

· 研究论文 ·

大豆TPS基因家族在盐胁迫下的表达变化及单倍型选择规律分析

曹婕^{1†}, 卢秋连^{1†}, 翟健平¹, 刘宝辉^{1,2}, 方超^{1,2}, 李世晨^{1,2*}, 苏彤^{1,2*}

¹广州大学生命科学学院/分子遗传与进化创新研究中心, 广州 510006

²广东省植物适应性与分子设计重点实验室, 广州 510006

摘要 海藻糖-6-磷酸合酶(trehalose-6-phosphate synthase, TPS)是合成海藻糖的关键酶, 已在多个物种中被报道参与调节光合作用、糖代谢、生长发育和逆境响应等生理过程。目前, TPS在大豆(*Glycine max*)中的报道极少。该文在大豆全基因组中鉴定了20个TPS基因及其包含的10种重要蛋白保守基序。启动子元件分析显示, 大豆TPS基因的启动子区富含大量胁迫响应元件; 盐胁迫处理后, 17个TPS基因的表达发生变化, 其中12个基因上调表达, 5个基因下调表达。对TPS进行单倍型和选择趋势分析, 发现TPS8、TPS13、TPS15、TPS17和TPS18存在2种主要的等位变异, 其中携带TPS15^{H2}、TPS13^{H2}、TPS17^{H2}和TPS18^{H2}的品种在栽培品种中大量富集, 受到强烈的人工选择。该研究揭示了大豆TPS基因家族的分子特征以及在盐胁迫下的表达模式和进化历史, 旨在为进一步解析大豆TPS基因的功能以及培育耐盐大豆品种提供理论依据和遗传材料。

关键词 大豆, 海藻糖-6-磷酸合酶, 表达分析, 耐盐, 人工选择

曹婕, 卢秋连, 翟健平, 刘宝辉, 方超, 李世晨, 苏彤 (2025). 大豆TPS基因家族在盐胁迫下的表达变化及单倍型选择规律分析. 植物学报 60, 172–185.

大豆(*Glycine max*)是全球广泛种植的重要经济作物, 为人类提供了超过四分之一的蛋白质和油分。然而, 随着土壤盐碱化问题日益严峻, 全球作物年产量平均减少50%–80%, 已成为制约农作物生长发育的主要因素之一(Hayes et al., 2019; Singh et al., 2021)。在盐胁迫环境下, 植物会经历脱水、代谢毒性、营养缺乏以及膜功能障碍等, 导致组织损伤和早衰等现象(Essah et al., 2003; Papiernik et al., 2005; Munns and Tester, 2008)。大豆是一种盐敏感作物, 深入研究大豆的耐盐机制, 筛选耐盐相关基因及其优异等位变异, 对于培育耐盐品种, 提高土地利用率具有重要意义。

盐胁迫会诱导植物细胞产生活性氧(reactive oxygen species, ROS), 引发氧化应激反应, 对细胞组分造成损伤。植物进化出多种防御机制来缓解ROS

引起的氧化胁迫。例如, 拟南芥(*Arabidopsis thaliana*) GIGANTEA同源基因E2的等位突变正向调控ROS清除相关基因的表达, 提高大豆的耐盐性(Dong et al., 2022)。此外, 一方面GmCONSTANS-like 1a通过抑制ROS的生成增强大豆的耐盐性(Xu et al., 2023)。另一方面, miR160a通过切割生长素反应因子(auxin-response factor, ARF) GmARF16的转录本促进大豆耐盐, 而GmARF16会激活编码bHLH转录因子的GmMYC2, 通过调控脯氨酸的生物合成降低大豆对盐胁迫的适应性(Wang et al., 2023)。GmSIN1 (Salt Induced NAC1)促进大豆根系生长并增强耐盐性, 有助于在盐胁迫下提高产量(Li et al., 2019)。

海藻糖-6-磷酸合酶(trehalose-6-phosphate synthase, TPS)是高等植物中合成海藻糖的关键酶, 在植物应对盐胁迫反应中发挥重要作用(Van Dijken et

收稿日期: 2024-07-22; 接受日期: 2024-12-14

基金项目: 广东省自然科学基金面上项目(No.2023A1515011668)、黑龙江省自然科学基金(No.LH2021C078)和河北省现代种业科技创新专项(No.22326316D)

† 共同第一作者

* 通讯作者。E-mail: lishichen@gzhu.edu.cn; sutong@gzhu.edu.cn

al., 2004)。已在拟南芥中鉴定出11个*TPS*基因, 分为2个亚群, I类*AtTPS1–AtTPS4*和II类*AtTPS5–AtTPS11* (Vandesteene et al., 2010; Yang et al., 2012)。I类基因编码的蛋白具有*TPS*催化活性, 在拟南芥中已证实可以恢复酵母*TPS*缺失突变体*tsps1*的海藻糖合成能力(Lunn, 2007; Ramon et al., 2009; Delorge et al., 2015)。而II类基因编码的蛋白与I类结构相似, 但并不具有*TPS*催化活性。水稻(*Oryza sativa*)中同样具有11个*TPS*基因, 它们已被证实可提高水稻对低温、脱水和盐的抗性(Li et al., 2011)。在盐处理条件下, 小麦(*Triticum aestivum*)和蒺藜苜蓿(*Medicago truncatula*)的*TPS*基因活性显著增强(El-Bashiti et al., 2005; Song et al., 2021)。甘薯(*Ipomoea batatas*) *IbTPS*在盐胁迫条件下促进耐逆相关基因的上调表达, 从而增强耐盐性(Jiang et al., 2014)。甜瓜(*Cucumis melo*) *CmTPS4*参与ABA信号转导, 调控植物对低温和盐碱胁迫的耐受性(Yuan et al., 2021)。在鳞叶卷柏(*Selaginella lepidophylla*)中, *SITPS1*响应盐和热胁迫(Zentella et al., 1999)。随着植物基因组数据库的建立, 越来越多物种的*TPS*基因被鉴定, 涵盖杨树(*Populus sp.*)、苹果(*Malus pumila*)、棉花(*Gossypium hirsutum*)、马铃薯(*Solanum tuberosum*)、小麦和苜蓿(*M. sativa*)等(Lunn, 2007; Yang et al., 2012; Xie et al., 2015; Mu et al., 2016; Du et al., 2017; Xu et al., 2017; Song et al., 2021)。然而, 对于大豆*TPS*基因家族的研究却极为有限, 其在盐胁迫响应中的作用并不清晰。

现代作物是通过野生亲缘种的驯化和遗传渐渗, 以及在新环境中的适应性分化而形成(夏正俊, 2017; Lu et al., 2022)。驯化的过程较为漫长, 且伴随有意识和无意识的人工选择, 导致驯化的植物与它们的野生祖先之间发生了剧烈的形态和生理变化(Hammer, 1984)。与野生大豆(*G. soja*)相比, 栽培大豆的耐盐性更弱(Li et al., 2024)。目前, 已在大豆中鉴定出多个响应盐胁迫的基因, 且有的耐盐基因等位变异在驯化过程中经历了人工选择。例如, 乙烯响应因子(ethylene response factor, ERF)是参与植物响应多种生物和非生物胁迫反应的重要转录因子。大豆中包含153个*ERF*家族基因, *ERF158^{H1}*、*ERF166^{H2}*和*ERF170^{H1}*单倍型是能够显著提高大豆耐盐性的自然等位变异。*ERF170^{H1}*在大豆驯化过程中受到微弱的

人工选择, *ERF158^{H1}*和*ERF166^{H2}*在驯化过程中逐渐丢失, 重新鉴定优异的等位变异可为培育耐盐大豆新品种提供重要的基因资源(高超升等, 2024)。脱水反应元件结合蛋白(dehydration responsive element binding protein, DREB)对植物的非生物胁迫反应至关重要。*DREB3a*和*DREB3b*的自然变异与大豆耐盐性的差异有关, 且*DREB3b*可能经历了自然选择和人工选择, 与栽培大豆的*DREB3b^{Ref}*相比, 来自野生大豆的*DREB3b^{39Del}*提高了耐盐性。*DREB3b*等位基因的鉴定为提高大豆的产量提供了重要信息(Hou et al., 2022)。

我国是大豆的主要消费国, 每年消费量超过 1×10^8 t, 然而国产大豆产量相对较低, 大部分需求严重依赖进口, 导致国家粮食安全风险突出。我国拥有广阔的盐碱地资源, 预计至少有 0.3×10^8 hm²具有开发利用潜力(胡炎等, 2023)。如果能通过分子育种改良和培育耐盐大豆品种, 不仅可以有效提高盐碱地大豆的产量和品质, 还能增强国家粮食安全保障能力。因此, 本研究在全基因组水平系统鉴定了大豆*TPS*基因, 对其保守基序、共线性和顺式作用元件进行了生物信息学分析。利用定量PCR分析了大豆*TPS*基因在盐胁迫下的表达模式。在此基础上, 结合大豆重测序数据对*TPS*的单倍型及选择规律进行分析, 以期获得受到选择的关键*TPS*自然变异, 为大豆*TPS*基因的功能鉴定及培育耐盐大豆品种提供优异的遗传资源。

1 材料与方法

1.1 植物材料与处理

供试材料为大豆品种Williams 82 (*Glycine max* (L.) Merr. cv. 'Williams 82') (以下简称Wm82), 在温度为25°C, 相对湿度为70%, 光照条件为长日照(光周期为16小时光照/8小时黑暗)的温室中进行种植(Dong et al., 2022)。具体操作方法: 首先将干净的大豆种子在蛭石中萌发5天, 然后转移到含有霍格兰营养液的培养箱中, 继续生长至V1发育时期(Fehr et al., 1971); 随后加入200 mmol·L⁻¹ NaCl作为盐胁迫处理, 对照组则继续在无NaCl的营养液中生长(Dong et al., 2022); 在盐胁迫处理第4小时分别取叶片和根。将收集的样品用铝箔包裹后放入液氮中, 于-80°C冰箱保存备用。每个样品均设3次生物学重复。整个培养过程中保持培养液中有充足的氧气供应, 以

促进根系呼吸。

1.2 大豆TPS基因家族的系统发育分析

为了获取大豆的TPS蛋白序列,本研究采用Phytozome网站(<https://phytozome-next.jgi.doe.gov/>)的BLASTP算法,以拟南芥TPS蛋白序列作为参考模板,根据最小序列同源性和 $E\text{-value}\leq 10e^{-20}$ 的标准,下载大豆TPS蛋白序列。为了扩展数据,本研究同时从Phytozome网站收集了菜豆(*Phaseolus vulgaris*)、苜蓿和百脉根(*Lotus japonicus*)的TPS蛋白序列。使用CLUSTALW算法对这些检索到的TPS序列进行多序列比对,并利用MEGA11软件的最大似然法(maximum likelihood method)构建系统发育树,通过1 000次bootstrap分析评估其可靠性(Tamura et al., 2021)。最后,使用在线工具iTOL (<https://itol.embl.de/index.shtml>)优化系统发育树(Letunic and Bork, 2021)。所有基因号见附表1。

1.3 蛋白保守基序分析

将所获得的大豆TPS蛋白序列导入MEME (<https://meme-suite.org/meme/tools/meme>)在线工具,进行保守基序预测和分析(Bailey et al., 2009)。将最大的保守基序设定为10,其它参数保持默认设置。将所获得的信息导入TBtools软件中,可视化MEME结果(Chen et al., 2023)。

1.4 共线性分析

从Phytozome网站下载大豆基因组fasta文件和相应的gff注释文件。使用TBtools软件中的MCScanX插件对这些文件进行处理和分析(Wang et al., 2012)。使用Circos功能将结果可视化(Krzywinski et al., 2009)。

1.5 顺式作用元件分析

从Phytozome网站下载大豆TPS基因上游2 Kb启动子序列,并使用PlantCARE (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>)在线工具对序列进行分析,以明确推测的顺式作用元件(Kelley et al., 2015)。然后,使用TBtools对识别到的顺式作用元件进行可视化(Chen et al., 2023)。顺式作用元件的详细信息见附表2。

1.6 RNA-seq和qRT-PCR

根据Dong等(2022)所述实验方法,分别取长日照下200 mmol·L⁻¹ NaCl处理和无NaCl处理大豆的叶片和根,并用康为超纯RNA提取试剂盒(CWBIO, 中国)提取总RNA。使用PrimeScript RT Reagent Kit with gDNA Eraser试剂盒(Takara, 日本)将1 μg RNA反转录成cDNA。采用实时荧光定量PCR试剂盒(Takara, Cat No.RR430)和Roche light Cyclser480仪器进行qRT-PCR。每个反应总体积为10 μL,含1 μL稀释5倍的cDNA, 0.2 μL 10 μmol·L⁻¹引物, 5 μL SYBR Green Mater Mix, 用ddH₂O补足至终体积10 μL。差异表达分析采用2^{-ΔΔCT}方法(Livak and Schmittgen, 2001)。实验设3次生物学重复。基因表达量分析以Tubulin作为内参基因并进行归一化处理。采用Excel进行双尾的Student's *t*检验,进行显著性分析。大豆TPS基因的qRT-PCR引物序列见附表3。

1.7 单倍型与驯化选择分析

根据大豆TPS基因的染色体位置,使用vcftools软件生成目标基因在559份材料中的单核苷酸多态性(SNP)注释文件和基因型文件(Su et al., 2024)。随后对目标区段的基因区域进行分类,选择外显子区作为主要研究对象,并对此区域内的SNP进行单倍型分析,比较野生大豆、农家品种和育成品种的组成。利用vcftools软件在559份大豆自然种质资源中,分析大豆TPS基因前后各1 Mb重测序数据中SNP的Fst(群体间遗传分化指数)和π(种群核苷酸多样性)值,以明确其驯化选择过程。

1.8 数据分析

实验数据采用Excel软件进行双尾的Student's *t*检验,*表示 $P<0.05$; **表示 $P<0.01$; N.S.表示无显著性差异。在qRT-PCR分析中,每个样本至少进行3次技术重复,以确保结果的可靠性。

2 结果与分析

2.1 大豆TPS基因家族成员的鉴定与蛋白保守基序分析

本研究基于已知的拟南芥TPS蛋白,在Phytozome网

站进行BLASTP搜索,以确定大豆基因组中的全部TPS蛋白。我们鉴定到21个大豆TPS蛋白,并按照拟南芥的命名规则和染色体顺序,将21个大豆TPS蛋白分别命名为GmTPS1–GmTPS21(附表1)(谢翎等,2014)。为探明大豆、拟南芥和其它豆科植物中TPS蛋白的系统发育关系,我们选取拟南芥(*At*)、大豆(*Gm*)、菜豆(*Pv*)、苜蓿(*Mt*)和百脉根(*Lj*)共71条TPS蛋白序列构建系统发育树(图1)。根据系统发育树的拓扑结构,我们发现与已报道的植物TPS特征相符,这些TPS蛋白可分为2类,分别为Clade I和Clade II(杜姣林等,2023)。Clade I包含AtTPS1–AtTPS4, GmTPS1–GmTPS4、GmTPS20, PvTPS1–PvTPS3, MtTPS1、MtTPS4、MtTPS10和MtTPS12, LjTPS1–LjTPS4,共20个TPS蛋白。Clade II包含其余51个TPS蛋白。

随后,我们利用MEME程序对大豆TPS蛋白的保守基序进行分析,共鉴定到10个motifs,除TPS21外,

所有大豆TPS蛋白均具有Motif 2和Motif 8(图2;附图1)。这些TPS蛋白主要分为5类。第一类包括所有Clade I类中的TPS1–TPS4和TPS20,具有6个蛋白保守基序,即Motif 1、Motif 2、Motif 5、Motif 7、Motif 8和Motif 9。Clade II中的TPS12和TPS19各自为一类,均包含7个蛋白保守基序,但其中存在2个motif的不同。第4类包括TPS5、TPS8–TPS10、TPS17和TPS18,具有9个蛋白保守基序。最后一类包括TPS6、TPS7、TPS11、TPS13–TPS16,具有9个蛋白保守基序,与第4类的区别在于其包含Motif 4,而非Motif 10。考虑到TPS21蛋白保守基序极少,缺少其它TPS常见的Motif 2和Motif 8,我们推测其可能是

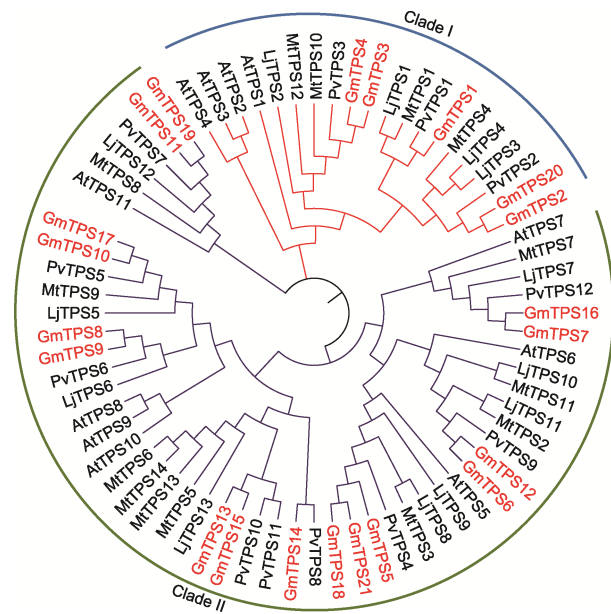


图1 拟南芥和豆科植物海藻糖-6-磷酸合酶(TPS)的系统发育树
拟南芥和豆科植物TPS蛋白分为2类(clade I和clade II)。红色的为大豆TPS蛋白。At: 拟南芥; Gm: 大豆; Pv: 菜豆; Mt: 苜蓿苜蓿; Lj: 百脉根

Figure 1 Phylogenetic tree of trehalose-6-phosphate synthase (TPS) from *Arabidopsis* and legumes
The TPS proteins from *Arabidopsis* and legumes are divided into two clades (clade I and clade II). The soybean TPS proteins are highlighted in red. At: *Arabidopsis thaliana*; Gm: *Glycine max*; Pv: *Phaseolus vulgaris*; Mt: *Medicago truncatula*; Lj: *Lotus japonicus*

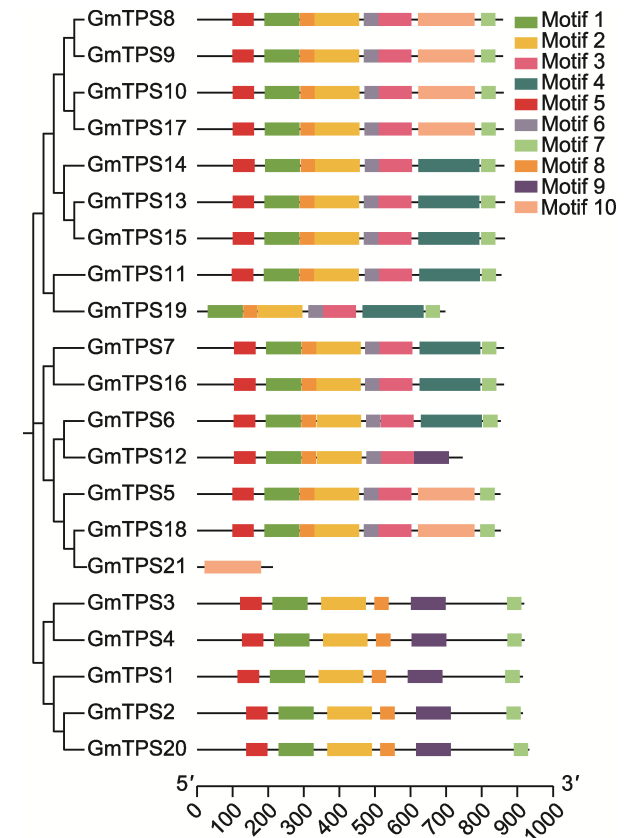


图2 大豆海藻糖-6-磷酸合酶(TPS)的蛋白保守基序
大豆TPS成员之间的系统发育关系与蛋白保守基序的分布,共鉴定到10个保守基序。底部刻度尺代表氨基酸序列长度(aa)。

Figure 2 Conserved protein motif of trehalose-6-phosphate synthase (TPS) in soybean
Phylogenetic relationships among TPS members and distribution of conserved protein motifs in soybean, ten motifs were identified. The bottom scale represents the amino acid sequence length (aa).

