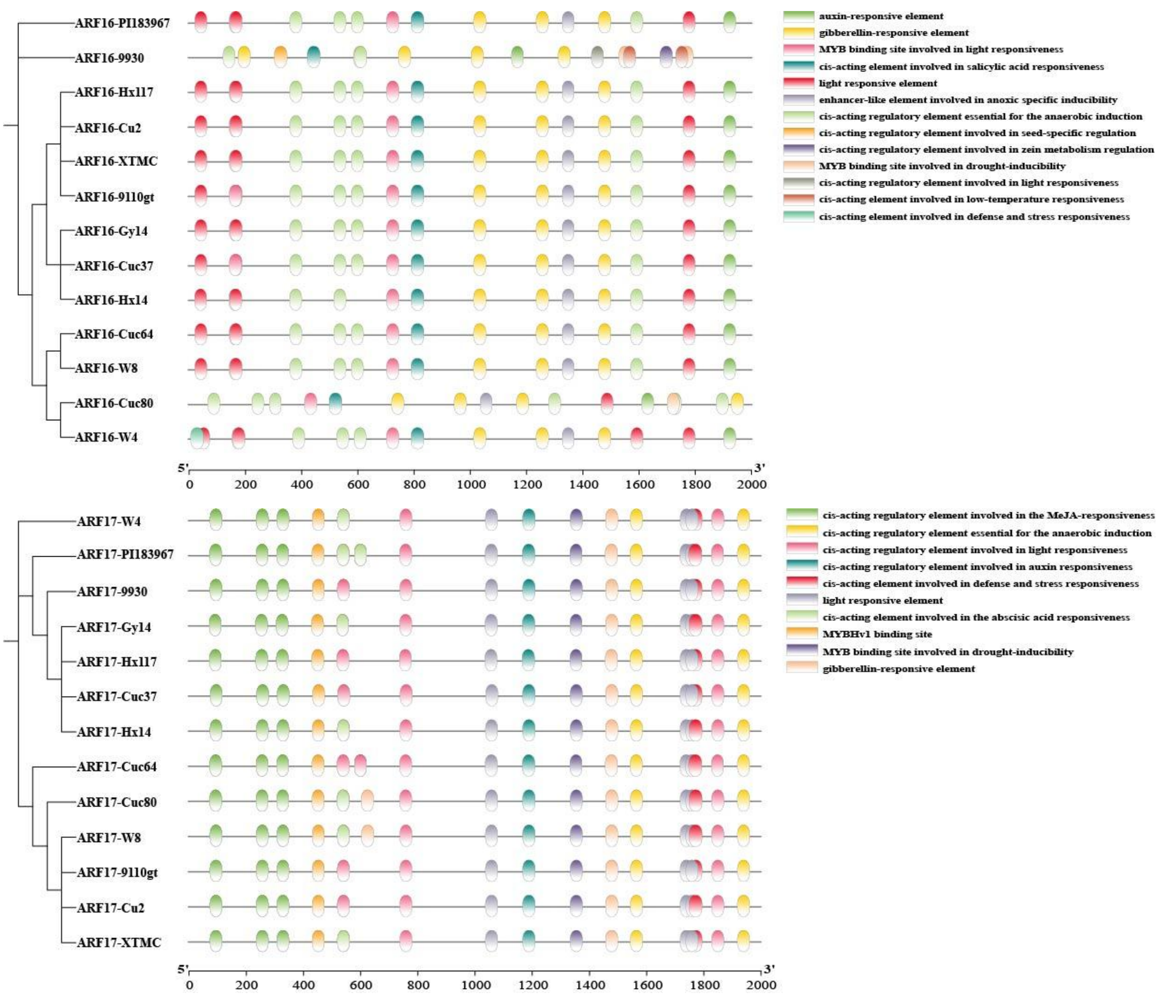
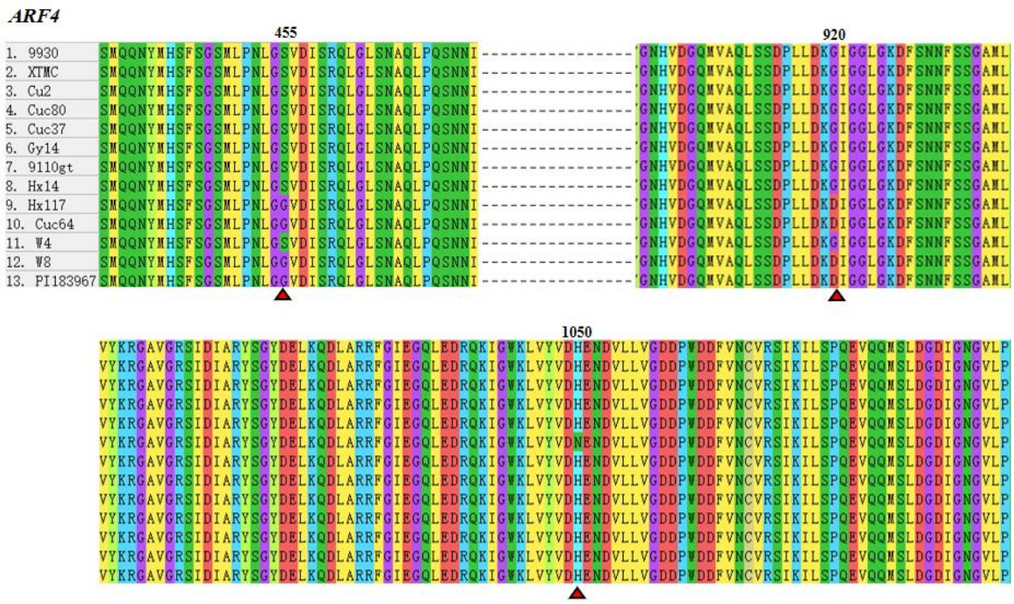
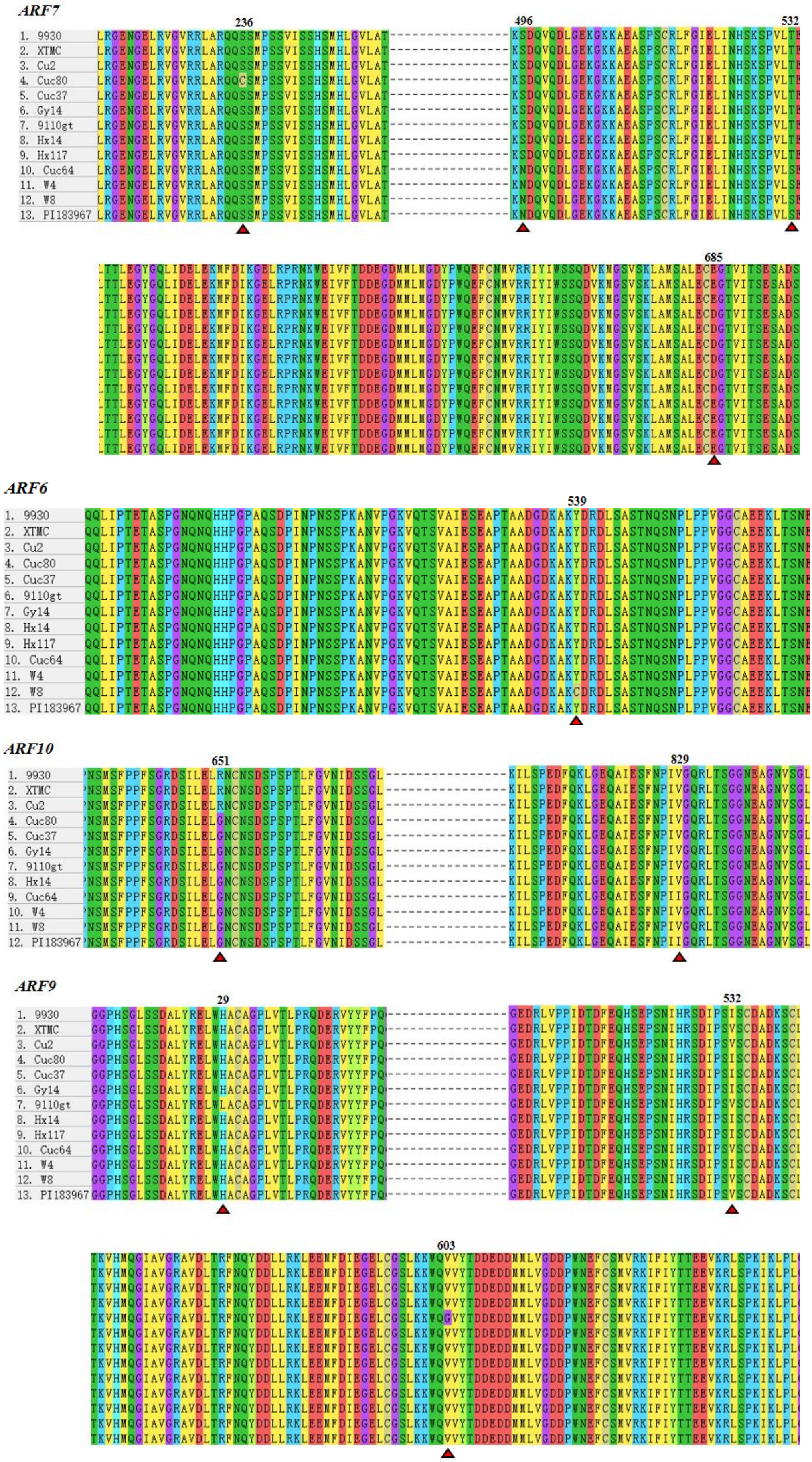


