**Investigation of aquaporins involved in the adaptation of Canavalia rosea to saline-alkaline soils and drought stress**

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**Additional file 1: Figure S1.** The structural features of the CrAQPs. **a** CrNIPs. **b** CrPIPs. **c** CrTIPs. **d** CrXIPs. **e** CrSIPs.

**Additional file 1: Figure S2.** The overexpression analyses of *CrPIP1;5* in the three transgenic Arabidopsis lines (*OX 1#*, *OX 5#*, and *OX 10#*). **a** RT-PCR and genomic DNA PCR analysis of *CrPIP1;5* in the transgenic Arabidopsis lines and WT plants. **b** Quantitative RT-PCR analysis of *CrPIP1;5* in the transgenic Arabidopsis lines and WT plants.

**Additional file 1: Figure S3.** Overexpression analyses of *CrPIP1;5* in the transgenic Arabidopsis lines (*OX 1#*, *OX 5#*, and *OX 10#*) and stress tolerance analyses of transgenic plants with regards to seed germination rates. **a** Photographs of the transgenic lines and WT seeds germinated on MS medium or MS medium with NaCl, NaCl plus NaHCO3 (pH 8.2), or mannitol for 7 d. **b‒d** The seed germination rates in WT and transgenic lines under NaCl (**b**), NaCl plus NaHCO3 (pH 8.2) (**c**), and mannitol (**d**) stresses after 7 d.

**Additional file 1: Figure S4.** Salt, salt-alkaline, and high osmotic stress analyses of the transgenic plants with *CrPIP1;5*’s overexpression based on seedling root lengths. Four-day-old seedlings were transplanted into MS medium containing NaCl, NaCl plus NaHCO3 (pH 8.2) or mannitol and then grown for 7 d before measuring the root length. **a** Photographs of the transgenic lines (*CrPIP1;5* *OX 1#*, *OX 5#*, and *OX 10#*) and WT seedlings on MS medium or MS medium with NaCl, NaCl plus NaHCO3 (pH 8.2), or mannitol; **b‒d** The seedling root lengths (mm) in WT and the transgenic lines under NaCl (**b**), NaCl plus NaHCO3 (pH 8.2) (**c**), or mannitol (**d**) stresses after 7 d. Error bars indicate the SD based on over three replicates (n ≥ 3). Asterisks indicate significant differences from the control (Student’ s *t*-test P values, \* p < 0.05 and \*\* p < 0.01).

**Additional file 1: Figure S5.** Subcellular localization of the CrPIP1;5 protein. Arabidopsis roots expressing 35S:GFP-CrPIP1;5 fusion proteins (upper two lines) and 35S:GFP (lower line) were observed under a laser scanning confocal microscope.