序列不完整基因, 导致蛋白功能缺失, 因此TPS21未纳入后续的大豆TPS分析中。

2.2 大豆TPS基因共线性分析

串联重复和片段重复是基因家族扩展的关键机制(Cannon et al., 2004)。一般而言, 串联重复指同一条染色体上出现2个相邻的同源基因, 通常它们之间不超过5个插入的基因。片段重复则导致基因组中存在大量重复的染色质区域(Wang et al., 2023)。为探究大豆TPS基因家族的扩展机制, 我们利用MCScanX程序对其进行共线性分析。结果显示, 在大豆基因组中, 有26对与大豆TPS基因相关的片段重复事件, 但并未发现串联重复的情况(图3)。这表明大豆TPS基因家族的扩增可能主要源自片段重复而非串联重复。

2.3 大豆TPS基因启动子区顺式作用元件分析

顺式作用元件在基因表达的转录调控中至关重要。为

探究大豆TPS基因可能参与的调控通路, 本研究提取了20个大豆TPS基因上游2 Kb启动子序列, 并采用PlantCARE程序分析顺式调控元件。结果显示, 在大豆TPS基因的启动子区共鉴定到16种顺式调控元件, TPS的启动子区域存在大量与环境胁迫相关的顺式作用元件和光响应元件, 暗示TPS基因可能参与大豆对环境变化的应答(图4)。此外, 与激素相关的元件数量也较多, 包括生长素、赤霉素、脱落酸和水杨酸等响应元件。TPS基因的顺式作用元件还与基因在胚乳、种子和根中的特异表达相关。

2.4 大豆TPS基因受盐胁迫调控

大豆TPS基因启动子区存在许多与环境胁迫应答相关的顺式作用元件。为了检测其是否受盐胁迫调控, 本研究对V1时期的Wm82植株进行盐处理, 并在处理后4小时分别取处理组和对照组的叶片和根进行定量PCR, 分析TPS在两组大豆样品中的表达变化。

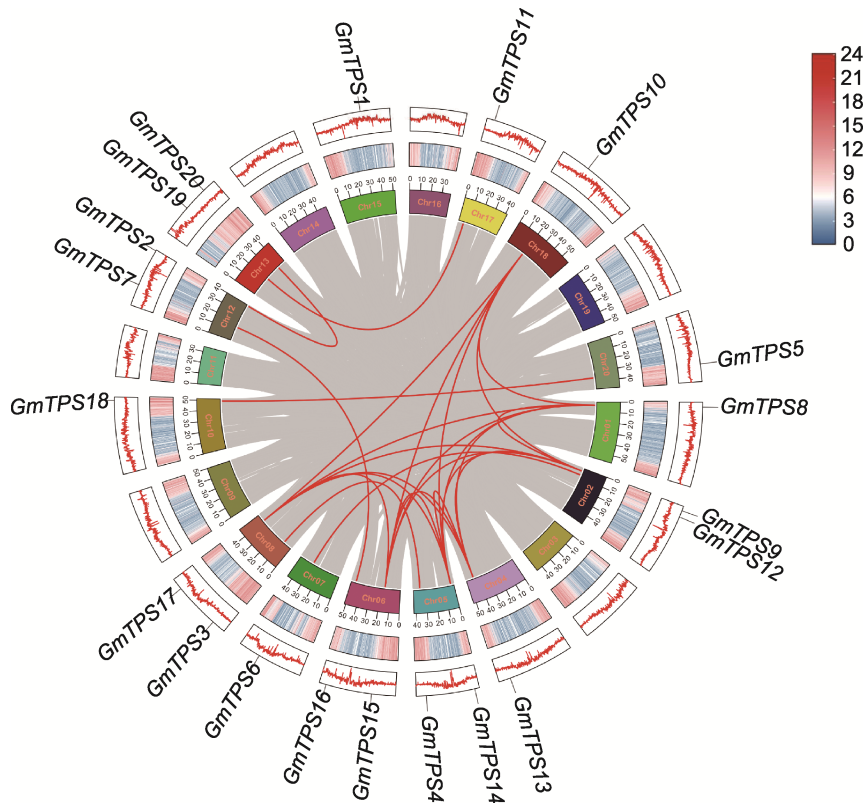


图3 大豆TPS基因的染色体分布及染色体间关系
红色曲线连接片段表示复制的基因对。

Figure 3 Chromosomal distribution and inter-chromosomal relationships of TPS genes in soybean
Red curves connecting pairs of genes indicate segmental duplications.

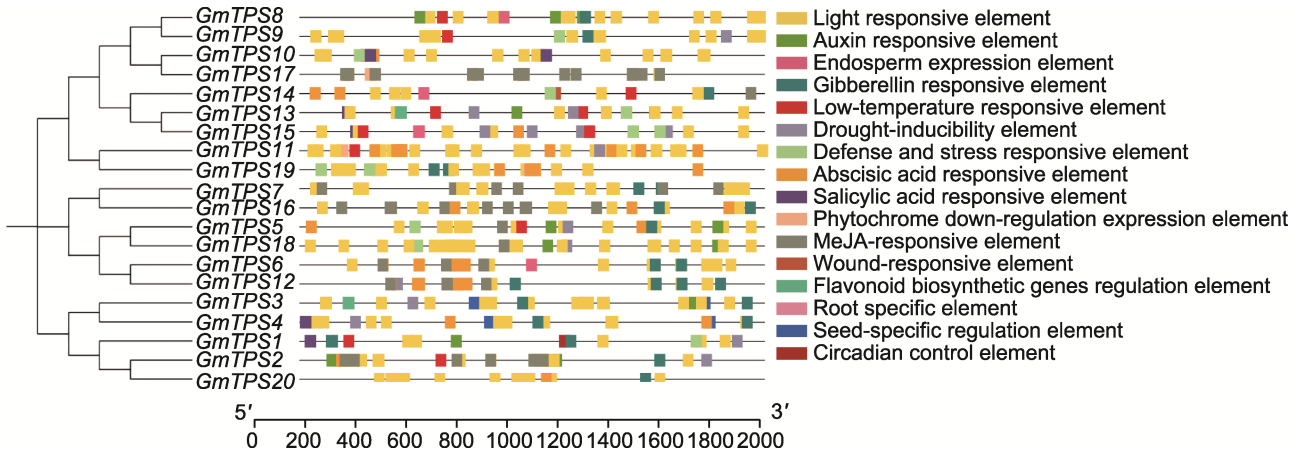


图4 大豆 *TPS* 基因的顺式作用元件

基因上游2 Kb启动子区域中的顺式作用元件被映射到每个 *TPS* 基因上, 以不同颜色标记不同类型的顺式作用元件, 右侧显示顺式作用元件类型。图中底部的刻度尺表示启动子序列的长度(bp)。

Figure 4 Cis-element analysis of *TPS* genes in soybean

The *cis*-elements in the 2 Kb upstream promoter regions of each *TPS* gene are mapped, with different types of *cis*-elements represented by different colors, as indicated on the right. The scale bars at the bottom of the figure represent the length of the promoter sequence (bp).

结果显示, 在20个 *TPS* 基因中, 除 *TPS3*、*TPS4* 和 *TPS14* 外, 其它基因的表达均在盐处理后发生显著变化。其中, 5个 *TPS* 基因在叶片或根中受盐胁迫负向调控, 12个 *TPS* 基因则表现出受盐胁迫诱导的表达模式。*TPS2* 和 *TPS5* 在盐处理后的叶片和根中下调表达, 而 *TPS1*、*TPS18* 和 *TPS20* 在盐处理后的根和叶片中表达模式不同, *TPS1* 在盐处理后的根中下调表达, 在叶片中无明显表达差异, 而 *TPS18* 和 *TPS20* 在盐处理后的叶片中下调表达, 在根中的表达量无显著变化, 说明它们在不同部位的调控机制可能不同, 导致仅在特定部位受到盐胁迫调控(图5; 附图2)。*TPS6*、*TPS7*、*TPS8*、*TPS9*、*TPS10*、*TPS11*、*TPS12*、*TPS13*、*TPS15*、*TPS16*、*TPS17* 和 *TPS19* 在盐处理后的叶片和根中表达量均显著增加, 说明它们在叶片和根中可能共享相似的上游调控信号, 或在地上部和地下部组织中可能具有类似的耐盐功能(图5; 附图2)。

2.5 大豆 *TPS* 基因的单倍型和驯化选择分析

在作物驯化和改良过程中, 通常将控制优异产量性状的基因组人为选择加以固定, 而忽略了对环境适应性相关基因组的选择和利用, 导致野生祖先的抗性位点变异在驯化过程中丢失。因此, 分析基因单倍型及各

单倍型的选择规律, 对于挖掘优异等位变异十分重要(Huang et al., 2022; Zhuang et al., 2022; Sun et al., 2023)。为挖掘大豆 *TPS* 基因的等位变异, 本研究分析了 *TPS* 基因的单倍型及其在驯化和改良过程中的选择规律。研究发现, *TPS3*、*TPS5*、*TPS6*、*TPS7*、*TPS9*、*TPS11*、*TPS12*、*TPS14*、*TPS16*、*TPS19* 和 *TPS20* 几乎没有非同义突变或者携带非同义突变的品种极少, 推测基因可能位于异染色质区, 不易发生突变, 或它们的功能较为保守, 突变可能会造成大豆生长缺陷或环境适应能力降低(附图3C, E–G, I, K, L, N, P, S, T)。*TPS1*、*TPS2*、*TPS4*、*TPS8*、*TPS10*、*TPS13*、*TPS15*、*TPS17* 和 *TPS18* 存在多种自然变异, 且携带每种自然变异的品种数目较多, 其中一些单倍型(如 *TPS1*^{H2}、*TPS2*^{H3}、*TPS4*^{H1}、*TPS4*^{H3}、*TPS10*^{H1} 和 *TPS10*^{H3}) 在栽培大豆中逐渐富集(附图3A, B, D, J)。值得注意的是, *TPS* 基因家族中存在受到强烈人工选择的基因, 包含 *TPS8*、*TPS13*、*TPS15*、*TPS17* 和 *TPS18*。在 *TPS8* 的编码序列中存在1个主要的多态性位点, 导致该基因被划分为2个主要单倍型。*TPS8*^{H1} 在核苷酸2 276位置的G-T置换导致氨基酸759处的色氨酸(W)被亮氨酸(L)所取代, 形成了 *TPS8*^{H2}。野生大豆主要携带H1, 在大豆驯化和改良过程中, *TPS8*^{H1}

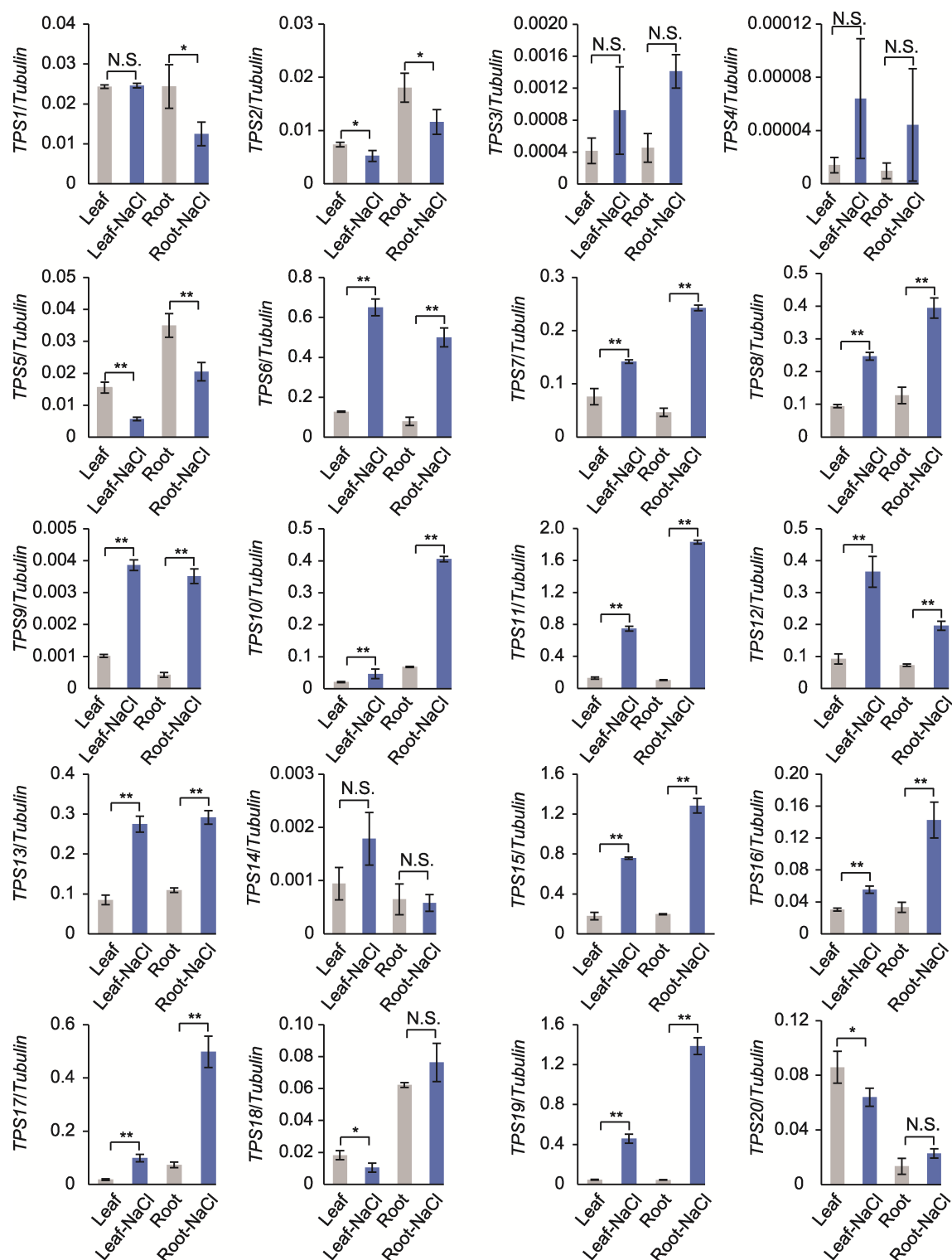


图5 qRT-PCR分析盐胁迫下 TPS 基因的表达量

图中从左至右, 从上至下依次为 $TPS1$ – $TPS20$ 。* $P < 0.05$; ** $P < 0.01$; N.S.表示无显著性差异。

Figure 5 qRT-PCR analysis of the TPS genes expression under salt stress

From left to right and top to bottom in the picture, they are $TPS1$ to $TPS20$ in sequence. * $P < 0.05$; ** $P < 0.01$; N.S. indicate no significant difference.

的频率逐渐下降, 而携带 *TPS8^{H2}* 的品种比例逐渐增加, 从野生大豆的12%增加到农家品种的41%, 再到栽培品种的59%, 表明 *TPS8^{H2}* 在大豆驯化与改良过

程中逐渐被优先选择(图6A, B)。 *TPS13* 主要存在2种自然变异, 其中 *TPS13^{H2}* 相对于 *TPS13^{H1}* 在多个位点发生非同义突变, 导致多个氨基酸发生改变(图6C)。

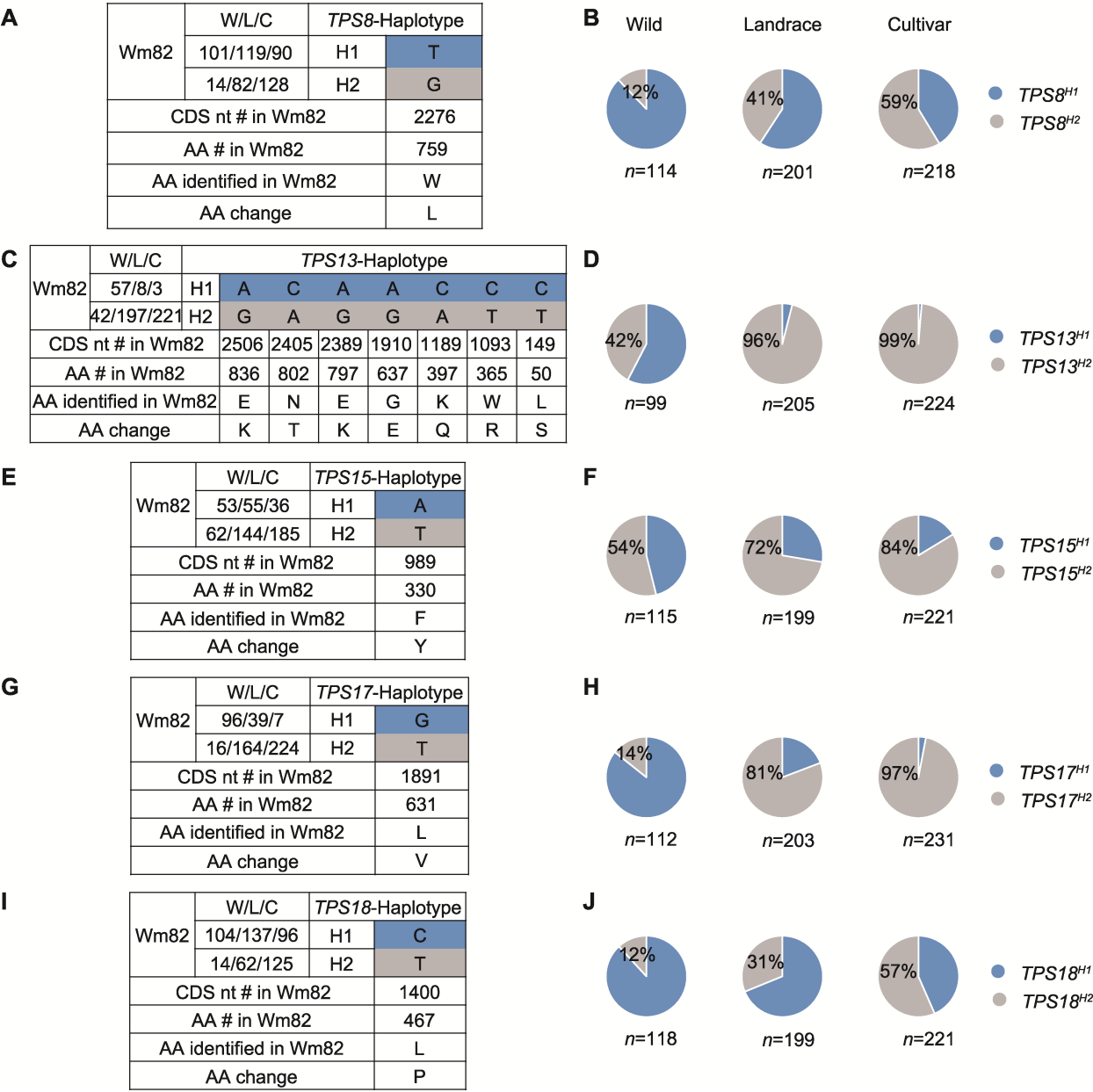


图6 受到人工选择的*TPS*基因
(A)、(C)、(E)、(G)和(I)分别代表*TPS8*、*TPS13*、*TPS15*、*TPS17*和*TPS18*的单倍型。图中所展示的为部分*TPS*重要位点的单倍型。
(B)、(D)、(F)、(H)和(J)分别代表不同等位变异在野生大豆(W)、农家品种(L)和栽培品种(C)中所占的比例。数据来自559份已测序的材料(121份野生大豆、207份农家品种和231份栽培品种)。

Figure 6 *TPS* genes subjected to intense artificial selection
(A), (C), (E), (G), and (I) represent the haplotypes of *TPS8*, *TPS13*, *TPS15*, *TPS17*, and *TPS18*, respectively. The figure shows several important *TPS* haplotypes. (B), (D), (F), (H), and (J) represent the proportions of different alleles in wild (W) soybeans, Landraces (L) and cultivated varieties (C), respectively. Data were obtained from 559 sequenced accessions (121 wild soybeans, 207 landraces, and 231 improved cultivars).