Figure S2. The cis-acting elements of *CsARF* genes in the pan-genome.

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496
532
685
ARF6
539
ARF10
651
829
ARF9
29
532
603

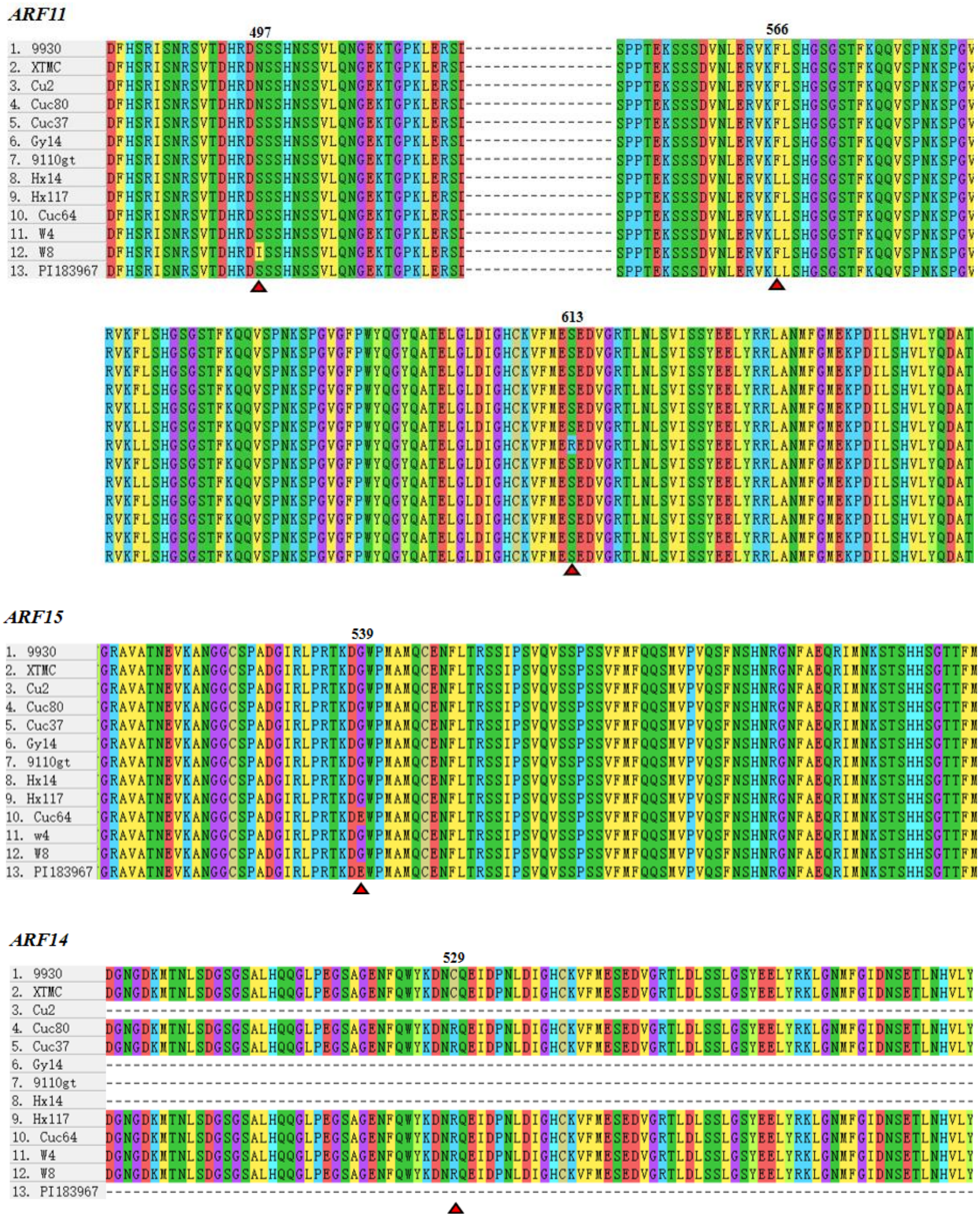


Figure S3. single amino acid differences with amino acid properties in the pan-genome.