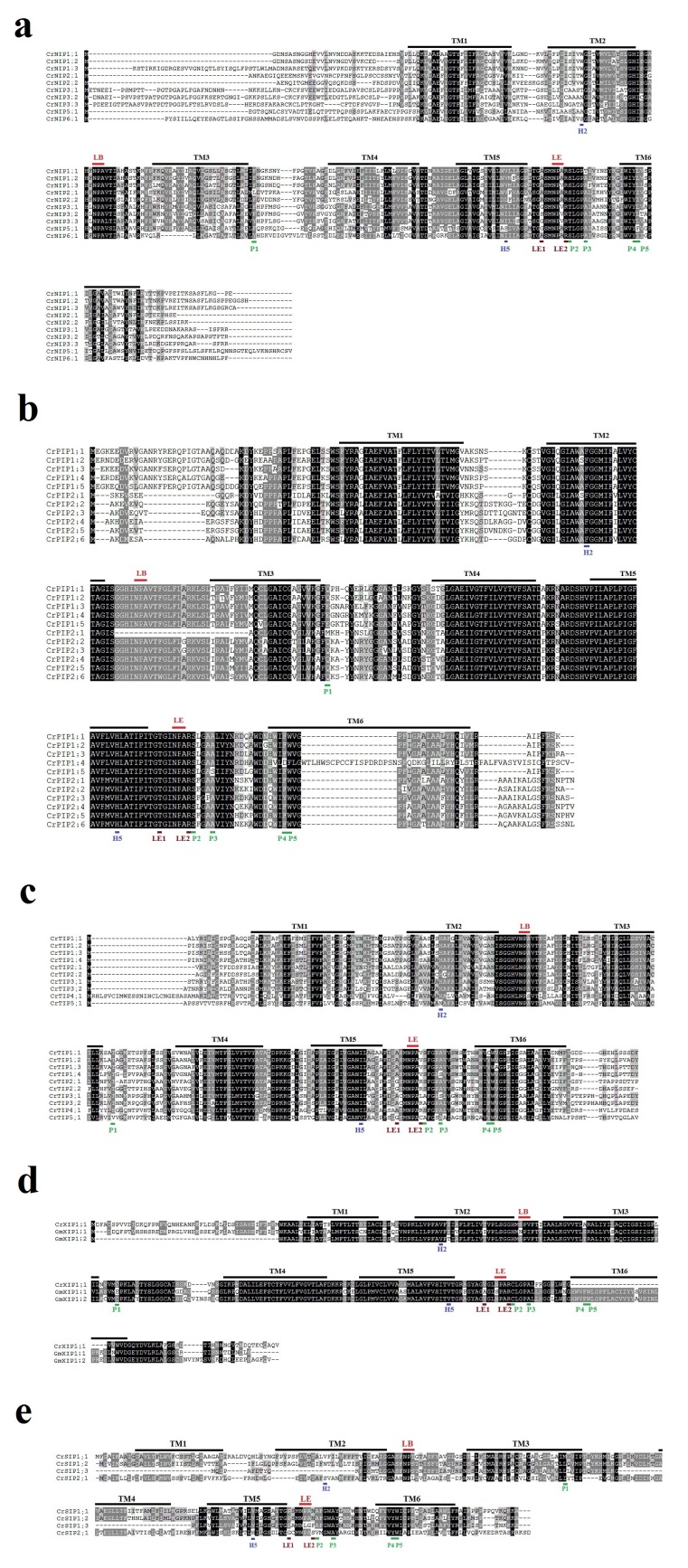
**Additional file 1: Table S1.** The obtained *CrAQP*s’ nucleotide and protein sequences information in this study.