TPS13^{H2}在野生大豆中的频率仅为42%，在农家品种和栽培品种中却被大量固定，这说明携带*TPS13*^{H2}的大豆品种可能更适合人类选择(图6D)。与*TPS15*^{H1}相比，*TPS15*^{H2}在核苷酸989位置上发生T-A置换，导致氨基酸从酪氨酸(Y)变为苯丙氨酸(F) (图6E)。携带*TPS15*^{H2}的品种从野生大豆中的54%到农家品种中的72%，再到栽培品种中的84%，说明这一变异在大豆驯化和改良过程中逐渐积累(图6F)。*TPS17*和*TPS18*均在其编码区存在1个主要的多态性位点，分别导致氨基酸发生改变，产生2种单倍型(图6G, I)。携带*TPS17*^{H2}和*TPS18*^{H2}的野生大豆占种群比例不到20%，但在农家品种和栽培品种中被大量固定，特别是携带*TPS17*^{H2}的栽培品种所占比例达97%，而携带*TPS18*^{H2}的农家品种所占比例较低，但在栽培品种中被大量选择，与*TPS8*^{H2}类似(图6H, J)。上述结果表明，大部分*TPS*基因几乎没有自然变异，表明它们在大豆生长发育中发挥重要且保守的功能，不可轻易突变。然而*TPS8*、*TPS13*、*TPS15*、*TPS17*和*TPS18*的自然变异在大豆驯化和改良过程中受到强烈的人工选择，这些变异可能与大豆在特定环境下的适应性及人类需求相关。进一步阐明这些重要变异的功能以及它们与盐胁迫响应的关系，对于培育具有高耐盐性的大豆品种具有重要意义。此外，*TPS*基因家族的Fst和Pi分析显示，20个大豆*TPS*基因并未处于驯化区间内，说明它们并非驯化基因(附图4)，但与单倍型分析结果一致，部分*TPS*基因受到强烈的人工选择，如*TPS17*基因所在区域范围内栽培大豆的多态性极低，与野生大豆中较高的多态性形成对比(附图4Q)。

3 讨论

耐盐性是植物适应盐碱地区的重要生态性状，但目前对大豆这一性状的研究较少，可应用的遗传材料极少。*TPS*是海藻糖合成的关键基因，在植物耐盐胁迫中发挥重要作用。本研究在大豆全基因组水平鉴定到20个*TPS*基因，包含10种不同的蛋白保守基序。共线性分析显示，26对片段重复事件扩展了大豆*TPS*基因家族。*TPS*基因家族的启动子区富含光响应元件、激素响应元件以及胁迫响应元件，表明*TPS*基因可能在大豆的开花、生物和非生物胁迫响应中发挥重要作用。基因表达分析显示，17个大豆*TPS*基因的表达在盐胁迫下受到不同程度的诱导或抑制，说明*TPS*基因

在大豆盐胁迫响应中可能发挥重要作用。单倍型和驯化选择分析发现，*TPS8*、*TPS13*、*TPS15*、*TPS17*和*TPS18*存在受到强烈人工选择的重要等位变异，进一步解析其在盐胁迫下的功能，对于揭示大豆耐盐机制，培育高耐盐性的大豆品种具有重要意义。

海藻糖是一种稳定的非还原性二糖，其合成、分解和调控不仅为植物体提供能量，而且在植物生长发育和抗逆过程中发挥重要作用(Lunn et al., 2014; Kosar et al., 2019; Paul et al., 2020)。*TPS*是高等植物合成海藻糖的关键酶，在多个物种中参与生物和非生物胁迫响应。本研究中顺式作用元件分析结果显示，*TPS*基因启动子区存在大量胁迫响应元件，暗示其在大豆盐胁迫调控中具有潜在作用。随后，通过盐处理大豆幼苗，系统检测了盐处理后*TPS*基因的表达变化。其中，*TPS2*和*TPS5*在盐处理后的叶片和根中显著下调表达，而*TPS6*、*TPS7*、*TPS8*、*TPS9*、*TPS10*、*TPS11*、*TPS12*、*TPS13*、*TPS15*、*TPS16*、*TPS17*和*TPS19*显著上调表达，说明大豆*TPS*基因受到不同机制调节，具有不同的功能(图5; 附图2)。

栽培大豆在6 000–9 000年前由野生大豆驯化而来，但由于长期的人工选择和群体遗传瓶颈效应，其遗传多样性远低于野生大豆(Hymowitz, 1970; Carter et al., 2004; Hyten et al., 2006; Lam et al., 2010)。这种遗传变异的减少导致一些关键基因或位点丢失，特别是与耐盐性相关的重要遗传变异(Hou et al., 2022)。在大豆种质资源中挖掘基因的等位变异，筛选关键变异类型，将丰富作物的遗传多样性，从而为作物分子育种提供重要遗传材料。本研究发现，*TPS8*、*TPS13*、*TPS15*、*TPS17*和*TPS18*的自然变异在大豆驯化和改良过程中受到强烈的人工选择，其中携带*TPS15*^{H2}、*TPS13*^{H2}、*TPS17*^{H2}和*TPS18*^{H2}的品种大量富集在栽培品种中(图6)。对这些等位变异功能的进一步解析有助于培育高耐盐性大豆品种。鉴于耐盐性并不被认为是大豆的驯化性状，目前尚不清楚为什么仅*TPS15*^{H2}、*TPS13*^{H2}、*TPS17*^{H2}和*TPS18*^{H2}在驯化过程中被强烈选择，推测这些基因可能处于许多驯化和农艺性状的热点区域，如开花时间、种子大小和株高。也有可能这些基因与控制大豆驯化性状的基因存在连锁，当人们在选择驯化基因时，同时也伴随着*TPS15*^{H2}、*TPS13*^{H2}、*TPS17*^{H2}和*TPS18*^{H2}等位基因型被选择。一般而言，大豆对盐的抗性与产量呈负相

关, 在驯化过程中人们倾向于选择提高产量的性状, 有可能这些 *TPS* 基因的选择对产量的影响较小, 导致它们也受到人工选择。

基因重复在植物中普遍存在, 为新基因功能提供了资源, 包括由重组、DNA重复和全基因组重复产生的串联和片段重复(Ramsey and Schemske, 1998; Wendel, 2000; Kondrashov et al., 2002; Conant and Wolfe, 2008)。本研究鉴定到来源于片段重复的20个大豆 *TPS* 基因, 这些基因主要分为2类(图1, 图2)。与其它物种中的 *TPS* 分类结果一致, Clade I 中的 *TPS* 具有相同的蛋白保守基序, 表明它们可能存在功能冗余。而Clade II 中的 *TPS* 不仅在蛋白保守基序上与Clade I 差异较大, 其内部蛋白基序差异也较大, 说明这些 *TPS* 在功能上可能存在分化。

作者贡献声明

曹婕和卢秋连: 完成实验和分析数据; 翟健平: 分析数据; 刘宝辉: 构思实验, 监督和审阅文稿; 方超: 指导论文修改; 李世晨和苏彤: 设计实验, 撰写和修改论文。

参考文献

- Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, Ren J, Li WW, Noble WS (2009). MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Res* **37**, W202–W208.
- Cannon SB, Mitra A, Baumgarten A, Young ND, May G (2004). The roles of segmental and tandem gene duplication in the evolution of large gene families in *Arabidopsis thaliana*. *BMC Plant Biol* **4**, 10.
- Carter TE Jr, Nelson RL, Sneller CH, Cui ZL (2004). Genetic diversity in soybean. In: Shibles RM, Harper JE, Wilson RF, Shoemaker RC, eds. Soybeans: Improvement, Production, and Uses, Vol. 16, 3rd edn. Madison: American Society of Agronomy. pp. 303–416.
- Chen CJ, Wu Y, Li JW, Wang X, Zeng ZH, Xu J, Liu YL, Feng JT, Chen H, He YH, Xia R (2023). TBtools-II: a "one for all, all for one" bioinformatics platform for biological big-data mining. *Mol Plant* **16**, 1733–1742.
- Conant GC, Wolfe KH (2008). Turning a hobby into a job: how duplicated genes find new functions. *Nat Rev Genet* **9**, 938–950.
- Delorge I, Figueroa C, Feil R, Lunn J, Van Dijck P (2015). Trehalose-6-phosphate synthase 1 is not the only active *TPS* in *Arabidopsis thaliana*. *Biochem J* **466**, 283–290.
- Dong LD, Hou ZH, Li HY, Li ZB, Fang C, Kong LP, Li YL, Du H, Li T, Wang LS, He ML, Zhao XH, Cheng Q, Kong FJ, Liu BH (2022). Agronomical selection on loss-of-function of *GIGANTEA* simultaneously facilitates soybean salt tolerance and early maturity. *J Integr Plant Biol* **64**, 1866–1882.
- Du JL, Lin XL, Ma YW, Chen JR, Chen HX, Li YF (2023). Research progress in plant *Trehalose-6-phosphate synthase* genes. *Plant Sci J* **41**, 411–420. (in Chinese)
- 杜姣林, 蔺新兰, 马豫皖, 陈己任, 陈海霞, 李玉帆 (2023). 植物海藻糖-6-磷酸合成酶基因研究进展. *植物科学学报* **41**, 411–420.
- Du LS, Qi SY, Ma JJ, Xing LB, Fan S, Zhang SW, Li YM, Shen YW, Zhang D, Han MY (2017). Identification of *TPS* family members in apple (*Malus × domestica* Borkh.) and the effect of sucrose sprays on *TPS* expression and floral induction. *Plant Physiol Bioch* **120**, 10–23.
- El-Bashiti T, Hamamcı H, Öktem HA, Yücel M (2005). Biochemical analysis of trehalose and its metabolizing enzymes in wheat under abiotic stress conditions. *Plant Sci* **169**, 47–54.
- Essah PA, Davenport R, Tester M (2003). Sodium influx and accumulation in *Arabidopsis*. *Plant Physiol* **133**, 307–318.
- Fehr WR, Caviness CE, Burmood DT, Pennington JS (1971). Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill. *Crop Sci* **11**, 929–931.
- Gao CS, Yuan JZ, Zhi JY, Cheng YH, Dong LD, Cheng Q (2024). Identification and domestication analysis of *ERF* salt tolerance genes in soybean. *J Plant Genet Resour* **25**, 30–38. (in Chinese)
- 高超升, 袁嘉志, 植健怡, 程玉汉, 董利东, 程群 (2024). 大豆 *ERF* 耐盐基因的鉴定和驯化分析. *植物遗传资源学报* **25**, 30–38.
- Hammer K (1984). Das domestikationssyndrom. *Die Kulturpflanze* **32**, 11–34.
- Hayes S, Pantazopoulou CK, Van Gelderen K, Reinen E, Tween AL, Sharma A, De Vries M, Prat S, Schuurink RC, Testerink C, Pierik R (2019). Soil salinity limits plant shade avoidance. *Curr Biol* **29**, 1669–1676.
- Hou ZH, Li YL, Cheng YH, Li WW, Li T, Du H, Kong FJ, Dong LD, Zheng DF, Feng NJ, Liu BH, Cheng Q (2022). Genome-wide analysis of *DREB* genes identifies a novel salt tolerance gene in wild soybean (*Glycine soja*). *Front Plant Sci* **13**, 821647.
- Hu Y, Yang F, Yang N, Jia W, Cui Y (2023). Analysis and prospects of saline-alkali land in China from the perspec-

- tive of utilization. *Chin J Soil Sci* **54**, 489–494. (in Chinese)
- 胡炎, 杨帆, 杨宁, 贾伟, 崔勇 (2023). 盐碱地资源分析及利用研究展望. *土壤通报* **54**, 489–494.
- Huang XH, Huang SW, Han B, Li JY (2022). The integrated genomics of crop domestication and breeding. *Cell* **185**, 2828–2839.
- Hymowitz T (1970). On the domestication of the soybean. *Econ Bot* **24**, 408–421.
- Hyten DL, Song QJ, Zhu YL, Choi IY, Nelson RL, Costa JM, Specht JE, Shoemaker RC, Cregan PB (2006). Impacts of genetic bottlenecks on soybean genome diversity. *Proc Natl Acad Sci USA* **103**, 16666–16671.
- Jiang T, Zhai H, Wang FB, Zhou HN, Si ZZ, He SZ, Liu QC (2014). Cloning and characterization of a salt tolerance-associated gene encoding trehalose-6-phosphate synthase in sweetpotato. *J Integr Plant Biol* **13**, 1651–1661.
- Kelley L, Mezulis S, Yates CM, Wass MN, Sternberg MJE (2015). The Phyre2 web portal for protein modeling, prediction and analysis. *Nature* **10**, 845–858.
- Kondrashov FA, Rogozin IB, Wolf YI, Koonin EV (2002). Selection in the evolution of gene duplications. *Genome Biol* **3**, research0008.
- Kosar F, Akram NA, Sadiq M, Al-Qurainy F, Ashraf M (2019). Trehalose: a key organic osmolyte effectively involved in plant abiotic stress tolerance. *J Plant Growth Regul* **38**, 606–618.
- Krzywinski M, Schein J, Birol İ, Connors J, Gascoyne R, Horsman D, Jones SJ, Marra MA (2009). Circos: an information aesthetic for comparative genomics. *Genome Res* **19**, 1639–1645.
- Lam HM, Xu X, Liu X, Chen WB, Yang GH, Wong FL, Li MW, He W, Qin N, Wang B, Li J, Jian M, Wang J, Shao GH, Wang J, Sun SSM, Zhang GY (2010). Resequencing of 31 wild and cultivated soybean genomes identifies patterns of genetic diversity and selection. *Nat Genet* **42**, 1053–1059.
- Letunic I, Bork P (2021). Interactive tree of life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Res* **49**, W293–W296.
- Li HW, Zang BS, Deng XW, Wang XP (2011). Overexpression of the trehalose-6-phosphate synthase gene *OsTPS1* enhances abiotic stress tolerance in rice. *Planta* **234**, 1007–1018.
- Li ML, Xue M, Ma HY, Feng P, Chen T, Sun XH, Li Q, Ding XD, Zhang SZ, Xiao JL (2024). Wild soybean (*Glycine soja*) transcription factor GsWRKY40 plays positive roles in plant salt tolerance. *Crop J* **12**, 766–775.
- Li S, Wang N, Ji DD, Zhang WX, Wang Y, Yu YC, Zhao SZ, Lyu M, You JJ, Zhang YY, Wang LL, Wang XF, Liu ZH, Tong JH, Xiao LT, Bai MY, Xiang FN (2019). A GmSIN1/GmNCED3s/GmRbohBs feed-forward loop acts as a signal amplifier that regulates root growth in soybean exposed to salt stress. *Plant Cell* **31**, 2107–2130.
- Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **25**, 402–408.
- Lu SJ, Fang C, Abe J, Kong FJ, Liu BH (2022). Current overview on the genetic basis of key genes involved in soybean domestication. *aBIOTECH* **3**, 126–139.
- Lunn JE (2007). Gene families and evolution of trehalose metabolism in plants. *Funct Plant Biol* **34**, 550–563.
- Lunn JE, Delorge I, Figueroa CM, Van Dijck P, Stitt M (2014). Trehalose metabolism in plants. *Plant J* **79**, 544–567.
- Mu M, Lu XK, Wang JJ, Wang DL, Yin ZJ, Wang S, Fan WL, Ye WW (2016). Genome-wide identification and analysis of the stress-resistance function of the TPS (trehalose-6-phosphate synthase) gene family in cotton. *BMC Genet* **17**, 54.
- Munns R, Tester M (2008). Mechanisms of salinity tolerance. *Annu Rev Plant Biol* **59**, 651–681.
- Papiernik SK, Grieve CM, Lesch SM, Yates SR (2005). Effects of salinity, imazethapyr, and chlorimuron application on soybean growth and yield. *Commun Soil Sci Plant Anal* **36**, 951–967.
- Paul MJ, Watson A, Griffiths CA (2020). Trehalose 6-phosphate signaling and impact on crop yield. *Biochem Soc Trans* **48**, 2127–2137.
- Ramon M, De Smet I, Vandesteene L, Naudts M, Leyman B, Van Dijck P, Rolland F, Beeckman T, Thevelein JM (2009). Extensive expression regulation and lack of heterologous enzymatic activity of the Class II trehalose metabolism proteins from *Arabidopsis thaliana*. *Plant Cell Environ* **32**, 1015–1032.
- Ramsey J, Schemske DW (1998). Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annu Rev Ecol Syst* **29**, 467–501.
- Singh M, Nara U, Kumar A, Choudhary A, Singh H, Thapa S (2021). Salinity tolerance mechanisms and their breeding implications. *J Genet Eng Biotechnol* **19**, 173.
- Song JB, Mao HY, Cheng J, Zhou Y, Chen RR, Zeng LM, Li H, Wang YH (2021). Identification of the trehalose-6-phosphate synthase gene family in *Medicago truncatula* and expression analysis under abiotic stresses. *Gene* **787**, 145641.