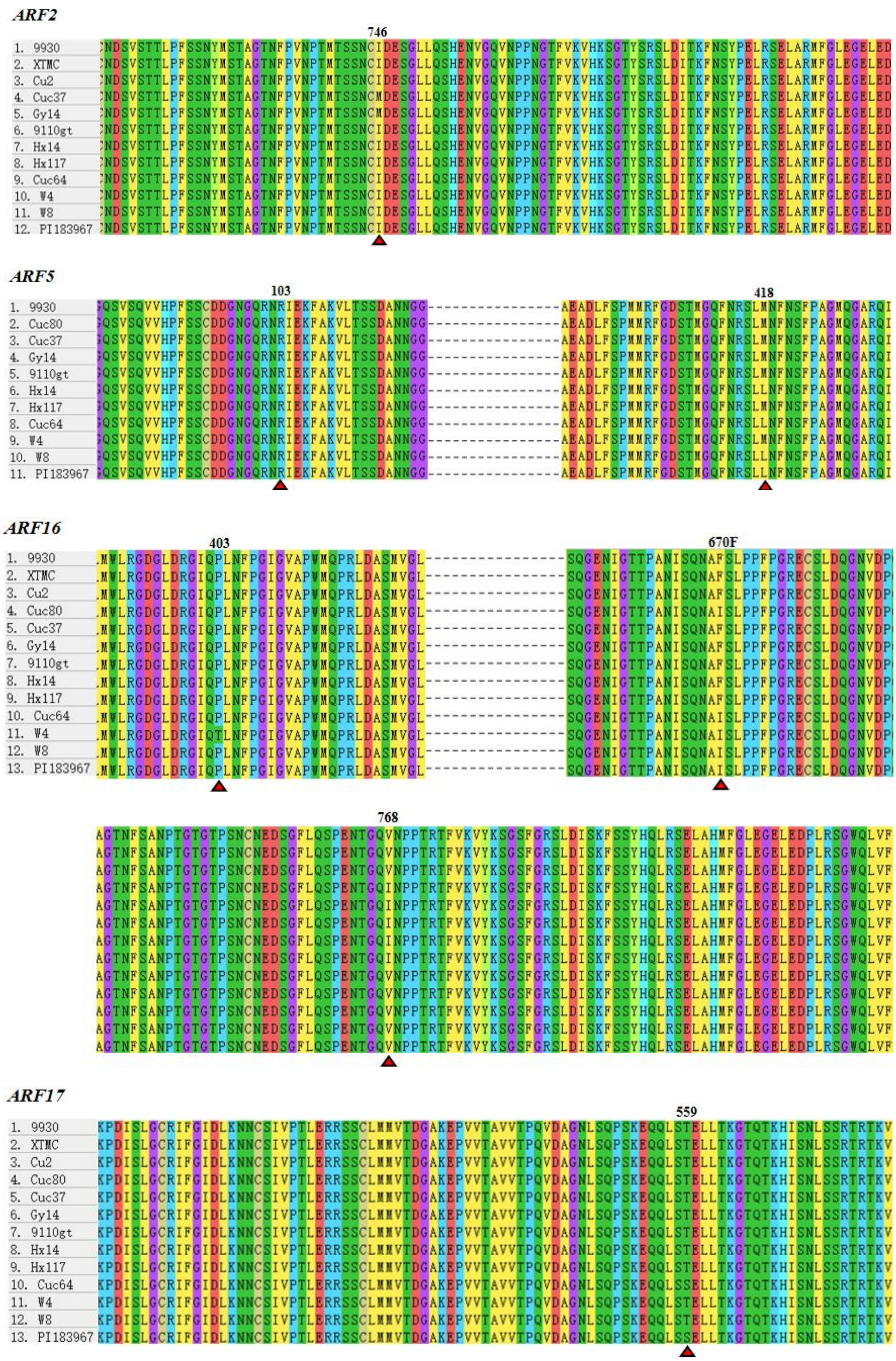
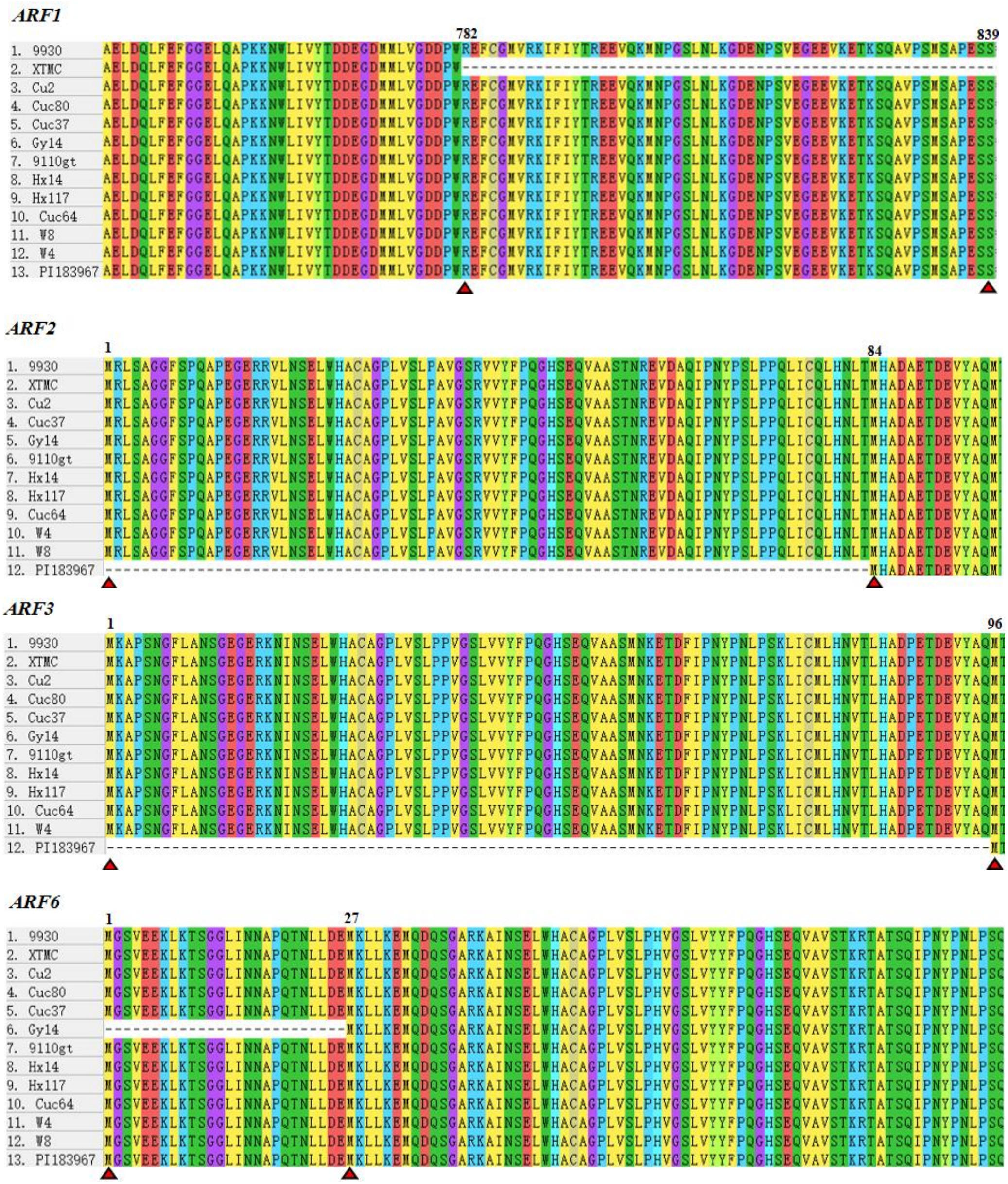
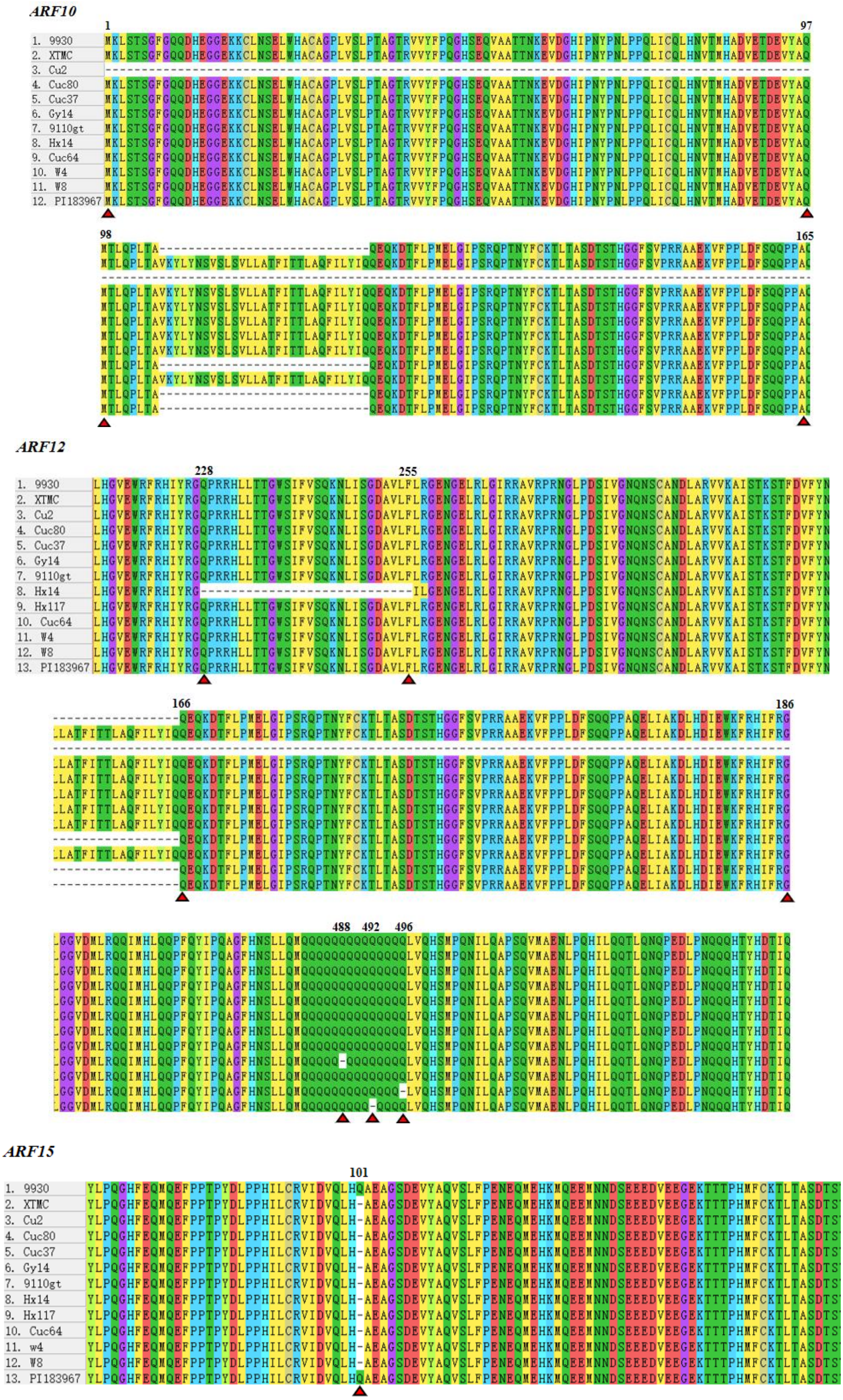


Figure S4. Single amino acid differences without changes in the amino acid properties in the pan-genome.