**Additional file 1: Table S2.** Primers' information used in this study.

**Additional file 1: Table S3.** The categories of *cis*-acting elements identified in the *CrAQP*s’ promoter regions.

**Additional file 1: Table S4.** The conserved motif sequences of the CrAQPs identified by the MEME web server.

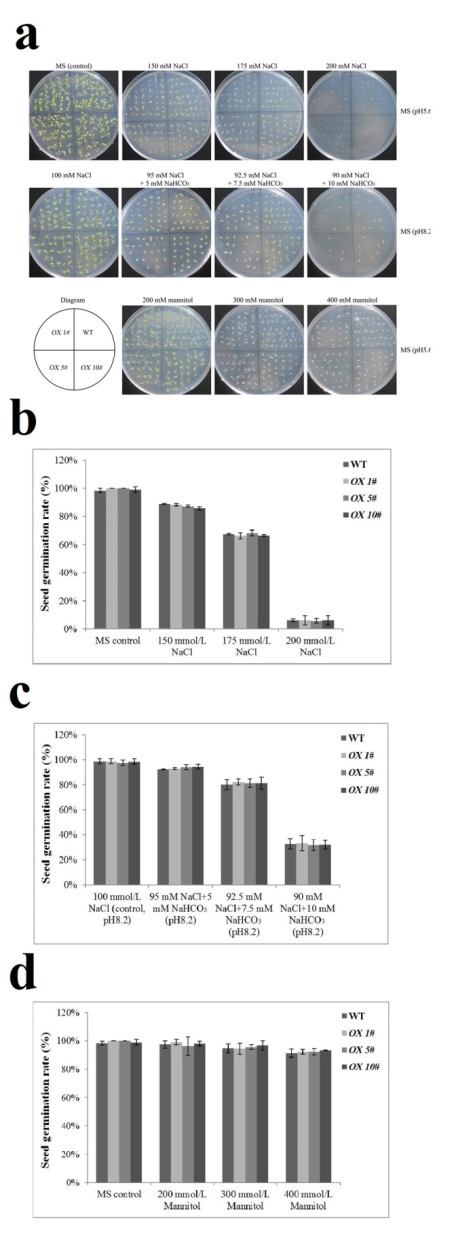
**Figure S1**



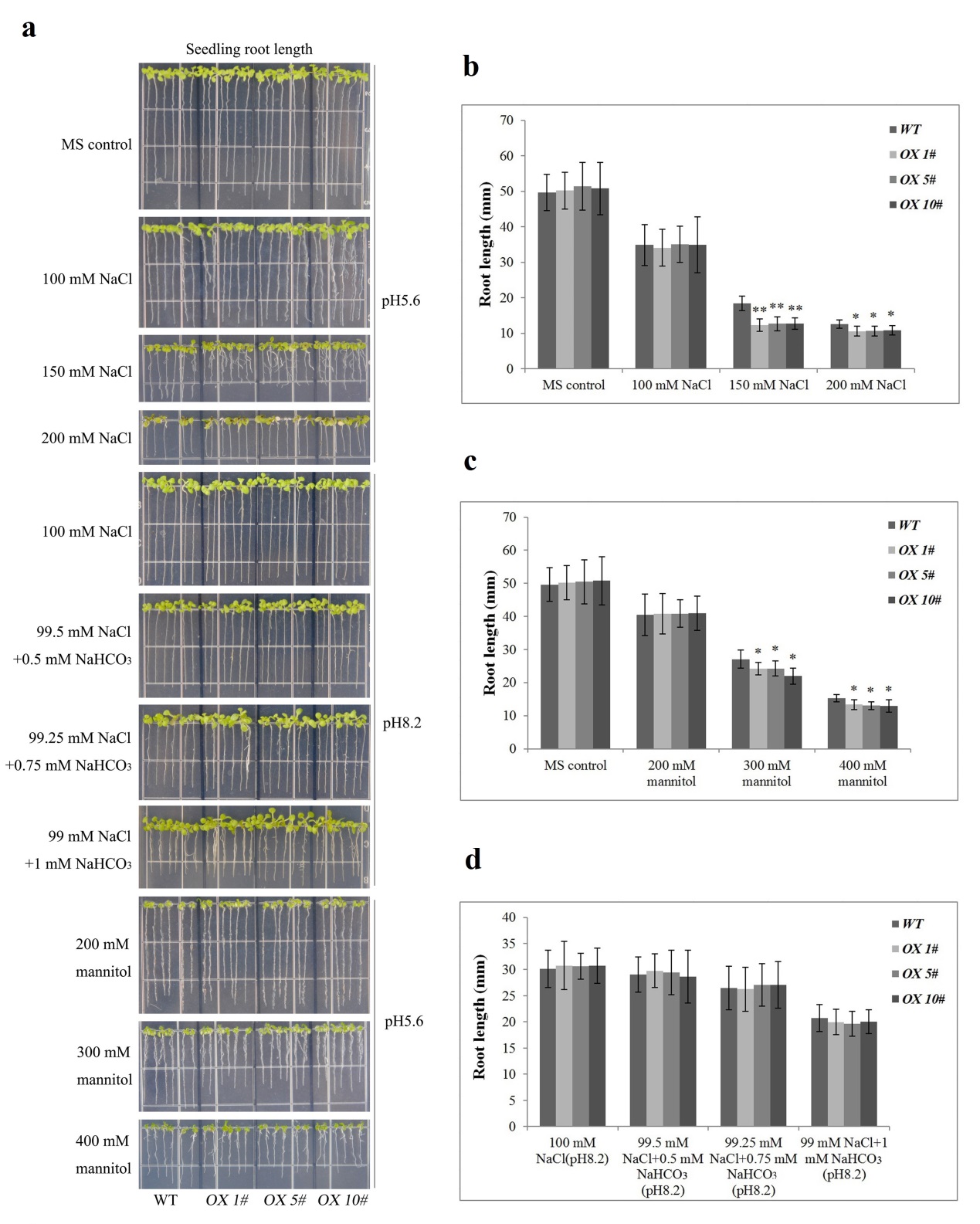
**Figure S2**



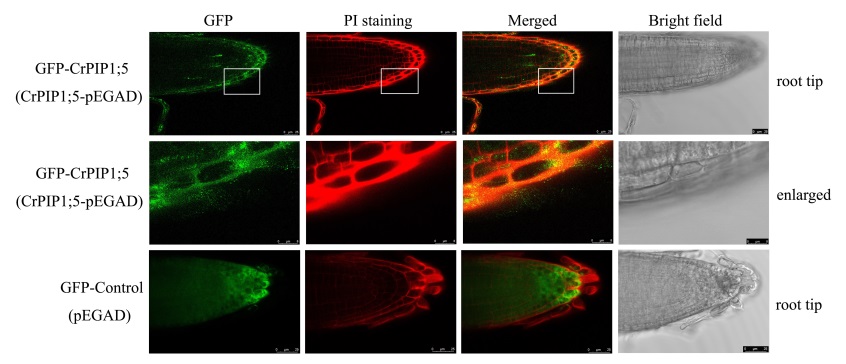
**Figure S3**



**Figure S4**



**Figure S5**



**Table S1**

See attached file.

**Table S2**

|  |  |  |
| --- | --- | --- |
| **Primer ID** | **Sequence (from 5′ to 3′)** | **Purpose** |
| CrPIP1;1RTF | TGTGAGAGTTGGTGCCAATAG | Primer pair for qRT-PCR of *CrPIP1;1* in *C. rosea* |
| CrPIP1;1RTR | CTGAAGGTGGCTCTTTGTAGTC |
| CrPIP1;2RTF | GTTCTTGGCACGCAAGTTATC | Primer pair for qRT-PCR of *CrPIP1;2* in *C. rosea* |