- Su T, Liu H, Wu YC, Wang JH, He FL, Li HY, Li SC, Wang LS, Li LX, Cao J, Lu QL, Zhao XH, Xiang HT, Lin C, Lu SJ, Liu BH, Kong FJ, Fang C (2024). Soybean hypocotyl elongation is regulated by a MYB33-SWEET11/21-GA-2ox8c module involving long-distance sucrose transport. *Plant Biotechnol J* **22**, 2859–2872.
- Sun XP, Xiang YL, Dou NN, Zhang H, Pei SR, Franco AV, Menon M, Monier B, Ferebee T, Liu T, Liu SY, Gao YC, Wang JB, Terzaghi W, Yan JB, Hearne S, Li L, Li F, Dai MQ (2023). The role of transposon inverted repeats in balancing drought tolerance and yield-related traits in maize. *Nat Biotechnol* **41**, 120–127.
- Tamura K, Stecher G, Kumar S (2021). MEGA11: molecular evolutionary genetics analysis version 11. *Mol Biol Evol* **38**, 3022–3027.
- Van Dijken AJH, Schluepmann H, Smeekens SCM (2004). *Arabidopsis* trehalose-6-phosphate synthase 1 is essential for normal vegetative growth and transition to flowering. *Plant Physiol* **135**, 969–977.
- Vandesteene L, Ramon M, Le Roy K, Van Dijck P, Roland F (2010). A single active trehalose-6-P synthase (TPS) and a family of putative regulatory TPS-like proteins in *Arabidopsis*. *Mol Plant* **3**, 406–419.
- Wang JH, Sun ZH, Liu H, Yue L, Wang F, Liu SR, Su BH, Liu BH, Kong FJ, Fang C (2023). Genome-wide identification and characterization of the soybean *Snf2* gene family and expression response to rhizobia. *Int J Mol Sci* **24**, 7250.
- Wang Y, Tang H, Debarry JD, Tan X, Li J, Wang X, Lee TH, Jin H, Marler B, Guo H, Kissinger JC, Paterson AH (2012). MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. *Nucleic Acids Res* **40**, e49.
- Wendel JF (2000). Genome evolution in polyploids. *Plant Mol Biol* **42**, 225–249.
- Xia ZJ (2017). Research progress in whole-genome analysis and cloning of genes underlying important agronomic traits in soybean. *Chin Bull Bot* **52**, 148–158. (in Chinese)
- 夏正俊 (2017). 大豆基因组解析与重要农艺性状基因克隆研究进展. *植物学报* **52**, 148–158.
- Xie DW, Wang XN, Fu LS, Sun J, Zheng W, Li ZF (2015). Identification of the trehalose-6-phosphate synthase gene family in winter wheat and expression analysis under conditions of freezing stress. *J Genet* **94**, 55–65.
- Xie L, Wang ZX, Huang B (2014). Genome-wide identification classification and expression of *TPS* family genes in soybean. *Chin J Oil Crop Sci* **36**, 160–167. (in Chinese)
- 谢翎, 汪章勋, 黄勃 (2014). 大豆 *TPS* 基因家族全基因组鉴定、分类与表达分析. *中国油料作物学报* **36**, 160–167.
- Xu CJ, Shan JM, Liu TM, Wang Q, Ji YJ, Zhang YT, Wang MY, Xia N, Zhao L (2023). CONSTANS-LIKE 1a positively regulates salt and drought tolerance in soybean. *Plant Physiol* **191**, 2427–2446.
- Xu YC, Wang YJ, Mattson N, Yang L, Jin QJ (2017). Genome-wide analysis of the *Solanum tuberosum* (potato) trehalose-6-phosphate synthase (TPS) gene family: evolution and differential expression during development and stress. *BMC Genomics* **18**, 926.
- Yang HL, Liu YJ, Wang CL, Zeng QY (2012). Molecular evolution of trehalose-6-phosphate synthase (TPS) gene family in *Populus*, *Arabidopsis* and rice. *PLoS One* **7**, e42438.
- Yuan GP, Liu JP, An GL, Li WH, Si WJ, Sun DX, Zhu YC (2021). Genome-wide identification and characterization of the trehalose-6-phosphate synthetase (TPS) gene family in watermelon (*Citrullus lanatus*) and their transcriptional responses to salt stress. *Int J Mol Sci* **23**, 276.
- Zentella R, Mascorro-Gallardo JO, Van Dijck P, Folch-Mallol J, Bonini B, Van Vaeck C, Gaxiola R, Covarrubias AA, Nieto-Sotelo J, Thevelein JM, Iturriaga G (1999). A *Selaginella lepidophylla* trehalose-6-phosphate synthase complements growth and stress-tolerance defects in a yeast *tps1* mutant. *Plant Physiol* **119**, 1473–1482.
- Zhuang YB, Wang XT, Li XC, Hu JM, Fan LC, Landis JB, Cannon SB, Grimwood J, Schmutz J, Jackson SA, Doyle JJ, Zhang XS, Zhang DJ, Ma JX (2022). Phylogenomics of the genus *Glycine* sheds light on polyploid evolution and life-strategy transition. *Nat Plants* **8**, 233–244.

Changes in the Expression of the Soybean *TPS* Gene Family Under Salt Stress and Haplotype Selection Analysis

Jie Cao^{1†}, Qiulian Lu^{1†}, Jianping Zhai¹, Baohui Liu^{1,2}, Chao Fang^{1,2}, Shichen Li^{1,2*}, Tong Su^{1,2*}

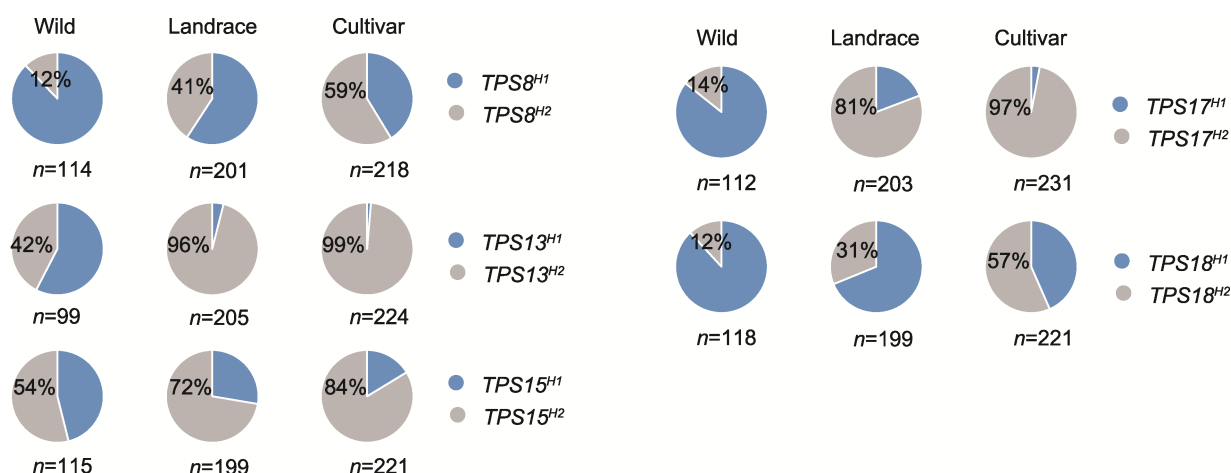
¹Innovative Research Center of Molecular Genetics and Evolution/College of Life Sciences, Guangzhou University, Guangzhou 510006, China; ²Guangdong Key Laboratory of Plant Adaptation and Molecular Design, Guangzhou 510006, China

INTRODUCTION: Trehalose-6-phosphate synthase (TPS) is a key enzyme involved in the synthesis of trehalose and has been reported to participate in regulating photosynthesis, carbohydrate metabolism, growth and development, and stress responses in various species. Currently, reports on *TPS* genes in soybean are scarce.

RATIONALE: TPS is a stable non-reducing disaccharide, whose synthesis, decomposition and regulation not only provide energy for plant, but also play an important role in plant growth and development and stress tolerance. The in-depth study of soybean *TPS* genes and its relationships with salt stress is of great significance in elucidating the molecular mechanism of soybean salt tolerance and improving soybean yield.

RESULTS: This study identified 20 soybean *TPS* genes and their associated 10 conserved protein motifs in the soybean genome. Molecular analysis of the promoter elements revealed that the *TPS* gene promoters are rich in stress-responsive elements. After salt stress treatment, the expression of 17 *TPS* genes changed, with 12 genes up-regulated and 5 genes down-regulated. Haplotype and selection analyses revealed two major allelic variations in *TPS8*, *TPS13*, *TPS15*, *TPS17*, and *TPS18*. Notably, variants carrying *TPS15*^{H2}, *TPS13*^{H2}, *TPS17*^{H2}, and *TPS18*^{H2} were significantly enriched in improved cultivars that underwent strong artificial selection.

CONCLUSION: This study reveals the molecular characteristics of the soybean *TPS* gene family, their expression patterns under salt stress, and their evolutionary history, providing a theoretical basis and genetic material for further elucidating the functions of soybean *TPS* genes and breeding salt-tolerant soybean varieties.



TPS genes were subjected to intense artificial selection. The natural variations of *TPS8*, *TPS13*, *TPS15*, *TPS17*, and *TPS18* have been subjected to strong artificial selection during soybean domestication and improvement, with the variants carrying *TPS15*^{H2}, *TPS13*^{H2}, *TPS17*^{H2}, and *TPS18*^{H2} being heavily enriched in improved cultivars.

Key words soybean, trehalose-6-phosphate synthase, expression analysis, salt tolerance, artificial selection

Cao J, Lu QL, Zhai JP, Liu BH, Fang C, Li SC, Su T (2025). Changes in the expression of the soybean *TPS* gene family under salt stress and haplotype selection analysis. *Chin Bull Bot* 60, 172–185.

† These authors contributed equally to this paper

* Authors for correspondence. E-mail: lishichen@gzhu.edu.cn; sutong@gzhu.edu.cn

附表1 拟南芥和豆科植物*TPS*基因家族

Appendix table 1 The *TPS* gene family in *Arabidopsis* and legumes

附表2 *TPS*基因家族顺式作用元件

Appendix table 2 Details of the *cis*-elements identified in the *TPS* gene family

附表3 用于*TPS*基因qRT-PCR分析的引物序列

Appendix table 3 Primer sequences of the *TPS* genes used for qRT-PCR

附图1 10个*TPS*基序的组成

Appendix figure 1 The composition of 10 *TPS* motifs

附图2 盐胁迫下*TPS*基因的表达变化

Appendix figure 2 Changes in the expression of *TPS* genes under salt stress

附图3 *TPS*基因单倍型

(A)–(O) 大豆*TPS*基因的单倍型。数据来自559份已测序材料(121份野生大豆、207份农家品种和231份栽培品种)。

Appendix figure 3 Analysis of *TPS* haplotypes

(A)–(O) Haplotypes of soybean *TPS* genes. The data were combined from 559 sequenced accessions (121 wild soybeans, 207 landraces and 231 improved cultivars).

附图4 在*TPS*基因周围2 Mb基因组区域内的野生大豆、农家品种和栽培品种的Fst和Pi

(A)–(T) 20个大豆*TPS*基因的Fst和Pi值。红色箭头表示*TPS*基因位置。数据来自559份已测序材料(121份野生大豆、207份农家品种和231份栽培品种)。

Appendix figure 4 Fst and Pi in wild soybeans, landraces and improved cultivars across the 2 Mb genomic region surrounding the *TPS*

(A)–(T) Fst and Pi of 20 soybean *TPS* genes. The red arrow represents the location of the *TPS*. The data were combined from 559 sequenced accessions (121 wild soybeans, 207 landraces and 231 improved cultivars).

<https://www.chinbullbotany.com/fileup/1647-3466/PDF/24-110-1.pdf>

(责任编辑: 朱亚娜)

通讯作者/团队简介

广州大学分子遗传与进化创新研究中心和广东省植物适应性与分子设计重点实验室

实验室以大豆为主要研究对象,收集了来自全世界的6 000多份野生大豆、农家品种和育成品种,并进行全基因组重测序。研究中心拥有高效快速大豆转基因和基因编辑平台、分子生物学和生物化学基因功能鉴定平台及高效的基因组学和生物信息学分析平台;还配备了完善的植物培养设施和多地田间试验基地。多次在*Nature Genetics*、*Nature Communications*、*Nature Plants*、*Proceedings of the National Academy of Sciences of the USA*、*Current Biology*、*Molecular Plant*、*Genome Biology*以及*Trends in Plant Science*等国际经典期刊上发表重要研究成果。

附表1 拟南芥和豆科TPS基因家族组成

Appendix table 1 The TPS gene family in *Arabidopsis* and legumes

物种 Species	参考基因组 Reference genome	基因名称 Gene Name	基因号 Gene ID
Arabidopsis	A.thaliana TAIR10	<i>AtTPS1</i>	AT1G78580
		<i>AtTPS2</i>	AT1G16980
		<i>AtTPS3</i>	AT1G17000
		<i>AtTPS4</i>	AT4G27550
		<i>AtTPS5</i>	AT4G17770
		<i>AtTPS6</i>	AT1G68020
		<i>AtTPS7</i>	AT1G06410
		<i>AtTPS8</i>	AT1G70290
		<i>AtTPS9</i>	AT1G23870
		<i>AtTPS10</i>	AT1G60140
		<i>AtTPS11</i>	AT2G18700
Soybean	G.max Wm82.a2.v1	<i>GmTPS1</i>	Glyma.15G210100
		<i>GmTPS2</i>	Glyma.12G234200
		<i>GmTPS3</i>	Glyma.08G120800
		<i>GmTPS4</i>	Glyma.05G163600
		<i>GmTPS5</i>	Glyma.20G120300
		<i>GmTPS6</i>	Glyma.07G172000
		<i>GmTPS7</i>	Glyma.12G129800
		<i>GmTPS8</i>	Glyma.01G031900
		<i>GmTPS9</i>	Glyma.02G033500
		<i>GmTPS10</i>	Glyma.18G136200
		<i>GmTPS11</i>	Glyma.17G067800
		<i>GmTPS12</i>	Glyma.02G086200
		<i>GmTPS13</i>	Glyma.04G180900
		<i>GmTPS14</i>	Glyma.05G036100
		<i>GmTPS15</i>	Glyma.06G184200
		<i>GmTPS16</i>	Glyma.06G275600
		<i>GmTPS17</i>	Glyma.08G288600
		<i>GmTPS18</i>	Glyma.10G270800
		<i>GmTPS19</i>	Glyma.13G092500
		<i>GmTPS20</i>	Glyma.13G264600
		<i>GmTPS21</i>	Glyma.07G169000
Phaseolus vulgaris	P.vulgaris v2.1	<i>PvTPS1</i>	Phvul.011G170300
		<i>PvTPS2</i>	Phvul.005G076500
		<i>PvTPS3</i>	Phvul.002G243600
		<i>PvTPS4</i>	Phvul.007G031800
		<i>PvTPS5</i>	Phvul.003G053000
		<i>PvTPS6</i>	Phvul.002G072400
		<i>PvTPS7</i>	Phvul.003G150400
		<i>PvTPS8</i>	Phvul.003G198800
		<i>PvTPS9</i>	Phvul.003G016300
		<i>PvTPS10</i>	Phvul.009G180300
		<i>PvTPS11</i>	Phvul.002G102300
		<i>PvTPS12</i>	Phvul.011G127700
Medicago	M.truncatula Mt4.0v	<i>MtTPS1</i>	Medtr0034s0170
		<i>MtTPS2</i>	Medtr1g032730
		<i>MtTPS3</i>	Medtr1g109620
		<i>MtTPS4</i>	Medtr2g073260
		<i>MtTPS5</i>	Medtr3g078210

	<i>MtTPS6</i>	<i>Medtr3g467030</i>
	<i>MtTPS7</i>	<i>Medtr4g080160</i>
	<i>MtTPS8</i>	<i>Medtr4g129270</i>
	<i>MtTPS9</i>	<i>Medtr8g063790</i>
	<i>MtTPS10</i>	<i>Medtr8g087930</i>
	<i>MtTPS11</i>	<i>Medtr8g105740</i>
	<i>MtTPS12</i>	<i>Medtr8g087910</i>
	<i>MtTPS13</i>	<i>Medtr3g467040</i>
	<i>MtTPS14</i>	<i>Medtr3g076810</i>
<i>Lotus japonicus</i> L.japonicus Lj1.0v1	<i>LjTPS1</i>	<i>Lj3g0024113</i>
	<i>LjTPS2</i>	<i>Lj4g0012591</i>
	<i>LjTPS3</i>	<i>Lj3g0025510</i>
	<i>LjTPS4</i>	<i>Lj3g0007787</i>
	<i>LjTPS5</i>	<i>Lj4g0012447</i>
	<i>LjTPS6</i>	<i>Lj2g0015842</i>
	<i>LjTPS7</i>	<i>Lj3g0019561</i>
	<i>LjTPS8</i>	<i>Lj5g0024862</i>
	<i>LjTPS9</i>	<i>Lj2g0022693</i>
	<i>LjTPS10</i>	<i>Lj4g0007903</i>
	<i>LjTPS11</i>	<i>Lj2g0018132</i>
	<i>LjTPS12</i>	<i>Lj4g0008825</i>
	<i>LjTPS13</i>	<i>Lj1g0010727</i>

附表2 TPS 基因家族顺式作用元件

Appendix table 2 Details of the cis-elements identified in TPS gene family

基因名 Gene Name	起始位置 Start position	终止位置 Stop position	顺式作用元件 Putative cis-elements
<i>GmTPS8</i>	1136	1185	Light responsive element
<i>GmTPS8</i>	1937	1984	Light responsive element
<i>GmTPS8</i>	1956	2006	Light responsive element
<i>GmTPS8</i>	807	856	Light responsive element
<i>GmTPS8</i>	536	584	Light responsive element
<i>GmTPS8</i>	1112	1160	Light responsive element
<i>GmTPS8</i>	1338	1387	Light responsive element
<i>GmTPS8</i>	494	540	Auxin responsive element
<i>GmTPS8</i>	1078	1124	Auxin responsive element
<i>GmTPS8</i>	1193	1239	Auxin responsive element
<i>GmTPS8</i>	856	903	Endosperm expression element
<i>GmTPS8</i>	1206	1253	Gibberellin responsive element
<i>GmTPS8</i>	591	637	Low-temperature responsive element
<i>GmTPS8</i>	1138	1187	Light responsive element
<i>GmTPS8</i>	1926	1975	Light responsive element
<i>GmTPS8</i>	659	705	Light responsive element
<i>GmTPS8</i>	1269	1315	Light responsive element
<i>GmTPS8</i>	1500	1546	Light responsive element
<i>GmTPS8</i>	1694	1740	Light responsive element
<i>GmTPS8</i>	1767	1815	Light responsive element
<i>GmTPS9</i>	1272	1318	Light responsive element
<i>GmTPS9</i>	1926	1975	Light responsive element
<i>GmTPS9</i>	1257	1303	Light responsive element
<i>GmTPS9</i>	1675	1721	Light responsive element
<i>GmTPS9</i>	1746	1794	Light responsive element
<i>GmTPS9</i>	1813	1859	Drought-inducibility element
<i>GmTPS9</i>	613	659	Low-temperature responsive element
<i>GmTPS9</i>	122	171	Light responsive element
<i>GmTPS9</i>	1149	1198	Light responsive element
<i>GmTPS9</i>	1094	1143	Defense and stress responsive element
<i>GmTPS9</i>	1217	1264	Gibberellin responsive element
<i>GmTPS9</i>	559	607	Light responsive element
<i>GmTPS9</i>	46	94	Light responsive element
<i>GmTPS9</i>	142	191	Light responsive element
<i>GmTPS9</i>	1147	1196	Light responsive element
<i>GmTPS9</i>	1937	1984	Light responsive element
<i>GmTPS9</i>	1956	2006	Light responsive element
<i>GmTPS9</i>	515	561	Light responsive element
<i>GmTPS12</i>	801	850	Light responsive element
<i>GmTPS12</i>	1498	1547	Light responsive element
<i>GmTPS12</i>	484	530	Light responsive element
<i>GmTPS12</i>	492	538	Light responsive element
<i>GmTPS12</i>	654	700	Light responsive element
<i>GmTPS12</i>	696	742	Light responsive element
<i>GmTPS12</i>	370	416	Light responsive element
<i>GmTPS12</i>	487	537.5	Light responsive element
<i>GmTPS12</i>	489	540	Light responsive element
<i>GmTPS12</i>	490	539	Light responsive element
<i>GmTPS12</i>	492	538	Light responsive element