ARF1
782
839
1. 9930
2. XTMC
3. Cu2
4. Cuc80
5. Cuc37
6. Gy14
7. 9110gt
8. Hx14
9. Hx117
10. Cuc64
11. W8
12. W4
13. PI183967
ARF2
1
84
1. 9930
2. XTMC
3. Cu2
4. Cuc37
5. Gy14
6. 9110gt
7. Hx14
8. Hx117
9. Cuc64
10. W4
11. W8
12. PI183967
ARF3
1
96
1. 9930
2. XTMC
3. Cu2
4. Cuc80
5. Cuc37
6. Gy14
7. 9110gt
8. Hx14
9. Hx117
10. Cuc64
11. W4
12. PI183967
ARF6
1
27
1. 9930
2. XTMC
3. Cu2
4. Cuc80
5. Cuc37
6. Gy14
7. 9110gt
8. Hx14
9. Hx117
10. Cuc64
11. W4
12. W8
13. PI183967



ARF10
1
97
1. 9930
2. XTMC
3. Cu2
4. Cuc80
5. Cuc37
6. Gy14
7. 9110gt
8. Hx14
9. Cuc64
10. W4
11. W8
12. PI183967
98
165
ARF12
228
255
1. 9930
2. XTMC
3. Cu2
4. Cuc80
5. Cuc37
6. Gy14
7. 9110gt
8. Hx14
9. Hx117
10. Cuc64
11. W4
12. W8
13. PI183967
166
186
488
492
496
ARF15
101
1. 9930
2. XTMC
3. Cu2
4. Cuc80
5. Cuc37
6. Gy14
7. 9110gt
8. Hx14
9. Hx117
10. Cuc64
11. w4
12. W8
13. PI183967