| CrPIP1;2RTR | CACCACCAAGCCTCTCATATT |
| CrPIP1;3RTF | CGGCATTAACCCAGCTAGAA | Primer pair for qRT-PCR of *CrPIP1;3* in *C. rosea* |
| CrPIP1;3RTR | AAGGGCAGCTCCAACAAA |
| CrPIP1;4RTF | AGGAGTCTAGGTGCTGCTATAA | Primer pair for qRT-PCR of *CrPIP1;4* in *C. rosea* |
| CrPIP1;4RTR | CGGATCACGATCTGGTGATATAAA |
| CrPIP1;5RTF | AGGGGTTAACCTTGTGAGCG | Primer pair for qRT-PCR of *CrPIP1;5* in *C. rosea* |
| CrPIP1;5RTR | TGGTCATCCCAGGCTTTGTC |
| CrPIP2;1RTF | CCGTCATAGGCCACAAGAAA | Primer pair for qRT-PCR of *CrPIP2;1* in *C. rosea* |
| CrPIP2;1RTR | GTGCAGTAGACGAGGACAAAG |
| CrPIP2;2RTF | CTGTGGCTGATGGCTACAATA | Primer pair for qRT-PCR of *CrPIP2;2* in *C. rosea* |
| CrPIP2;2RTR | CTTAGGATCAGTGGCAGAGAAG |
| CrPIP2;3RTF | AGCAACCCTTCTCTTCCTTTAC | Primer pair for qRT-PCR of *CrPIP2;3* in *C. rosea* |
| CrPIP2;3RTR | CGTCACATTCGGTGTTACCT |
| CrPIP2;4RTF | TGCCACACTTCTCTTCCTTTAC | Primer pair for qRT-PCR of *CrPIP2;4* in *C. rosea* |