<i>GmTPS12</i>	652	701	Light responsive element
<i>GmTPS12</i>	654	700	Light responsive element
<i>GmTPS12</i>	369	414	MeJA-responsive element
<i>GmTPS12</i>	614	659	MeJA-responsive element
<i>GmTPS12</i>	780	825	MeJA-responsive element
<i>GmTPS12</i>	1618	1665	Light responsive element
<i>GmTPS12</i>	398	444	Drought-inducibility element
<i>GmTPS12</i>	610	656	Drought-inducibility element
<i>GmTPS12</i>	904	951	Gibberellin responsive element
<i>GmTPS12</i>	1511	1558	Gibberellin responsive element
<i>GmTPS12</i>	1622	1669	Gibberellin responsive element
<i>GmTPS12</i>	1788	1835	Gibberellin responsive element
<i>GmTPS12</i>	371	416	Abscisic acid responsive element
<i>GmTPS12</i>	484	529	Abscisic acid responsive element
<i>GmTPS12</i>	490	539	Abscisic acid responsive element
<i>GmTPS12</i>	492	538	Abscisic acid responsive element
<i>GmTPS12</i>	493	538	Abscisic acid responsive element
<i>GmTPS12</i>	652	701	Abscisic acid responsive element
<i>GmTPS12</i>	654	700	Abscisic acid responsive element
<i>GmTPS12</i>	655	700	Abscisic acid responsive element
<i>GmTPS12</i>	694	743	Abscisic acid responsive element
<i>GmTPS12</i>	697	742	Abscisic acid responsive element
<i>GmTPS12</i>	803	851	Light responsive element
<i>GmTPS12</i>	1730	1779	Light responsive element
<i>GmTPS12</i>	1732	1780	Light responsive element
<i>GmTPS12</i>	369	414	MeJA-responsive element
<i>GmTPS12</i>	614	659	MeJA-responsive element
<i>GmTPS12</i>	780	825	MeJA-responsive element
<i>GmTPS13</i>	1887	1934	Light responsive element
<i>GmTPS13</i>	182	231	Salicylic acid responsive element
<i>GmTPS13</i>	561	607	Low-temperature responsive element
<i>GmTPS13</i>	1195	1241	Low-temperature responsive element
<i>GmTPS13</i>	1382	1431	Defense and stress responsive element
<i>GmTPS13</i>	1503	1552	Defense and stress responsive element
<i>GmTPS13</i>	1096	1141	Abscisic acid responsive element
<i>GmTPS13</i>	1298	1343	Abscisic acid responsive element
<i>GmTPS13</i>	392	442	Light responsive element
<i>GmTPS13</i>	727	773	Drought-inducibility element
<i>GmTPS13</i>	1154	1200	Drought-inducibility element
<i>GmTPS13</i>	193	241	Light responsive element
<i>GmTPS13</i>	912	958	Auxin responsive element
<i>GmTPS13</i>	1507	1553	Light responsive element
<i>GmTPS13</i>	1602	1648	Light responsive element
<i>GmTPS13</i>	1095	1141	Light responsive element
<i>GmTPS13</i>	1297	1343	Light responsive element
<i>GmTPS13</i>	410	461	flavonoid biosynthetic genes regulation element
<i>GmTPS14</i>	149	197	Light responsive element
<i>GmTPS14</i>	150	196	Light responsive element
<i>GmTPS14</i>	150	196	Light responsive element
<i>GmTPS14</i>	1737	1784	Gibberellin responsive element
<i>GmTPS14</i>	1076	1125	Wound-responsive element
<i>GmTPS14</i>	385	433	Light responsive element
<i>GmTPS14</i>	1919	1964	MeJA-responsive element
<i>GmTPS14</i>	1690	1739	Light responsive element

<i>GmTPS14</i>	511	558	Endosperm expression element
<i>GmTPS14</i>	1054	1103	Defense and stress responsive element
<i>GmTPS14</i>	43	90	Abscisic acid responsive element
<i>GmTPS14</i>	150	196	Abscisic acid responsive element
<i>GmTPS14</i>	151	196	Abscisic acid responsive element
<i>GmTPS14</i>	1403	1449	Low-temperature responsive element
<i>GmTPS14</i>	1919	1964	MeJA-responsive element
<i>GmTPS14</i>	303	349	Light responsive element
<i>GmTPS14</i>	434	480	Light responsive element
<i>GmTPS14</i>	1276	1322	Light responsive element
<i>GmTPS4</i>	26	72	Light responsive element
<i>GmTPS4</i>	1898	1944	Light responsive element
<i>GmTPS4</i>	1322	1371	Light responsive element
<i>GmTPS4</i>	1316	1366	Light responsive element
<i>GmTPS4</i>	1896	1946	Light responsive element
<i>GmTPS4</i>	625	671	Light responsive element
<i>GmTPS4</i>	1728	1774	Light responsive element
<i>GmTPS4</i>	285	331	Light responsive element
<i>GmTPS4</i>	1029	1078	Light responsive element
<i>GmTPS4</i>	794	842	Seed-specific regulation element
<i>GmTPS4</i>	1742	1790	Seed-specific regulation element
<i>GmTPS4</i>	48	94	Light responsive element
<i>GmTPS4</i>	81	127	Light responsive element
<i>GmTPS4</i>	350	396	Light responsive element
<i>GmTPS4</i>	832	878	Light responsive element
<i>GmTPS4</i>	860	906	Light responsive element
<i>GmTPS4</i>	1	50	Salicylic acid responsive element
<i>GmTPS4</i>	217	263	Drought-inducibility element
<i>GmTPS4</i>	838	887	Light responsive element
<i>GmTPS4</i>	866	915	Light responsive element
<i>GmTPS4</i>	625	670	Abscisic acid responsive element
<i>GmTPS4</i>	1729	1774	Abscisic acid responsive element
<i>GmTPS4</i>	1002	1049	Gibberellin responsive element
<i>GmTPS4</i>	1903	1950	Gibberellin responsive element
<i>GmTPS15</i>	1530	1576	Light responsive element
<i>GmTPS15</i>	488	539	Endosperm expression element
<i>GmTPS15</i>	611	660	Light responsive element
<i>GmTPS15</i>	807	854	Light responsive element
<i>GmTPS15</i>	919	965	Light responsive element
<i>GmTPS15</i>	72	118	Light responsive element
<i>GmTPS15</i>	1650	1696	Light responsive element
<i>GmTPS15</i>	217	266	Salicylic acid responsive element
<i>GmTPS15</i>	1210	1256	Light responsive element
<i>GmTPS15</i>	1887	1934	Light responsive element
<i>GmTPS15</i>	228	276	Light responsive element
<i>GmTPS15</i>	774	820	Drought-inducibility element
<i>GmTPS15</i>	978	1024	Drought-inducibility element
<i>GmTPS15</i>	1189	1235	Drought-inducibility element
<i>GmTPS15</i>	1560	1606	Drought-inducibility element
<i>GmTPS15</i>	250	296	Low-temperature responsive element
<i>GmTPS15</i>	1225	1271	Low-temperature responsive element
<i>GmTPS15</i>	1411	1460	Defense and stress responsive element
<i>GmTPS15</i>	1526	1575	Defense and stress responsive element
<i>GmTPS15</i>	920	965	Abscisic acid responsive element

<i>GmTPS16</i>	1868	1915	Light responsive element
<i>GmTPS16</i>	722	771	Light responsive element
<i>GmTPS16</i>	601	646	MeJA-responsive element
<i>GmTPS16</i>	785	830	MeJA-responsive element
<i>GmTPS16</i>	875	920	MeJA-responsive element
<i>GmTPS16</i>	1531	1576	MeJA-responsive element
<i>GmTPS16</i>	366	419	MeJA-responsive element
<i>GmTPS16</i>	947	1000	MeJA-responsive element
<i>GmTPS16</i>	158	204	MeJA-responsive element
<i>GmTPS16</i>	1254	1301	MeJA-responsive element
<i>GmTPS16</i>	645	691	Light responsive element
<i>GmTPS16</i>	1407	1453	Light responsive element
<i>GmTPS16</i>	1824	1870	Light responsive element
<i>GmTPS16</i>	645	691	Light responsive element
<i>GmTPS16</i>	1824	1870	Light responsive element
<i>GmTPS16</i>	75	121	Light responsive element
<i>GmTPS16</i>	1069	1115	Light responsive element
<i>GmTPS16</i>	1078	1124	Light responsive element
<i>GmTPS16</i>	1105	1151	Light responsive element
<i>GmTPS16</i>	1321	1367	Light responsive element
<i>GmTPS16</i>	601	646	MeJA-responsive element
<i>GmTPS16</i>	785	830	MeJA-responsive element
<i>GmTPS16</i>	875	920	MeJA-responsive element
<i>GmTPS16</i>	1531	1576	MeJA-responsive element
<i>GmTPS16</i>	1544	1593	Light responsive element
<i>GmTPS16</i>	1524	1571	Gibberellin responsive element
<i>GmTPS16</i>	1916	1963	Gibberellin responsive element
<i>GmTPS16</i>	645	691	Abscisic acid responsive element
<i>GmTPS16</i>	646	691	Abscisic acid responsive element
<i>GmTPS16</i>	1407	1452	Abscisic acid responsive element
<i>GmTPS16</i>	1824	1870	Abscisic acid responsive element
<i>GmTPS16</i>	1825	1870	Abscisic acid responsive element
<i>GmTPS16</i>	506	555	Light responsive element
<i>GmTPS16</i>	720	769	Light responsive element
<i>GmTPS16</i>	722	772	Light responsive element
<i>GmTPS6</i>	336	382	Light responsive element
<i>GmTPS6</i>	489	539	Light responsive element
<i>GmTPS6</i>	491	537	Light responsive element
<i>GmTPS6</i>	645	696	Light responsive element
<i>GmTPS6</i>	646	695	Light responsive element
<i>GmTPS6</i>	648	694	Light responsive element
<i>GmTPS6</i>	489	539	Light responsive element
<i>GmTPS6</i>	491	537	Light responsive element
<i>GmTPS6</i>	648	694	Light responsive element
<i>GmTPS6</i>	689	735	Light responsive element
<i>GmTPS6</i>	490	539	Light responsive element
<i>GmTPS6</i>	1494	1543	Light responsive element
<i>GmTPS6</i>	791	840	Light responsive element
<i>GmTPS6</i>	1616	1663	Light responsive element
<i>GmTPS6</i>	335	380	MeJA-responsive element
<i>GmTPS6</i>	608	653	MeJA-responsive element
<i>GmTPS6</i>	770	815	MeJA-responsive element
<i>GmTPS6</i>	1283	1331	Light responsive element
<i>GmTPS6</i>	974	1021	Endosperm expression element

<i>GmTPS6</i>	793	841	Light responsive element
<i>GmTPS6</i>	1728	1777	Light responsive element
<i>GmTPS6</i>	1730	1778	Light responsive element
<i>GmTPS6</i>	1507	1554	Gibberellin responsive element
<i>GmTPS6</i>	1620	1667	Gibberellin responsive element
<i>GmTPS6</i>	337	382	Abscisic acid responsive element
<i>GmTPS6</i>	491	537	Abscisic acid responsive element
<i>GmTPS6</i>	492	537	Abscisic acid responsive element
<i>GmTPS6</i>	646	695	Abscisic acid responsive element
<i>GmTPS6</i>	648	694	Abscisic acid responsive element
<i>GmTPS6</i>	649	694	Abscisic acid responsive element
<i>GmTPS6</i>	687	736	Abscisic acid responsive element
<i>GmTPS6</i>	690	735	Abscisic acid responsive element
<i>GmTPS6</i>	335	380	MeJA-responsive element
<i>GmTPS6</i>	608	653	MeJA-responsive element
<i>GmTPS6</i>	770	815	MeJA-responsive element
<i>GmTPS6</i>	1771	1817	Light responsive element
<i>GmTPS6</i>	1773	1819	Light responsive element
<i>GmTPS6</i>	1833	1879	Light responsive element
<i>GmTPS6</i>	203	249	Light responsive element
<i>GmTPS3</i>	329	375	Light responsive element
<i>GmTPS3</i>	766	812	Light responsive element
<i>GmTPS3</i>	794	840	Light responsive element
<i>GmTPS3</i>	1169	1215	Light responsive element
<i>GmTPS3</i>	728	776	Seed-specific regulation element
<i>GmTPS3</i>	1720	1768	Seed-specific regulation element
<i>GmTPS3</i>	963	1012	Light responsive element
<i>GmTPS3</i>	1217	1265	Light responsive element
<i>GmTPS3</i>	1201	1247	Abscisic acid responsive element
<i>GmTPS3</i>	1202	1247	Abscisic acid responsive element
<i>GmTPS3</i>	1707	1752	Abscisic acid responsive element
<i>GmTPS3</i>	1827	1873	Abscisic acid responsive element
<i>GmTPS3</i>	1828	1873	Abscisic acid responsive element
<i>GmTPS3</i>	936	983	Gibberellin responsive element
<i>GmTPS3</i>	1903	1950	Gibberellin responsive element
<i>GmTPS3</i>	537	585	Light responsive element
<i>GmTPS3</i>	87	137	Light responsive element
<i>GmTPS3</i>	772	821	Light responsive element
<i>GmTPS3</i>	800	849	Light responsive element
<i>GmTPS3</i>	464	510	Drought-inducibility element
<i>GmTPS3</i>	1668	1719	Auxin responsive element
<i>GmTPS3</i>	93	139	Light responsive element
<i>GmTPS3</i>	1629	1675	Light responsive element
<i>GmTPS3</i>	1201	1247	Light responsive element
<i>GmTPS3</i>	1827	1873	Light responsive element
<i>GmTPS3</i>	1201	1247	Light responsive element
<i>GmTPS3</i>	1706	1752	Light responsive element
<i>GmTPS3</i>	1827	1873	Light responsive element
<i>GmTPS3</i>	1286	1335	Light responsive element
<i>GmTPS3</i>	1217	1266	Light responsive element
<i>GmTPS3</i>	1280	1330	Light responsive element
<i>GmTPS3</i>	185	236	flavonoid biosynthetic genes regulation element
<i>GmTPS17</i>	925	975	Light responsive element
<i>GmTPS17</i>	280	331	tochrome down-regulation expression element

<i>GmTPS17</i>	189	235	Light responsive element
<i>GmTPS17</i>	720	766	Light responsive element
<i>GmTPS17</i>	1523	1569	Light responsive element
<i>GmTPS17</i>	1408	1453	MeJA-responsive element
<i>GmTPS17</i>	1527	1572	MeJA-responsive element
<i>GmTPS17</i>	1117	1164	MeJA-responsive element
<i>GmTPS17</i>	174	220	MeJA-responsive element
<i>GmTPS17</i>	183	229	MeJA-responsive element
<i>GmTPS17</i>	919	967	MeJA-responsive element
<i>GmTPS17</i>	1451	1497	MeJA-responsive element
<i>GmTPS17</i>	190	235	MeJA-responsive element
<i>GmTPS17</i>	720	765	MeJA-responsive element
<i>GmTPS17</i>	940	990	MeJA-responsive element
<i>GmTPS17</i>	941	990	MeJA-responsive element
<i>GmTPS17</i>	301	350	MeJA-responsive element
<i>GmTPS17</i>	1167	1213	MeJA-responsive element
<i>GmTPS17</i>	744	793	MeJA-responsive element
<i>GmTPS17</i>	1408	1453	MeJA-responsive element
<i>GmTPS17</i>	1527	1572	MeJA-responsive element
<i>GmTPS18</i>	870	920	Light responsive element
<i>GmTPS18</i>	857	902	MeJA-responsive element
<i>GmTPS18</i>	623	670	Light responsive element
<i>GmTPS18</i>	624	670	Light responsive element
<i>GmTPS18</i>	915	961	Light responsive element
<i>GmTPS18</i>	1775	1822	Auxin responsive element
<i>GmTPS18</i>	334	380	Light responsive element
<i>GmTPS18</i>	556	602	Light responsive element
<i>GmTPS18</i>	602	648	Light responsive element
<i>GmTPS18</i>	676	722	Light responsive element
<i>GmTPS18</i>	1509	1555	Light responsive element
<i>GmTPS18</i>	1920	1966	Light responsive element
<i>GmTPS18</i>	483	532	Defense and stress responsive element
<i>GmTPS18</i>	1590	1635	Abscisic acid responsive element
<i>GmTPS18</i>	1127	1173	Drought-inducibility element
<i>GmTPS18</i>	1045	1091	Auxin responsive element
<i>GmTPS18</i>	1497	1547	Light responsive element
<i>GmTPS18</i>	1682	1729	Light responsive element
<i>GmTPS18</i>	447	493	Light responsive element
<i>GmTPS18</i>	1107	1153	Light responsive element
<i>GmTPS18</i>	649	697	Light responsive element
<i>GmTPS18</i>	1288	1336	Light responsive element
<i>GmTPS18</i>	857	902	MeJA-responsive element
<i>GmTPS18</i>	709	756	Light responsive element
<i>GmTPS18</i>	1799	1846	Light responsive element
<i>GmTPS18</i>	23	69	Light responsive element
<i>GmTPS18</i>	167	213	Light responsive element
<i>GmTPS18</i>	1589	1635	Light responsive element
<i>GmTPS7</i>	45	90	Abscisic acid responsive element
<i>GmTPS7</i>	71	116	Abscisic acid responsive element
<i>GmTPS7</i>	668	713	Abscisic acid responsive element
<i>GmTPS7</i>	685	731	Abscisic acid responsive element
<i>GmTPS7</i>	686	731	Abscisic acid responsive element
<i>GmTPS7</i>	1827	1873	Abscisic acid responsive element
<i>GmTPS7</i>	1828	1873	Abscisic acid responsive element