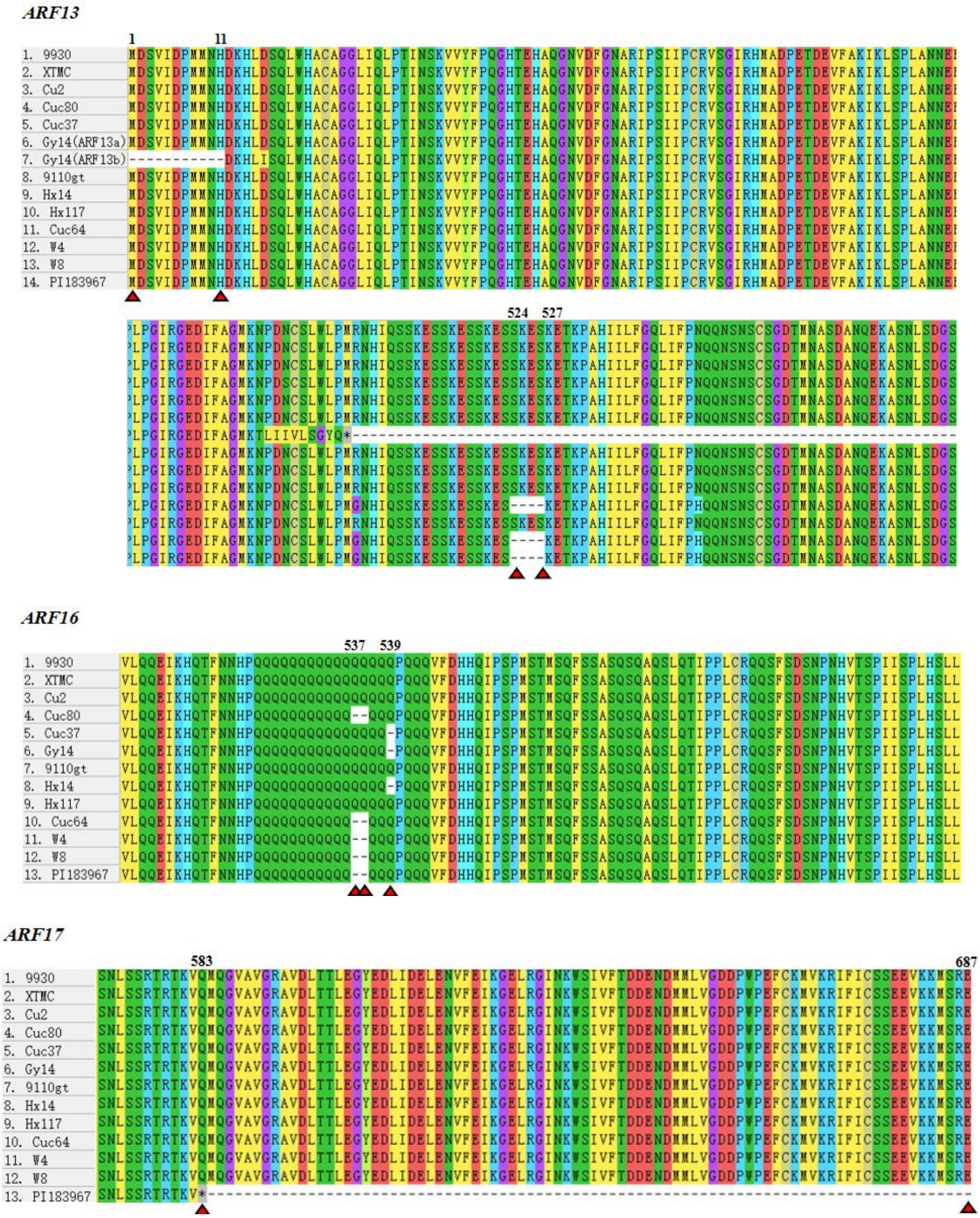
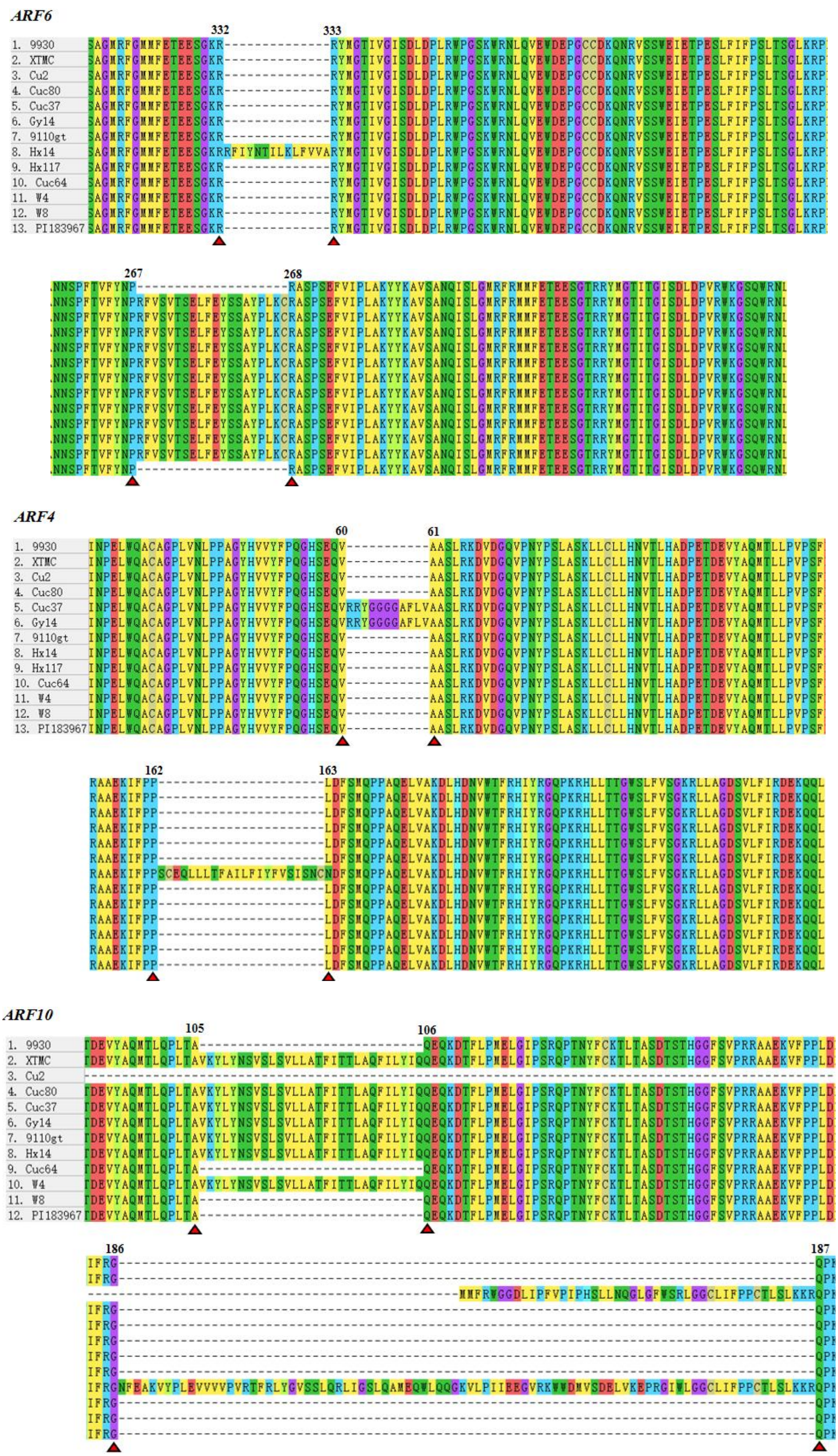
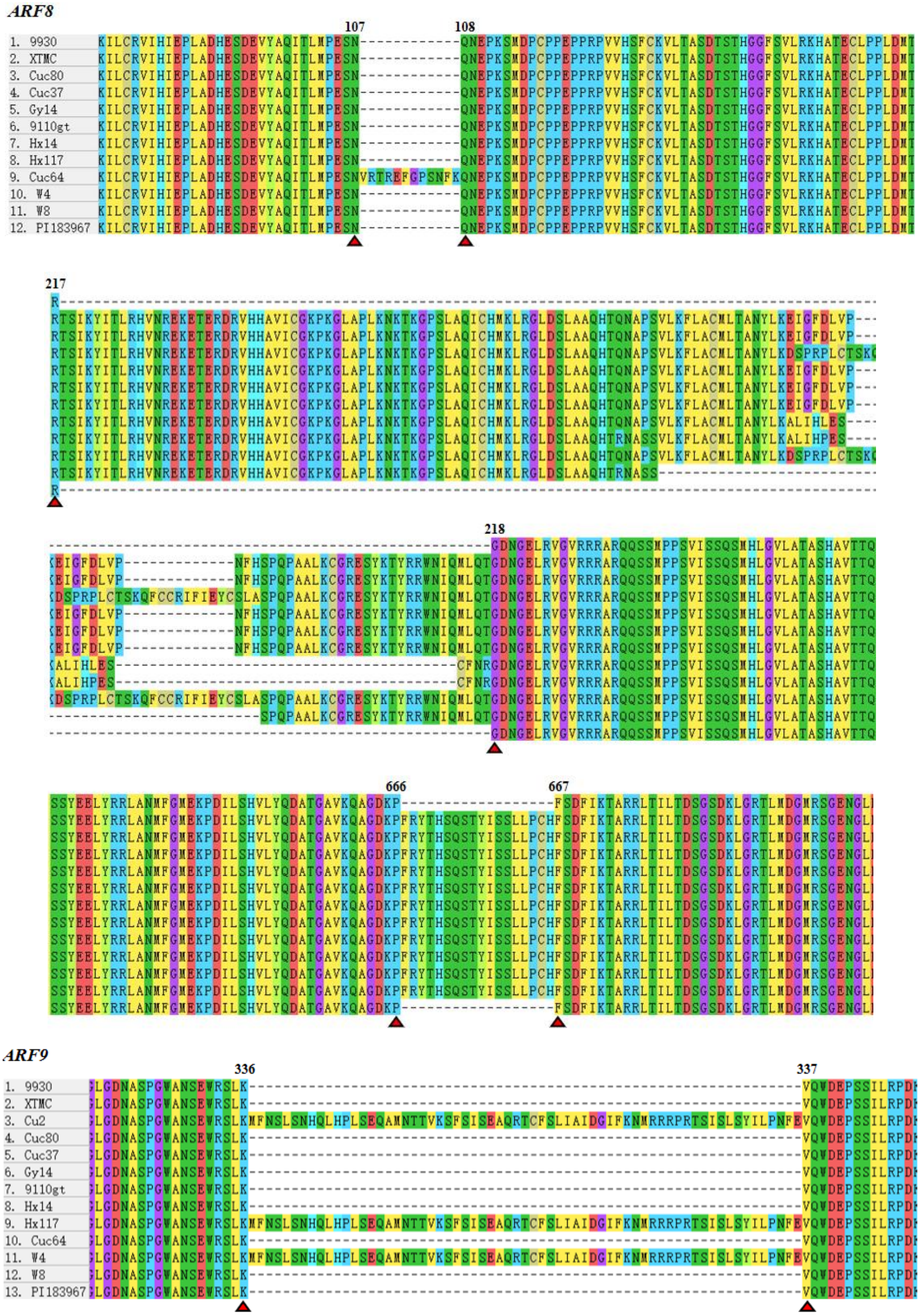