| CrPIP2;4RTR | CAACACCACCACAAACATCAC |
| CrPIP2;5RTF | GCAGTGACATTTGGGTTGTTT | Primer pair for qRT-PCR of *CrPIP2;5* in *C. rosea* |
| CrPIP2;5RTR | CCAACCCAACTCCACAGATAG |
| CrPIP2;6RTF | GGAAAGTGTCGCTGGTTAGA | Primer pair for qRT-PCR of *CrPIP2;6* in *C. rosea* |
| CrPIP2;6RTR | CTCCTGCGTATCTGTTGTAGTAG |
| CrEF-αRTF | GACCTTCTTCGTTTCTCGCA | Primer pair for qRT-PCR of reference gene CrEF-α in *C. rosea* |
| CrEF-αRTR | CGAACCTCTCAATCACACGC |
| CrPIP1;5OXF | GGCAGCGGCCGAATTC ATGGAGGGGAAGGAACAGGA | Primer pair for cloning the full-length CDS of *CrPIP2;3* and construction of *CrPIP1;5*-pEGAD, *Eco*RI and *Bam*HI sites were underlined |
| CrPIP1;5OXR | CAGTTATCTAGGATCC TTACTTGGACTTGAAAGGAATGG |
| AtACT2RTF | GGTAACATTGTGCTCAGTGGTGG | Primer pair for qRT-PCR of reference gene *AtActin2* (*At3g18780*) in Arabidopsis |
| AtACT2RTF | AACGACCTTAATCTTCATGCTGC |