<i>GmTPS7</i>	1533	1580	Gibberellin responsive element
<i>GmTPS7</i>	760	809	Light responsive element
<i>GmTPS7</i>	762	812	Light responsive element
<i>GmTPS7</i>	1319	1366	Light responsive element
<i>GmTPS7</i>	73	118	MeJA-responsive element
<i>GmTPS7</i>	643	688	MeJA-responsive element
<i>GmTPS7</i>	825	870	MeJA-responsive element
<i>GmTPS7</i>	917	962	MeJA-responsive element
<i>GmTPS7</i>	1540	1585	MeJA-responsive element
<i>GmTPS7</i>	1780	1825	MeJA-responsive element
<i>GmTPS7</i>	252	298	Light responsive element
<i>GmTPS7</i>	1097	1143	Light responsive element
<i>GmTPS7</i>	1106	1152	Light responsive element
<i>GmTPS7</i>	1136	1182	Light responsive element
<i>GmTPS7</i>	1332	1378	Light responsive element
<i>GmTPS7</i>	1891	1937	Light responsive element
<i>GmTPS7</i>	229	277	Light responsive element
<i>GmTPS7</i>	1436	1483	Gibberellin responsive element
<i>GmTPS7</i>	44	90	Light responsive element
<i>GmTPS7</i>	71	117	Light responsive element
<i>GmTPS7</i>	666	715	Light responsive element
<i>GmTPS7</i>	667	713	Light responsive element
<i>GmTPS7</i>	685	731	Light responsive element
<i>GmTPS7</i>	1827	1873	Light responsive element
<i>GmTPS7</i>	685	731	Light responsive element
<i>GmTPS7</i>	1827	1873	Light responsive element
<i>GmTPS7</i>	73	118	MeJA-responsive element
<i>GmTPS7</i>	643	688	MeJA-responsive element
<i>GmTPS7</i>	825	870	MeJA-responsive element
<i>GmTPS7</i>	917	962	MeJA-responsive element
<i>GmTPS7</i>	1540	1585	MeJA-responsive element
<i>GmTPS7</i>	1780	1825	MeJA-responsive element
<i>GmTPS7</i>	1228	1276	Light responsive element
<i>GmTPS7</i>	673	720	Light responsive element
<i>GmTPS7</i>	1867	1914	Light responsive element
<i>GmTPS7</i>	762	811	Light responsive element
<i>GmTPS2</i>	316	365	Light responsive element
<i>GmTPS2</i>	664	713	Light responsive element
<i>GmTPS2</i>	665	715	Light responsive element
<i>GmTPS2</i>	138	188	Light responsive element
<i>GmTPS2</i>	155	201	Light responsive element
<i>GmTPS2</i>	1013	1059	Light responsive element
<i>GmTPS2</i>	1043	1089	Light responsive element
<i>GmTPS2</i>	1057	1103	Light responsive element
<i>GmTPS2</i>	173	219	Light responsive element
<i>GmTPS2</i>	191	237	Light responsive element
<i>GmTPS2</i>	212	258	Light responsive element
<i>GmTPS2</i>	800	846	Light responsive element
<i>GmTPS2</i>	1007	1053	Light responsive element
<i>GmTPS2</i>	1011	1060	Light responsive element
<i>GmTPS2</i>	1013	1059	Light responsive element
<i>GmTPS2</i>	1025	1071	Light responsive element
<i>GmTPS2</i>	1041	1090	Light responsive element
<i>GmTPS2</i>	1055	1104	Light responsive element

<i>GmTPS2</i>	1057	1103	Light responsive element
<i>GmTPS2</i>	498	547.5	Light responsive element
<i>GmTPS2</i>	172	217	MeJA-responsive element
<i>GmTPS2</i>	214	259	MeJA-responsive element
<i>GmTPS2</i>	654	699	MeJA-responsive element
<i>GmTPS2</i>	799	844	MeJA-responsive element
<i>GmTPS2</i>	985	1030	MeJA-responsive element
<i>GmTPS2</i>	1027	1072	MeJA-responsive element
<i>GmTPS2</i>	115	161	Auxin responsive element
<i>GmTPS2</i>	1083	1129	Auxin responsive element
<i>GmTPS2</i>	242	290	Light responsive element
<i>GmTPS2</i>	1728	1774	Drought-inducibility element
<i>GmTPS2</i>	585	631	Low-temperature responsive element
<i>GmTPS2</i>	314	364	Light responsive element
<i>GmTPS2</i>	316	364	Light responsive element
<i>GmTPS2</i>	665	715	Light responsive element
<i>GmTPS2</i>	666	714	Light responsive element
<i>GmTPS2</i>	1526	1573	Gibberellin responsive element
<i>GmTPS2</i>	156	201	Abscisic acid responsive element
<i>GmTPS2</i>	174	219	Abscisic acid responsive element
<i>GmTPS2</i>	192	237	Abscisic acid responsive element
<i>GmTPS2</i>	212	257	Abscisic acid responsive element
<i>GmTPS2</i>	801	846	Abscisic acid responsive element
<i>GmTPS2</i>	1007	1052	Abscisic acid responsive element
<i>GmTPS2</i>	1011	1060	Abscisic acid responsive element
<i>GmTPS2</i>	1013	1059	Abscisic acid responsive element
<i>GmTPS2</i>	1014	1059	Abscisic acid responsive element
<i>GmTPS2</i>	1025	1070	Abscisic acid responsive element
<i>GmTPS2</i>	1043	1088	Abscisic acid responsive element
<i>GmTPS2</i>	1057	1103	Abscisic acid responsive element
<i>GmTPS2</i>	1058	1103	Abscisic acid responsive element
<i>GmTPS2</i>	1649	1695	Light responsive element
<i>GmTPS2</i>	172	217	MeJA-responsive element
<i>GmTPS2</i>	214	259	MeJA-responsive element
<i>GmTPS2</i>	654	699	MeJA-responsive element
<i>GmTPS2</i>	799	844	MeJA-responsive element
<i>GmTPS2</i>	985	1030	MeJA-responsive element
<i>GmTPS2</i>	1027	1072	MeJA-responsive element
<i>GmTPS2</i>	1071	1118	Light responsive element
<i>GmTPS2</i>	1072	1118	Light responsive element
<i>GmTPS19</i>	1081	1129	Light responsive element
<i>GmTPS19</i>	555	602	Gibberellin responsive element
<i>GmTPS19</i>	617	664	Gibberellin responsive element
<i>GmTPS19</i>	68	114	Light responsive element
<i>GmTPS19</i>	468	514	Light responsive element
<i>GmTPS19</i>	744	790	Light responsive element
<i>GmTPS19</i>	773	819	Light responsive element
<i>GmTPS19</i>	950	996	Light responsive element
<i>GmTPS19</i>	990	1039	Light responsive element
<i>GmTPS19</i>	967	1017	Root specific element
<i>GmTPS19</i>	329	375	Light responsive element
<i>GmTPS19</i>	835	884	Light responsive element
<i>GmTPS19</i>	989	1039	Light responsive element
<i>GmTPS19</i>	991	1037	Light responsive element

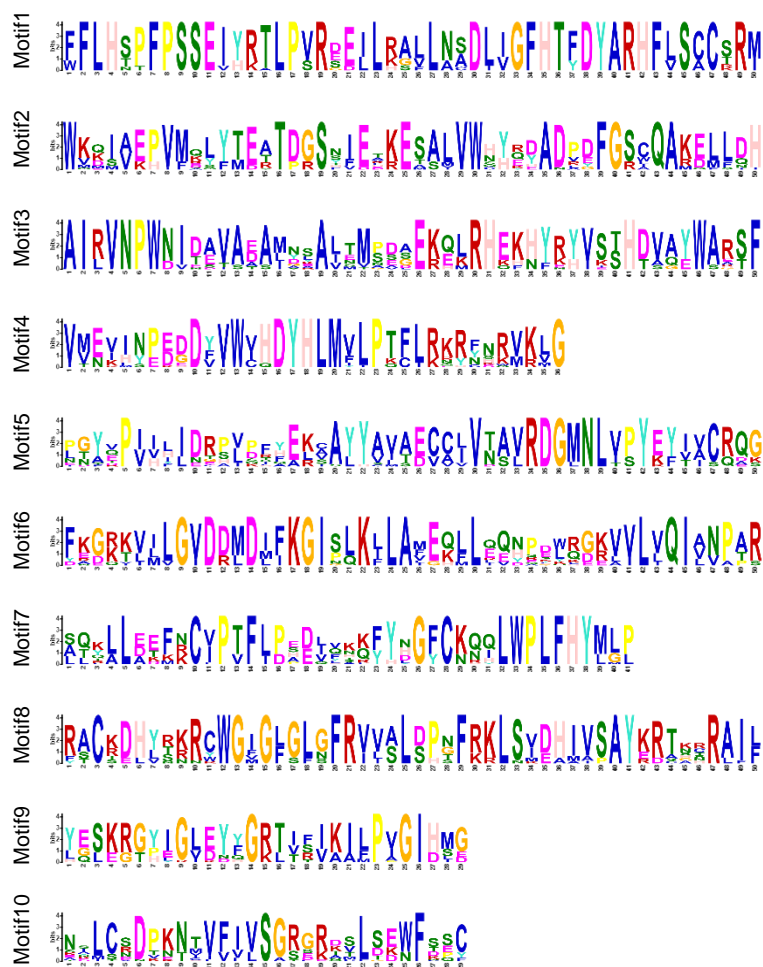
<i>GmTPS19</i>	1691	1737	Light responsive element
<i>GmTPS19</i>	837	883	Light responsive element
<i>GmTPS19</i>	970	1016	Light responsive element
<i>GmTPS19</i>	991	1037	Light responsive element
<i>GmTPS19</i>	135	181	Light responsive element
<i>GmTPS19</i>	139	185	Light responsive element
<i>GmTPS19</i>	172	218	Light responsive element
<i>GmTPS19</i>	196	242	Light responsive element
<i>GmTPS19</i>	640	686	Light responsive element
<i>GmTPS19</i>	307	355	Light responsive element
<i>GmTPS19</i>	1216	1264	Light responsive element
<i>GmTPS19</i>	838	883	Abscisic acid responsive element
<i>GmTPS19</i>	970	1015	Abscisic acid responsive element
<i>GmTPS19</i>	991	1037	Abscisic acid responsive element
<i>GmTPS19</i>	992	1037	Abscisic acid responsive element
<i>GmTPS19</i>	1691	1736	Abscisic acid responsive element
<i>GmTPS19</i>	68	117	Defense and stress responsive element
<i>GmTPS19</i>	277	326	Defense and stress responsive element
<i>GmTPS20</i>	1527	1573	Light responsive element
<i>GmTPS20</i>	379	432	Light responsive element
<i>GmTPS20</i>	1039	1085	Light responsive element
<i>GmTPS20</i>	320	366	Light responsive element
<i>GmTPS20</i>	369	415	Light responsive element
<i>GmTPS20</i>	373	419	Light responsive element
<i>GmTPS20</i>	429	475	Light responsive element
<i>GmTPS20</i>	580	626	Light responsive element
<i>GmTPS20</i>	911	957	Light responsive element
<i>GmTPS20</i>	938	984	Light responsive element
<i>GmTPS20</i>	968	1014	Light responsive element
<i>GmTPS20</i>	1062	1108	Light responsive element
<i>GmTPS20</i>	1465	1512	Gibberellin responsive element
<i>GmTPS20</i>	1039	1084	Abscisic acid responsive element
<i>GmTPS20</i>	816	864	Light responsive element
<i>GmTPS1</i>	1281	1329	Light responsive element
<i>GmTPS1</i>	1703	1751	Light responsive element
<i>GmTPS1</i>	1116	1165	Circadian control element
<i>GmTPS1</i>	651	697	Auxin responsive element
<i>GmTPS1</i>	478	526	Light responsive element
<i>GmTPS1</i>	441	491	Light responsive element
<i>GmTPS1</i>	1143	1190	Gibberellin responsive element
<i>GmTPS1</i>	1808	1855	Light responsive element
<i>GmTPS1</i>	22	71	Salicylic acid responsive element
<i>GmTPS1</i>	1860	1906	Drought-inducibility element
<i>GmTPS1</i>	1682	1731	Defense and stress responsive element
<i>GmTPS1</i>	113	165	Gibberellin responsive element
<i>GmTPS1</i>	188	234	Low-temperature responsive element
<i>GmTPS11</i>	300	346	Light responsive element
<i>GmTPS11</i>	395	441	Light responsive element
<i>GmTPS11</i>	416	462	Light responsive element
<i>GmTPS11</i>	1054	1100	Light responsive element
<i>GmTPS11</i>	1320	1366	Light responsive element
<i>GmTPS11</i>	1445	1491	Light responsive element
<i>GmTPS11</i>	1690	1736	Light responsive element
<i>GmTPS11</i>	414	464	Light responsive element

<i>GmTPS11</i>	416	462	Light responsive element
<i>GmTPS11</i>	1445	1491	Light responsive element
<i>GmTPS11</i>	159	210	tochrome down-regulation expression element
<i>GmTPS11</i>	1511	1560	Light responsive element
<i>GmTPS11</i>	1595	1644	Light responsive element
<i>GmTPS11</i>	415	464	Light responsive element
<i>GmTPS11</i>	392	442	Root specific element
<i>GmTPS11</i>	1617	1664	Light responsive element
<i>GmTPS11</i>	33	81	Light responsive element
<i>GmTPS11</i>	1511	1560	Light responsive element
<i>GmTPS11</i>	328	374	Light responsive element
<i>GmTPS11</i>	375	421	Light responsive element
<i>GmTPS11</i>	1425	1471	Light responsive element
<i>GmTPS11</i>	1248	1301	Light responsive element
<i>GmTPS11</i>	131	179	Light responsive element
<i>GmTPS11</i>	737	785	Light responsive element
<i>GmTPS11</i>	1275	1323	Light responsive element
<i>GmTPS11</i>	1968	2016	Light responsive element
<i>GmTPS11</i>	214	260	Low-temperature responsive element
<i>GmTPS11</i>	301	346	Abscisic acid responsive element
<i>GmTPS11</i>	395	440	Abscisic acid responsive element
<i>GmTPS11</i>	416	462	Abscisic acid responsive element
<i>GmTPS11</i>	417	462	Abscisic acid responsive element
<i>GmTPS11</i>	1054	1099	Abscisic acid responsive element
<i>GmTPS11</i>	1321	1366	Abscisic acid responsive element
<i>GmTPS11</i>	1445	1491	Abscisic acid responsive element
<i>GmTPS11</i>	1446	1491	Abscisic acid responsive element
<i>GmTPS11</i>	1691	1736	Abscisic acid responsive element
<i>GmTPS11</i>	920	968	Light responsive element
<i>GmTPS11</i>	1268	1314	Drought-inducibility element
<i>GmTPS11</i>	57	103	Light responsive element
<i>GmTPS11</i>	472	518	Light responsive element
<i>GmTPS11</i>	626	672	Light responsive element
<i>GmTPS11</i>	642	688	Light responsive element
<i>GmTPS11</i>	947	993	Light responsive element
<i>GmTPS11</i>	1122	1168	Light responsive element
<i>GmTPS11</i>	1363	1409	Light responsive element
<i>GmTPS10</i>	64	110	Light responsive element
<i>GmTPS10</i>	297	343	Light responsive element
<i>GmTPS10</i>	291	337	Auxin responsive element
<i>GmTPS10</i>	1476	1523	Light responsive element
<i>GmTPS10</i>	1293	1339	Light responsive element
<i>GmTPS10</i>	90	138	Light responsive element
<i>GmTPS10</i>	447	495	Light responsive element
<i>GmTPS10</i>	1556	1603	Light responsive element
<i>GmTPS10</i>	263	309	Low-temperature responsive element
<i>GmTPS10</i>	1013	1063	Light responsive element
<i>GmTPS10</i>	1014	1063	Light responsive element
<i>GmTPS10</i>	298	343	Abscisic acid responsive element
<i>GmTPS10</i>	233	282	Defense and stress responsive element
<i>GmTPS10</i>	997	1045	Light responsive element
<i>GmTPS10</i>	942	990	Light responsive element
<i>GmTPS10</i>	828	877	Light responsive element
<i>GmTPS10</i>	1553	1600	Light responsive element

<i>GmTPS10</i>	1554	1600	Light responsive element
<i>GmTPS10</i>	544	590	Light responsive element
<i>GmTPS10</i>	1712	1758	Light responsive element
<i>GmTPS10</i>	1722	1768	Light responsive element
<i>GmTPS10</i>	280	329	Salicylic acid responsive element
<i>GmTPS10</i>	1037	1086	Salicylic acid responsive element
<i>GmTPS5</i>	1059	1105	Auxin responsive element
<i>GmTPS5</i>	1683	1730	Light responsive element
<i>GmTPS5</i>	1498	1548	Light responsive element
<i>GmTPS5</i>	1302	1350	Light responsive element
<i>GmTPS5</i>	850	895	MeJA-responsive element
<i>GmTPS5</i>	405	451	Light responsive element
<i>GmTPS5</i>	1112	1158	Light responsive element
<i>GmTPS5</i>	696	743	Light responsive element
<i>GmTPS5</i>	1800	1847	Light responsive element
<i>GmTPS5</i>	611	658	Light responsive element
<i>GmTPS5</i>	612	658	Light responsive element
<i>GmTPS5</i>	908	954	Light responsive element
<i>GmTPS5</i>	1776	1823	Auxin responsive element
<i>GmTPS5</i>	850	895	MeJA-responsive element
<i>GmTPS5</i>	590	636	Light responsive element
<i>GmTPS5</i>	664	710	Light responsive element
<i>GmTPS5</i>	1510	1556	Light responsive element
<i>GmTPS5</i>	1920	1966	Light responsive element
<i>GmTPS5</i>	472	521	Defense and stress responsive element
<i>GmTPS5</i>	26	73	Abscisic acid responsive element
<i>GmTPS5</i>	1449	1498	Abscisic acid responsive element
<i>GmTPS5</i>	1491	1538	Gibberellin responsive element
<i>GmTPS5</i>	932	978	Low-temperature responsive element
<i>GmTPS5</i>	1132	1178	Drought-inducibility element