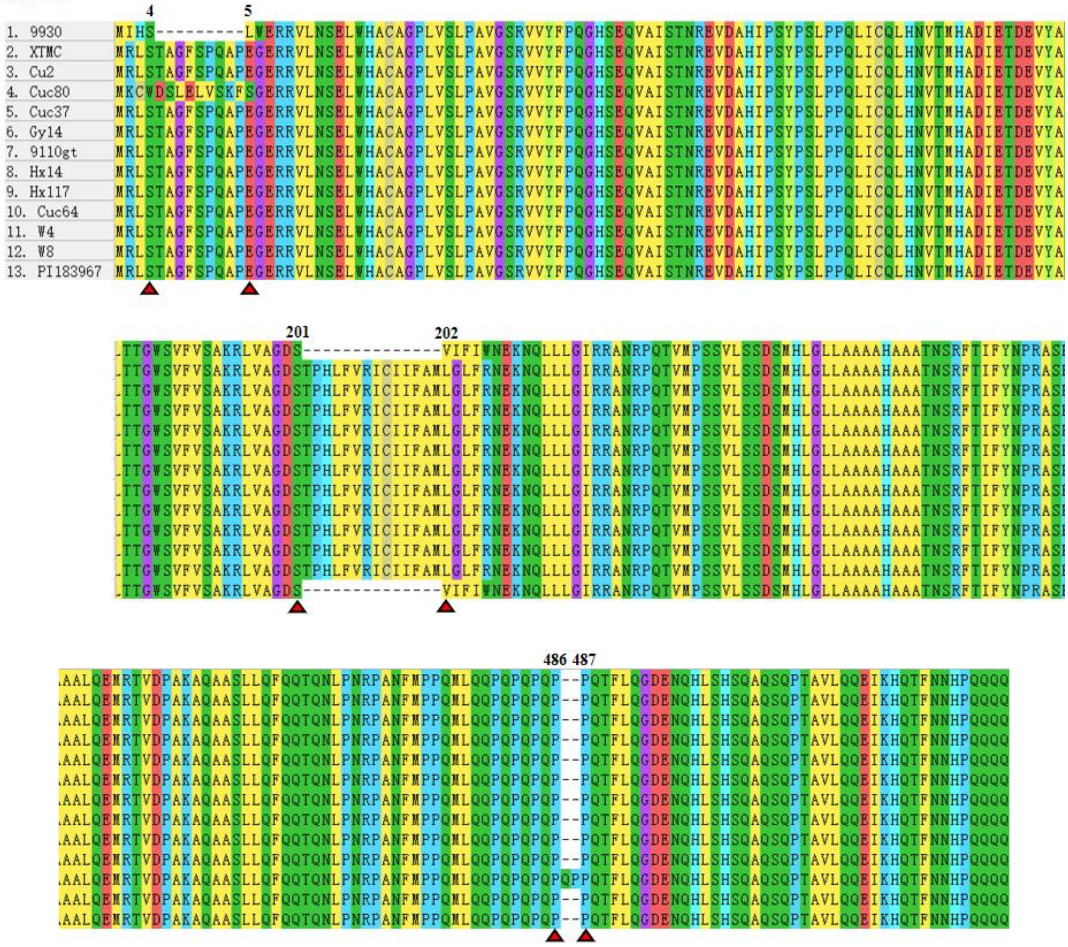
Figure S5. The absence of amino acid fragments in the pan-genome.


ARF6
332
333
1. 9930
2. XTMC
3. Cu2
4. Cuc80
5. Cuc37
6. Gy14
7. 9110gt
8. Hx14
9. Hx117
10. Cuc64
11. W4
12. W8
13. PI183967
267
268
ARF4
60
61
1. 9930
2. XTMC
3. Cu2
4. Cuc80
5. Cuc37
6. Gy14
7. 9110gt
8. Hx14
9. Hx117
10. Cuc64
11. W4
12. W8
13. PI183967
162
163
ARF10
105
106
1. 9930
2. XTMC
3. Cu2
4. Cuc80
5. Cuc37
6. Gy14
7. 9110gt
8. Hx14
9. Cuc64
10. W4
11. W8
12. PI183967
186
187



ARF8
107
108
1. 9930
2. XTMC
3. Cuc80
4. Cuc37
5. Gy14
6. 9110gt
7. Hx14
8. Hx117
9. Cuc64
10. W4
11. W8
12. PI183967
217
218
666
667
ARF9
336
337
1. 9930
2. XTMC
3. Cu2
4. Cuc80
5. Cuc37
6. Gy14
7. 9110gt
8. Hx14
9. Hx117
10. Cuc64
11. W4
12. W8
13. PI183967

ARF16


4
5
1. 9930
2. XTMC
3. Cu2
4. Cuc80
5. Cuc37
6. Gy14
7. 9110gt
8. Hx14
9. Hx117
10. Cuc64
11. W4
12. W8
13. PI183967
201
202
486 487