**Table S3**

See attached file.

**Table S4**

|  |  |  |  |
| --- | --- | --- | --- |
| **Group** | **Motif** | **E-value** | **Consensus sequence** |
| CrNIP | 8 | 1.1e-149 | E:\文档20200424备份\生态构建项目相关资料\海刀豆项目\海刀豆基因家族分析\海刀豆目标基因家族\海刀豆等植物物种的水孔蛋白基因家族序列汇总\CmAQP的MEME分析\CmAQP--MEME---所有成员同时10motifs分析\logo-8.jpg  RSDEATSVDLLQKVLAEFIGTYIFVFAGCGSAIA |
| 2 | 9.6e-335 | E:\文档20200424备份\生态构建项目相关资料\海刀豆项目\海刀豆基因家族分析\海刀豆目标基因家族\海刀豆等植物物种的水孔蛋白基因家族序列汇总\CmAQP的MEME分析\CmAQP--MEME---所有成员同时10motifs分析\logo-2.jpg  LFVLVYSTAHISGGHINPAVTF |
| 6 | 1.1e-276 | E:\文档20200424备份\生态构建项目相关资料\海刀豆项目\海刀豆基因家族分析\海刀豆目标基因家族\海刀豆等植物物种的水孔蛋白基因家族序列汇总\CmAQP的MEME分析\CmAQP--MEME---所有成员同时10motifs分析\logo-6.jpg  GLFLGRKFSLLRAILYIIAQLLGSICA |
| 7 | 5.1e-181 | E:\文档20200424备份\生态构建项目相关资料\海刀豆项目\海刀豆基因家族分析\海刀豆目标基因家族\海刀豆等植物物种的水孔蛋白基因家族序列汇总\CmAQP的MEME分析\CmAQP--MEME---所有成员同时10motifs分析\logo-7.jpg  VGAGQALVLEIIITFGLMFTVYAVATDPK |
| 1 | 1.3e-365 | E:\文档20200424备份\生态构建项目相关资料\海刀豆项目\海刀豆基因家族分析\海刀豆目标基因家族\海刀豆等植物物种的水孔蛋白基因家族序列汇总\CmAQP的MEME分析\CmAQP--MEME---所有成员同时10motifs分析\logo-1.jpg  IGFAVFLNILAAGPITGASMNPARS |
| 4 | 1.4e-370 | E:\文档20200424备份\生态构建项目相关资料\海刀豆项目\海刀豆基因家族分析\海刀豆目标基因家族\海刀豆等植物物种的水孔蛋白基因家族序列汇总\CmAQP的MEME分析\CmAQP--MEME---所有成员同时10motifs分析\logo-4.jpg  ANVSNAWDDHWIYWVGPFIGAALAALYYQ |
| CrPIP | 3 | 1.1e-312 | E:\文档20200424备份\生态构建项目相关资料\海刀豆项目\海刀豆基因家族分析\海刀豆目标基因家族\海刀豆等植物物种的水孔蛋白基因家族序列汇总\CmAQP的MEME分析\CmAQP--MEME---所有成员同时10motifs分析\logo-3.jpg  KDYHDPPPAPLFDPEELTSWSFYRAGIAEFVATLLFLYITVLTVIGYKKQ |
| 10 | 5.9e-061 | E:\文档20200424备份\生态构建项目相关资料\海刀豆项目\海刀豆基因家族分析\海刀豆目标基因家族\海刀豆等植物物种的水孔蛋白基因家族序列汇总\CmAQP的MEME分析\CmAQP--MEME---所有成员同时10motifs分析\logo-10.jpg  CSGVGILGIAWAFGG |
| 2 | 9.6e-335 | E:\文档20200424备份\生态构建项目相关资料\海刀豆项目\海刀豆基因家族分析\海刀豆目标基因家族\海刀豆等植物物种的水孔蛋白基因家族序列汇总\CmAQP的MEME分析\CmAQP--MEME---所有成员同时10motifs分析\logo-2.jpg  LFVLVYSTAHISGGHINPAVTF |
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| 9 | 2.7e-079 | E:\文档20200424备份\生态构建项目相关资料\海刀豆项目\海刀豆基因家族分析\海刀豆目标基因家族\海刀豆等植物物种的水孔蛋白基因家族序列汇总\CmAQP的MEME分析\CmAQP--MEME---所有成员同时10motifs分析\logo-9.jpg  FQKAYYNRYGGGANSVSDGYS |
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| 1 | 1.3e-365 | E:\文档20200424备份\生态构建项目相关资料\海刀豆项目\海刀豆基因家族分析\海刀豆目标基因家族\海刀豆等植物物种的水孔蛋白基因家族序列汇总\CmAQP的MEME分析\CmAQP--MEME---所有成员同时10motifs分析\logo-1.jpg  IGFAVFLNILAAGPITGASMNPARS |
| 4 | 1.4e-370 | E:\文档20200424备份\生态构建项目相关资料\海刀豆项目\海刀豆基因家族分析\海刀豆目标基因家族\海刀豆等植物物种的水孔蛋白基因家族序列汇总\CmAQP的MEME分析\CmAQP--MEME---所有成员同时10motifs分析\logo-4.jpg  ANVSNAWDDHWIYWVGPFIGAALAALYYQ |
| CrTIP | 8 | 1.1e-149 | E:\文档20200424备份\生态构建项目相关资料\海刀豆项目\海刀豆基因家族分析\海刀豆目标基因家族\海刀豆等植物物种的水孔蛋白基因家族序列汇总\CmAQP的MEME分析\CmAQP--MEME---所有成员同时10motifs分析\logo-8.jpg  RSDEATSVDLLQKVLAEFIGTYIFVFAGCGSAIA |
| 2 | 9.6e-335 | E:\文档20200424备份\生态构建项目相关资料\海刀豆项目\海刀豆基因家族分析\海刀豆目标基因家族\海刀豆等植物物种的水孔蛋白基因家族序列汇总\CmAQP的MEME分析\CmAQP--MEME---所有成员同时10motifs分析\logo-2.jpg  LFVLVYSTAHISGGHINPAVTF |
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| 7 | 5.1e-181 | E:\文档20200424备份\生态构建项目相关资料\海刀豆项目\海刀豆基因家族分析\海刀豆目标基因家族\海刀豆等植物物种的水孔蛋白基因家族序列汇总\CmAQP的MEME分析\CmAQP--MEME---所有成员同时10motifs分析\logo-7.jpg  VGAGQALVLEIIITFGLMFTVYAVATDPK |
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| 4 | 1.4e-370 | E:\文档20200424备份\生态构建项目相关资料\海刀豆项目\海刀豆基因家族分析\海刀豆目标基因家族\海刀豆等植物物种的水孔蛋白基因家族序列汇总\CmAQP的MEME分析\CmAQP--MEME---所有成员同时10motifs分析\logo-4.jpg  ANVSNAWDDHWIYWVGPFIGAALAALYYQ |
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