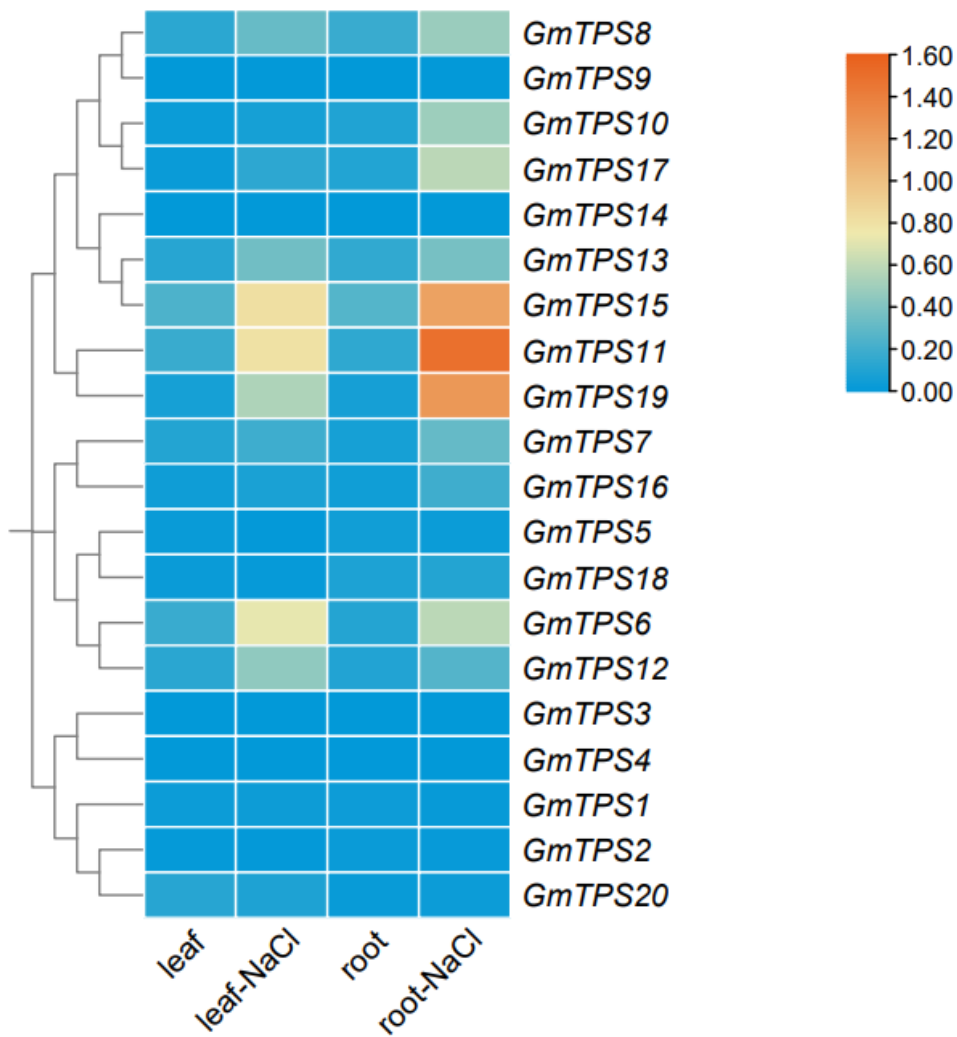
附表3 *TPS* 基因的qRT-PCR引物Appendix table 3 The primer sequences of *TPS* genes used for qRT-PCR in this study

基因名称 Gene name	正向引物 Forward primer (5'-3')	反向引物 Reverse primer (5'-3')
<i>GmTPS1</i>	CGAGCTGTTGGTGTACAAAGGG	GGTGATGTCATGGACTTACTGTGC
<i>GmTPS2</i>	CGTGCACTTGACCTTCCTCAAGTC	GAACAACCTTTATCATGCCAACCAG
<i>GmTPS3</i>	GGATTCCATACCTATGATTATGCC	CTTCAGGGAGTTCAAGGGCTTGAG
<i>GmTPS4</i>	GCCAAAGGCAAGATGAGAGGC	TGCAGTAGCCATTATAGTACTGG
<i>GmTPS5</i>	GGGAAACTTGTGTTCCAGTACCC	CTCACACCCTGAGGTTTTACTTCC
<i>GmTPS6</i>	ACACGCCTACTTTCTGCCATGC	TGCAAATACCTCGGCCTTTGGAG
<i>GmTPS7</i>	ACTTCTTGAGATGGTCCCACAATC	GAACCATCAGTCGCCTCTGTATAG
<i>GmTPS8</i>	ATCATCAATATGCAGACCCCGAC	GAAAGAACCTTTTCAGCTACCCAG
<i>GmTPS9</i>	GCTTTGGTCTGGCATCATCAAG	GTGGATTGCCATCATTAAACCATAG
<i>GmTPS10</i>	TCATTGTTAGTGGGAGGGGGAG	GTTGCTTCTGTATACAACCTGCATC
<i>GmTPS11</i>	AACAATAAACAAGACTCCAAGCAG	CATTTTCCATTCAAAATCAGTCGC
<i>GmTPS12</i>	TAACGGTCAAGAGTGGCCAGAG	AGTCCCTTGCTAACACCCTGTG
<i>GmTPS13</i>	CATTGTAAAGCACCCAGTCCC	GTCTCTACAGAGGAACCGTCTGTG
<i>GmTPS14</i>	TGGGAAATGAACCATAACAAGTACC	CCTTTAGTAATCCCCAGTGACTTG
<i>GmTPS15</i>	GTGACGCCGATCCTGATTTTC	ATTTGTGGTGCTGAGGATGAGG
<i>GmTPS16</i>	GAGATGGTCTCACGGTGGAGAATG	GCCATCAGTCGCCTCTGTATATTG
<i>GmTPS17</i>	CCGTCTTAAATGCTCTATGTAACG	TTTTCCAATCAAGGTCTGCAGAC
<i>GmTPS18</i>	TTCATTCTGTTTATTATCTTTTGG	CCTTGAAACCATTTTGTATGATGGC
<i>GmTPS19</i>	CACGGTTACTTCACTAGGTGGAC	CTATATGTTGTCCCCTTATAACCC
<i>GmTPS20</i>	TCGCATCAATGGCAGATTTGGAG	AAATCAAGAGAGCGATCCAGGTG



附图 1 10个TPS Motif的组成

Appendix figure 1 The composition of 10 TPS motifs



附图 2 盐处理下 *TPS* 基因的表达变化

Appendix figure 2 The expression changes of *TPS* genes under salt treatment

A

Wm82	W/L/C	TPS1-Haplotype				
	25/43/35	H1	T	C	A	C
	3/0/0	H2	G	T	A	C
	0/1/0	H3	G	C	G	C
	83/149/154	H4	G	C	A	C
	3/8/23	H5	G	C	A	T
CDS nt #in Wm82		123	1672	1903	2521	
AA # in Wm82		41	558	635	841	
AA identified in Wm82		M	R	N	S	
AA change		I	W	D	P	

B

Wm82	W/L/C	TPS2-Haplotype					
	12/27/8	H1	G	A	T	G	A
	2/0/0	H2	A	G	T	C	A
	33/0/0	H3	A	G	T	G	A
	6/0/0	H4	A	A	TAAC	G	A
	2/0/0	H5	A	A	T	G	G
	51/174/218	H6	A	A	T	G	A
CDS nt #in Wm82		166	239	2468-2469	2585	2668	
AA # in Wm82		56	80	823	862	890	
AA identified in Wm82		I	E	V	R	T	
AA change		V	G	VT	P	A	

C

Wm82	W/L/C	TPS3-Haplotype						
	1/2/0	H1	A	A	G	G	C	G
	1/0/0	H2	C	G	G	G	C	G
	1/0/0	H3	C	A	T	G	C	G
	1/0/0	H4	C	A	G	A	C	G
	11/23/12	H5	C	A	G	A	C	G
	8/3/0	H6	C	A	G	G	C	A
	86/170/213	H7	C	A	G	G	C	G
CDS nt #in Wm82		215	611	829	1156	1974	2533	2726
AA # in Wm82		72	204	277	386	658	845	909
AA identified in Wm82		T	K	A	D	I	R	R
AA change		K	R	S	N	M	S	H

D

Wm82	W/L/C	TPS4-Haplotype										
	0/1/0	H1	A	G	G	G	A	G	A	A	G	G
	13/51/86	H2	A	G	G	G	G	G	A	A	G	G
	0/2/0	H3	C	T	G	G	G	G	A	A	G	G
	11/11/0	H4	C	G	A	G	G	G	A	A	G	G
	1/0/0	H5	C	G	G	T	G	G	A	A	G	G
	1/0/0	H6	C	G	G	G	G	C	A	A	G	G
	0/1/9	H7	C	G	G	G	G	G	C	G	C	C
	4/24/70	H8	C	G	G	G	G	G	A	G	C	C
	57/44/87	H9	C	G	G	G	G	G	A	A	G	G
CDS nt #in Wm82		116	183	314	321	820	1015	1656	1886	1887	1897	
AA # in Wm82		39	61	105	107	274	339	552	629	629	633	
AA identified in Wm82		A	L	G	E	E	E	E	K	K	V	
AA change		E	F	D	D	K	Q	D	R	N	L	

附图 3 *TPS*单倍型分析

A~O 大豆 *TPS*基因的单倍型。数据来自559份已测序的材料(121份野生大豆、207份农家品种和231份栽培品种)。

Appendix figure 3 The analysis of *TPS* haplotypes

A~O Haplotypes of soybean *TPS* genes. Data are combined from 559 sequenced accessions (121 wild soybeans, 207 landraces and 231 improved cultivars).

E

Wm82	W/L/C	TPS5-Haplotype	
	2/0/0	H1	G A
	1/0/0	H2	A G
	116/207/231	H3	A A
CDS nt #in Wm82		883	2135
AA # in Wm82		295	712
AA identified in Wm82		T	E
AA change		A	G

F

Wm82	W/L/C	TPS6-Haplotype	
	3/0/0	H1	A
	118/207/231	H2	G
CDS nt #in Wm82		970	
AA # in Wm82		324	
AA identified in Wm82		E	
AA change		K	

G

Wm82	W/L/C	TPS7-Haplotype	
	0/2/0	H1	A
	121/204/231	H2	G
CDS nt #in Wm82		454	
AA # in Wm82		152	
AA identified in Wm82		G	
AA change		S	

H

Wm82	W/L/C	TPS8-Haplotype							
	3/0/0	H1	T	C	A	T	G	T	G T
	0/7/0	H2	G	T	A	T	G	T	G T
	1/0/0	H3	G	C	T	T	G	G	G T
	1/0/0	H4	G	C	A	G	G	T	G T
	1/0/0	H5	G	CC	A	T	A	T	G T
	2/0/0	H6	G	C	A	T	G	T	T T
	93/112/90	H7	G	C	A	T	G	T	G T
	14/82/128	H8	G	C	A	T	G	T	G G
CDS nt #in Wm82		207	652	688	751	881	1802	1804	2276
AA # in Wm82		69	218	230	251	294	601	602	759
AA identified in Wm82		L	R	S	L	R	I	V	W
AA change		F	W	C	V	H	S	L	L

附图 3 *TPS*单倍型分析
A~O 大豆 *TPS*基因的单倍型。数据来自559份已测序的材料(121份野生大豆、207份农家品种和231份栽培品种)。
Appendix figure 3 The analysis of *TPS* haplotypes
A~O Haplotypes of soybean *TPS* genes. Data are combined from 559 sequenced accessions (121 wild soybeans, 207 landraces and 231 improved cultivars).

I

Wm82	W/L/C	TPS9-Haplotype			
	3/0/0	H1	C	G	TCTA
	3/0/0	H2	T	A	TCTA
	0/1/0	H3	T	G	A
	112/205/231	H4	T	G	TCTA
CDS nt #in Wm82			1430	881	405-407
AA # in Wm82			477	294	135-136
AA identified in Wm82			V	R	
AA change			A	H	nf del

J

Wm82	W/L/C	TPS10-Haplotype													
	17/0/0	H1	C	A	C	T	C	C	A	G	G	A	C	A	C
	0/1/0	H2	T	TGA	C	T	C	C	A	G	G	A	C	A	C
	0/0/15	H3	T	A	T	CT	C	C	A	G	G	A	C	A	C
	10/75/83	H4	T	A	T	T	C	C	A	G	G	A	C	A	C
	2/0/0	H5	T	A	C	T	T	C	A	G	C	A	C	A	C
	1/0/0	H6	T	A	C	T	C	T	A	A	G	A	C	A	C
	6/35/7	H7	T	A	C	T	C	C	G	G	G	A	C	A	C
	3/0/0	H8	T	A	C	T	C	C	A	A	G	T	C	A	C
	1/0/0	H9	T	A	C	T	C	C	A	A	G	A	C	G	C
	32/1/1	H10	T	A	C	T	C	C	A	A	G	A	C	A	C
	6/0/0	H11	T	A	C	T	C	C	A	G	C	A	C	A	C
	0/1/0	H12	T	A	C	T	C	C	A	G	G	A	TC	A	C
	1/0/0	H13	T	A	C	T	C	C	A	G	G	A	C	A	T
	30/77/103	H14	T	A	C	T	C	C	A	G	G	A	C	A	C
CDS nt #in Wm82		2545	2064-2065	1778	1668	1610	1558	1345	1111	1066	945	408	250	113	
AA # in Wm82		849	689-670	593	557	537	520	449	371	356	315	137	84	38	
AA identified in Wm82		H	M	A	W	A	R	K	V	V	L	L	S	S	
AA change		Y	stopgain	V	fs ins	V	C	E	I	L	F	fs ins	G	L	

K

Wm82	W/L/C	TPS11-Haplotype				
	4/0/0	H1	A	C	A	A
	6/0/0	H2	G	T	A	A
	9/0/0	H3	G	C	G	A
	2/0/0	H4	G	C	A	G
	94/207/231	H5	G	C	A	A
	CDS nt #in Wm82		1960	1555	1175	1166
AA # in Wm82		654	519	392	389	
AA identified in Wm82		G	H	E	N	
AA change		S	Y	G	S	

L

Wm82	W/L/C	TPS12-Haplotype					
	1/18/2	H1	T	A	C	A	G
	0/2/0	H2	C	C	C	A	G
	1/0/0	H3	C	A	T	A	T
	6/0/0	H4	C	A	C	G	G
	113/187/227	H5	C	A	C	A	G
CDS nt #in Wm82		2045	2000	1541	1513	1092	
AA # in Wm82		682	667	514	505	365	
AA identified in Wm82		A	Y	S	I	Q	
AA change		V	S	L	V	H	

附图 3 *TPS*单倍型分析
 A~O 大豆 *TPS*基因的单倍型。数据来自559份已测序的材料(121份野生大豆、207份农家品种和231份栽培品种)。
Appendix figure 3 The analysis of *TPS* haplotypes
 A~O Haplotypes of soybean *TPS* genes. Data are combined from 559 sequenced accessions (121 wild soybeans, 207 landraces and 231 improved cultivars).

M

Wm82	W/L/C		TPS13-Haplotype																	
	3/0/0	H1	A	A	C	A	C	A	G	G	C	G	C	A	C	C	G	A	A	C
	1/0/0	H2	A	C	C	A	C	A	G	G	C	G	C	A	C	C	C	A	A	C
	13/0/0	H3	A	C	C	A	C	A	G	G	C	G	C	A	C	C	G	A	A	C
	1/0/0	H4	A	C	C	A	C	G	*	G	C	G	C	A	A	C	G	A	A	C
	10/6/3	H5	A	C	C	A	C	G	G	G	C	G	C	A	A	C	G	A	A	C
	7/0/0	H6	A	C	C	A	C	G	G	G	C	G	C	A	C	C	G	A	A	C
	1/0/0	H7	A	C	C	A	C	G	G	G	A	A	C	A	C	G	G	A	G	C
	21/2/0	H8	A	C	C	A	C	G	G	G	A	G	C	A	C	G	G	A	G	C
	0/1/0	H9	G	C	A	G	T	G	G	G	A	G	T	A	C	C	G	A	A	T
	4/0/0	H10	G	C	A	G	C	G	T	G	A	G	T	A	C	C	G	A	A	T
	5/0/0	H11	G	C	A	G	C	G	G	A	A	G	T	A	C	C	G	A	A	T
	1/0/0	H12	G	C	A	G	C	G	G	G	A	G	T	G	C	C	G	A	A	T
	1/0/0	H13	G	C	A	G	C	G	G	G	A	G	T	A	C	C	G	G	A	T
	31/196/221	H14	G	C	A	G	C	G	G	G	A	G	T	A	C	C	G	A	A	T
CDS nt #in Wm82			2506	2481	2405	2389	2168	1910	1890-1891	1381	1189	1094	1093	1079	1069	928	514	344	179	149
AA # in Wm82			836	827	802	797	723	637	630	461	397	365	365	360	357	310	172	115	60	50
AA identified in Wm82			E	D	N	E	A	G	V	E	K	W	W	Q	Q	L	D	D	K	L
AA change			K	E	T	K	V	E	fs del	K	Q	stopgain	R	R	K	V	H	G	R	S

N

Wm82	W/L/C	TPS14-Haplotype									
	19/0/0	H1	A	A	C	C	C	A	T	G	A
	3/14/53	H2	G	G	C	C	G	A	T	G	A
	2/0/0	H3	G	A	G	C	G	A	T	G	A
	1/0/0	H4	G	A	C	G	G	A	T	G	A
	2/0/0	H5	G	A	C	C	C	A	T	G	A
	1/0/0	H6	G	A	C	C	G	G	T	G	A
	7/6/4	H7	G	A	C	C	G	A	C	G	A
	1/0/0	H8	G	A	C	C	G	A	T	T	A
	4/0/0	H9	G	A	C	C	G	A	T	G	G
	73/183/160	H10	G	A	C	C	G	A	T	G	A
CDS nt #in Wm82		2564	2305	1785	1718	1619	904	791	154	109	
AA # in Wm82		855	769	595	573	540	302	264	52	37	
AA identified in Wm82		G	T	F	P	S	I	F	A	M	
AA change		E	A	L	R	T	V	S	S	V	

O

Wm82	W/L/C		TPS15-Haplotype				
	2/45/1	H1	C	C	A	G	G
	4/5/4	H2	A	T	T	G	G
	1/0/0	H3	A	C	A	A	G
	50/10/35	H4	A	C	A	G	G
	1/0/0	H5	A	C	T	G	A
	57/139/181	H6	A	C	T	G	G
CDS nt #in Wm82			128	172	989	1420	1429
AA # in Wm82			43	58	330	474	477
AA identified in Wm82			K	R	F	A	V
AA change			T	C	Y	T	I

P

Wm82	W/L/C		TPS16-Haplotype		
	1/0/0	H1	G	C	A
	0/1/0	H2	T	T	A
	2/0/0	H3	T	C	T
	118/206/231	H4	T	C	A
CDS nt #in Wm82			62	466	1594
AA # in Wm82			21	156	532
AA identified in Wm82			G	R	T
AA change			V	W	S

附图 3 *TPS*单倍型分析
A~O 大豆 *TPS*基因的单倍型。数据来自559份已测序的材料(121份野生大豆、207份农家品种和231份栽培品种)。
Appendix figure 3 The analysis of *TPS* haplotypes
A~O Haplotypes of soybean *TPS* genes. Data are combined from 559 sequenced accessions (121 wild soybeans, 207 landraces and 231 improved cultivars).