ARF12


453
454
1. 9930
2. XTMC
3. Cu2
4. Cuc80
5. Cuc37
6. Gy14
7. 9110gt
8. Hx14
9. Hx117
10. Cuc64
11. W4
12. W8
13. PI183967

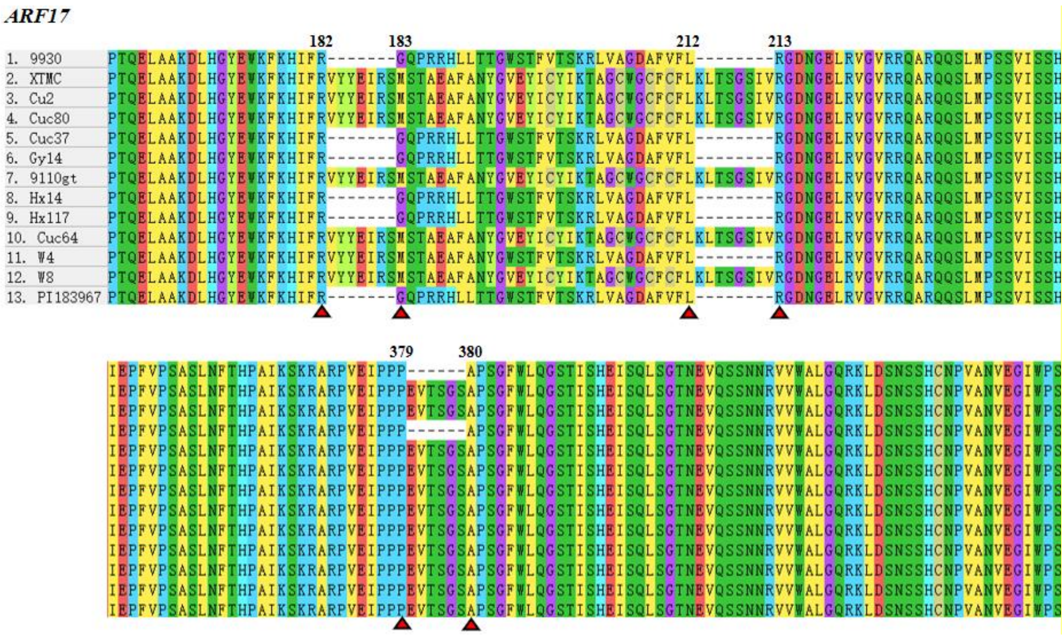


Figure S6. Insertions of amino acid fragments in the pan-genome.

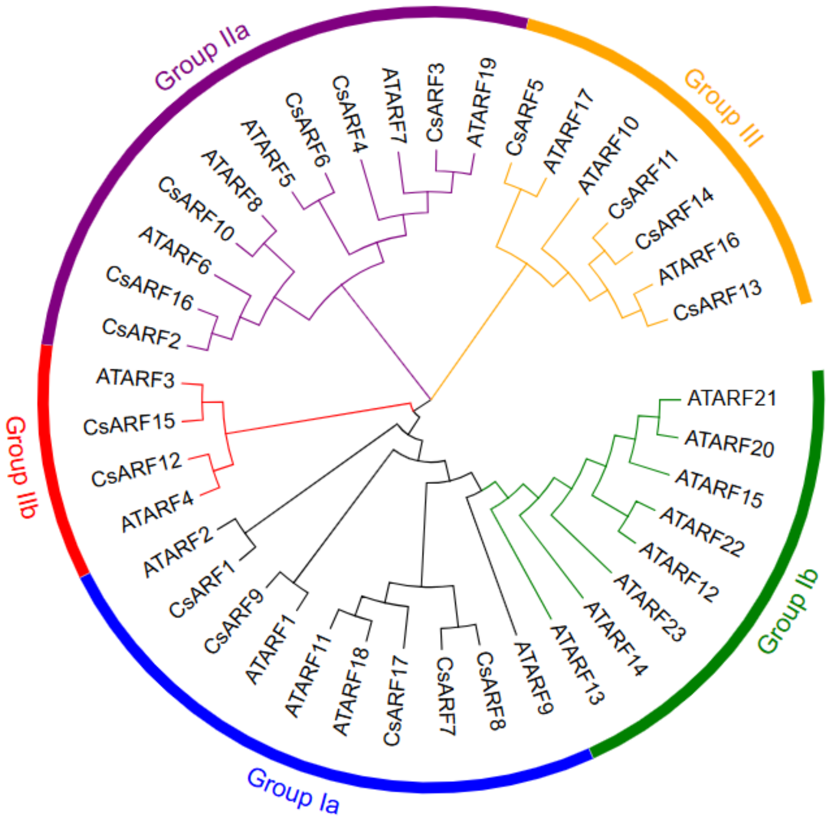


Figure S7. The phylogenetic tree of ARF proteins from cucumber variety 9930 and Arabidopsis genomes.

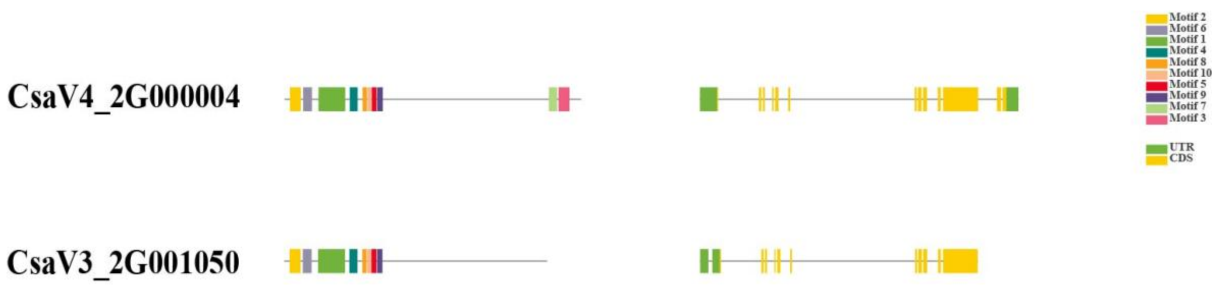


Figure S8. The conserved protein motifs, and gene structure of CsaV4_2G000004 and CsaV3_2G001050.