Q

Wm82	W/L/C	TPS17-Haplotype					
	1/0/0	H1	C	C	G	G	C
	15/0/0	H2	G	G	G	G	C
	0/1/0	H3	G	C	A	G	C
	0/1/0	H4	G	C	G	A	C
	1/0/0	H5	G	C	G	G	G
	79/38/7	H6	G	C	G	G	C
	16/163/224	H7	G	C	G	G	C
CDS nt #in Wm82		244	296	512	1030	1502	1891
AA # in Wm82		82	99	171	344	501	631
AA identified in Wm82		V	S	R	D	A	L
AA change		L	C	H	N	G	V

R

Wm82	W/L/C	TPS18-Haplotype	
	1/2/0	H1	C
	103/135/96	H2	C
	14/62/125	H3	T
CDS nt #in Wm82		1400	599
AA # in Wm82		467	200
AA identified in Wm82		L	F
AA change		P	Y

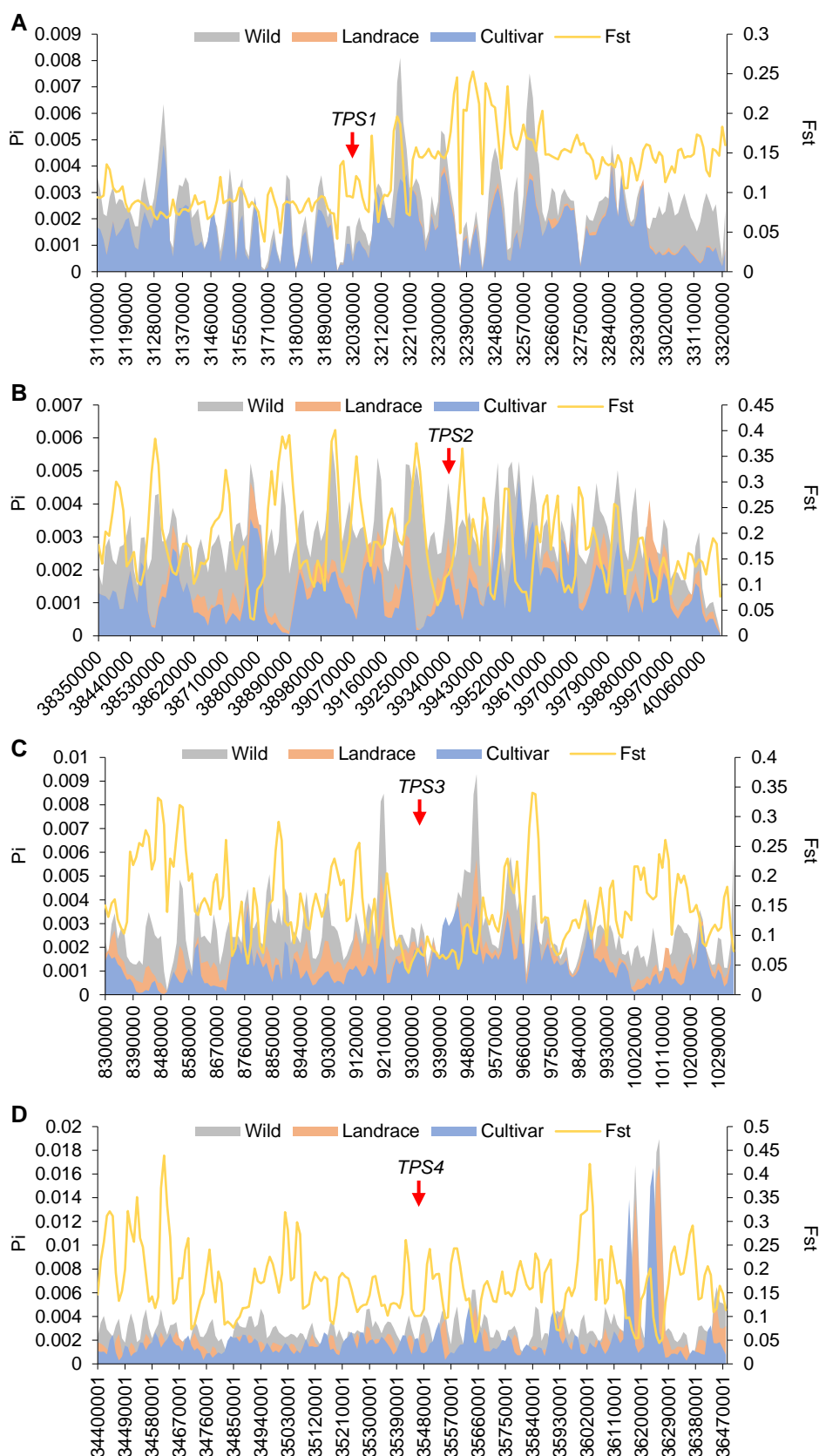
S

Wm82	W/L/C	TPS19-Haplotype				
	3/0/0	H1	A	G	G	C
	3/0/0	H2	G	T	G	C
	0/11/1	H3	G	G	A	C
	0/7/3	H4	G	G	G	T
	1/0/0	H5	G	G	G	C
	109/183/225	H6	G	G	G	C
CDS nt #in Wm82		250	488	1175	1613	1820
AA # in Wm82		84	163	392	538	607
AA identified in Wm82		V	R	R	T	L
AA change		I	M	K	I	H

T

Wm82	W/L/C	TPS20-Haplotype			
	1/0/0	H1	A	C	A
	0/1/0	H2	G	T	A
	3/0/0	H3	G	C	T
	2/0/0	H4	G	C	A
	114/206/231	H5	G	C	A
CDS nt #in Wm82		1936	1744	1561	1313
AA # in Wm82		646	582	521	438
AA identified in Wm82		V	R	T	R
AA change		I	W	S	K

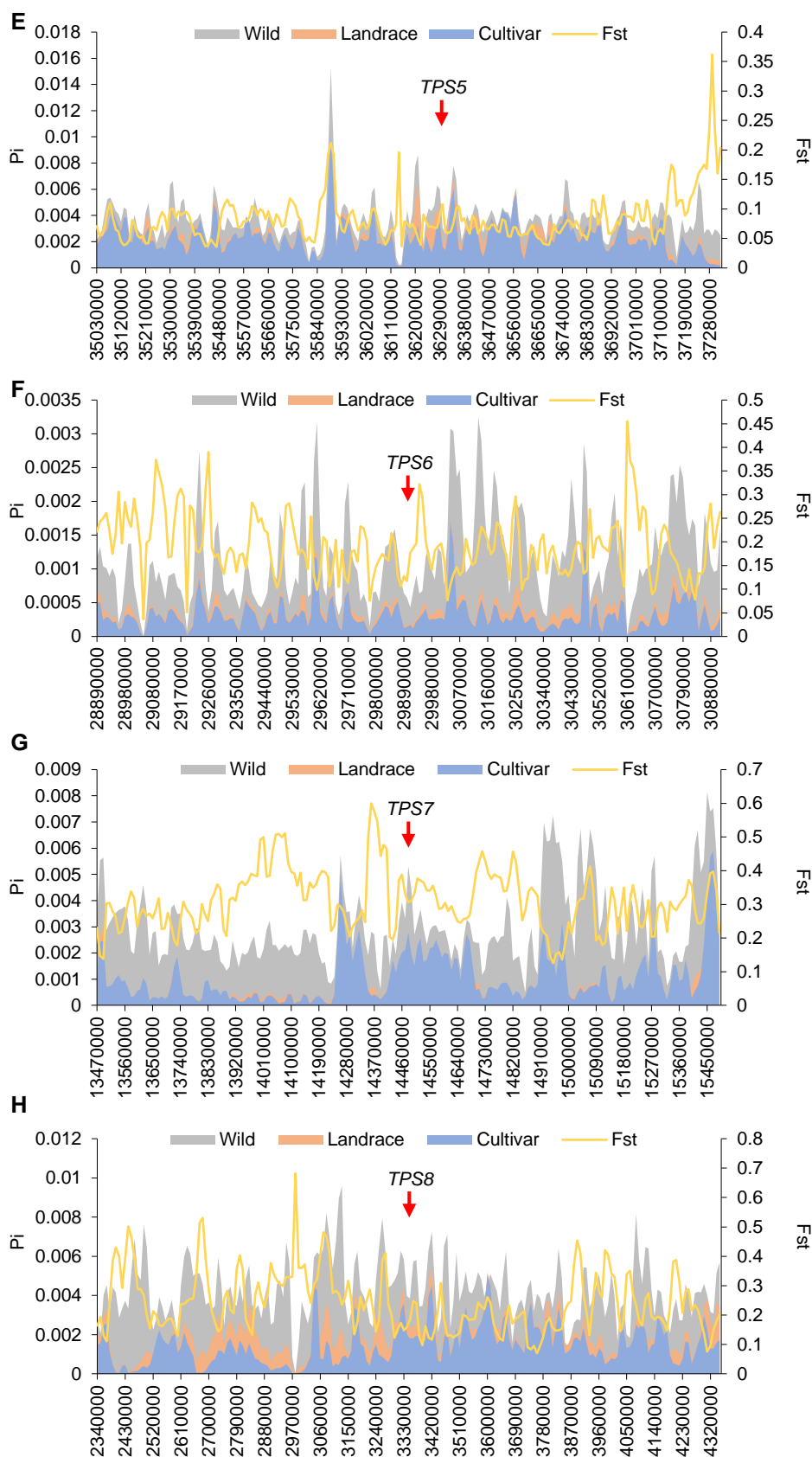
附图 3 *TPS*单倍型分析
A~O 大豆*TPS*基因的单倍型。数据来自559份已测序的材料(121份野生大豆、207份农家品种和231份栽培品种)。
Appendix figure 3 The analysis of *TPS* haplotypes
A~O Haplotypes of soybean *TPS* genes. Data are combined from 559 sequenced accessions (121 wild soybeans, 207 landraces and 231 improved cultivars).



附图 4 在 *TPS* 基因周围 2-Mb 基因组区域内的野生豆、农家品种和栽培品种的 *Fst* 和 *Pi* 值。A~T 20 个大豆 *TPS* 基因的 *Fst* 和 *Pi* 值。红色箭头表示 *TPS* 基因位置。数据来自 559 份已测序的材料 (121 份野生大豆、207 份农家品种和 231 份栽培品种)。

Appendix figure 4 *Fst* and *Pi* in wild soybeans, landraces and improved cultivars across the 2-Mb genomic region surrounding *TPS*

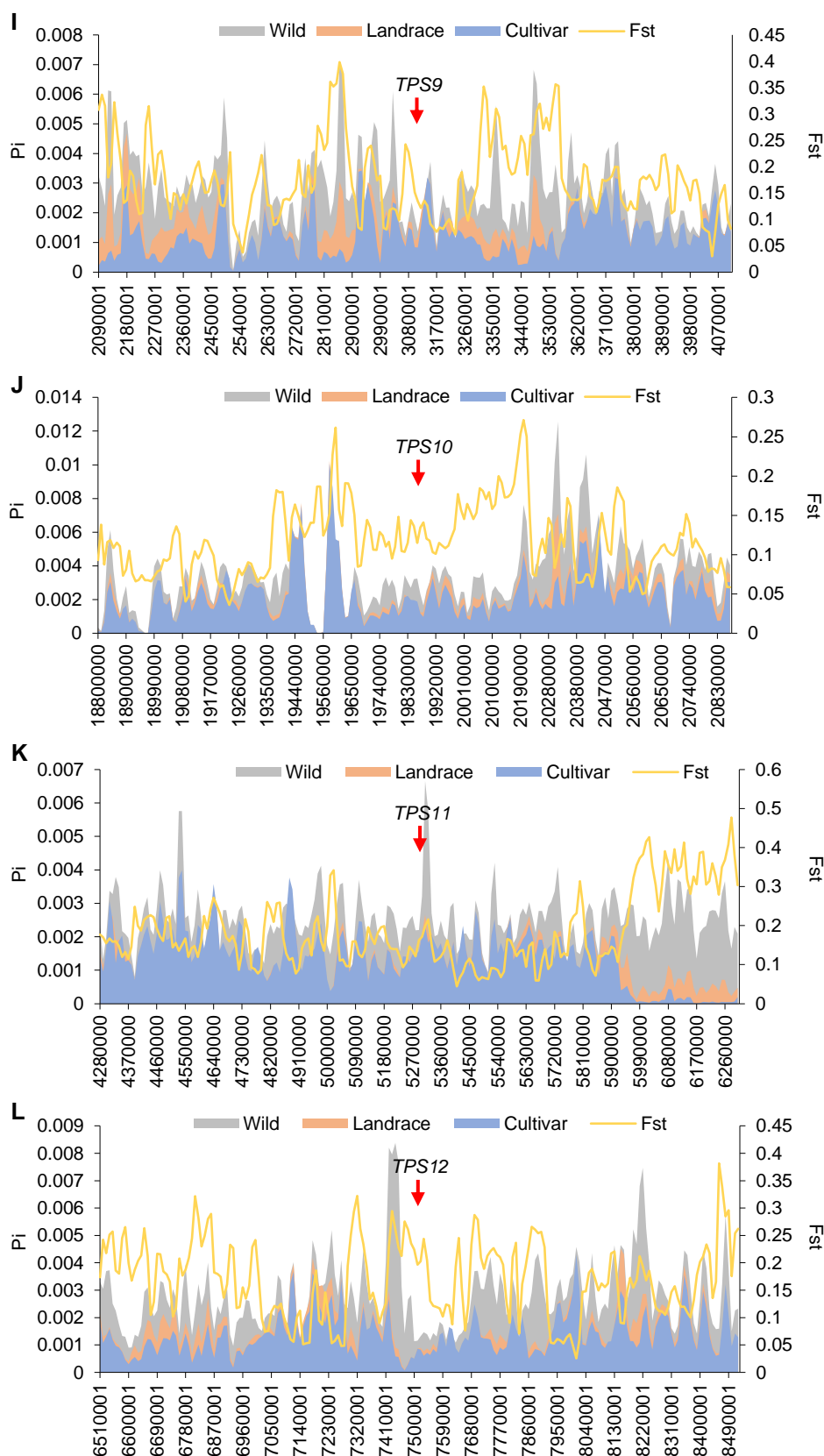
A~T *Fst* and *Pi* of 20 soybean *TPS* genes. The red arrow represented the location of *TPS*. Data are combined from 559 sequenced accessions (121 wild soybeans, 207 landraces and 231 improved cultivars).



附图 4 在 TPS 基因周围 2-Mb 基因组区域内的野生豆、农家品种和栽培品种的 F_{st} 和 P_i A~T 20 个大豆 TPS 基因的 F_{st} 和 P_i 值。红色箭头表示 TPS 基因位置。数据来自 559 份已测序的材料 (121 份野生大豆、207 份农家品种和 231 份栽培品种)。

Appendix figure 4 F_{st} and P_i in wild soybeans, landraces and improved cultivars across the 2-Mb genomic region surrounding TPS

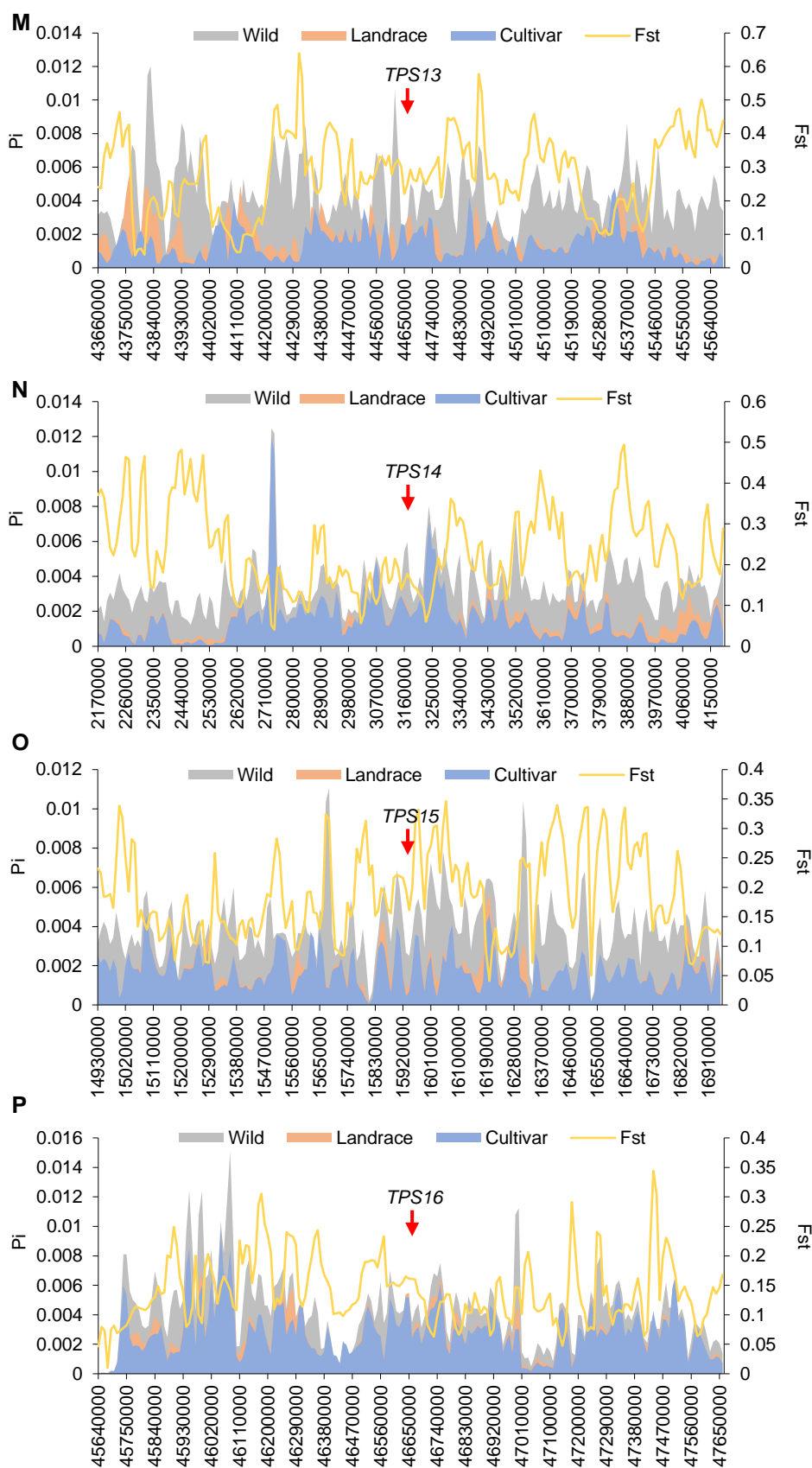
A~T F_{st} and P_i of 20 soybean TPS genes. The red arrow represented the location of TPS . Data are combined from 559 sequenced accessions (121 wild soybeans, 207 landraces and 231 improved cultivars).



附图 4 在 *TPS* 基因周围 2-Mb 基因组区域内的野生豆、农家品种和栽培品种的 Fst 和 Pi
A~T 20 个大豆 *TPS* 基因的 Fst 和 Pi 值。红色箭头表示 *TPS* 基因位置。数据来自 559 份已测序的材料(121 份野生大豆、207 份农家品种和 231 份栽培品种)。

Appendix figure 4 Fst and Pi in wild soybeans, landraces and improved cultivars across the 2-Mb genomic region surrounding *TPS*

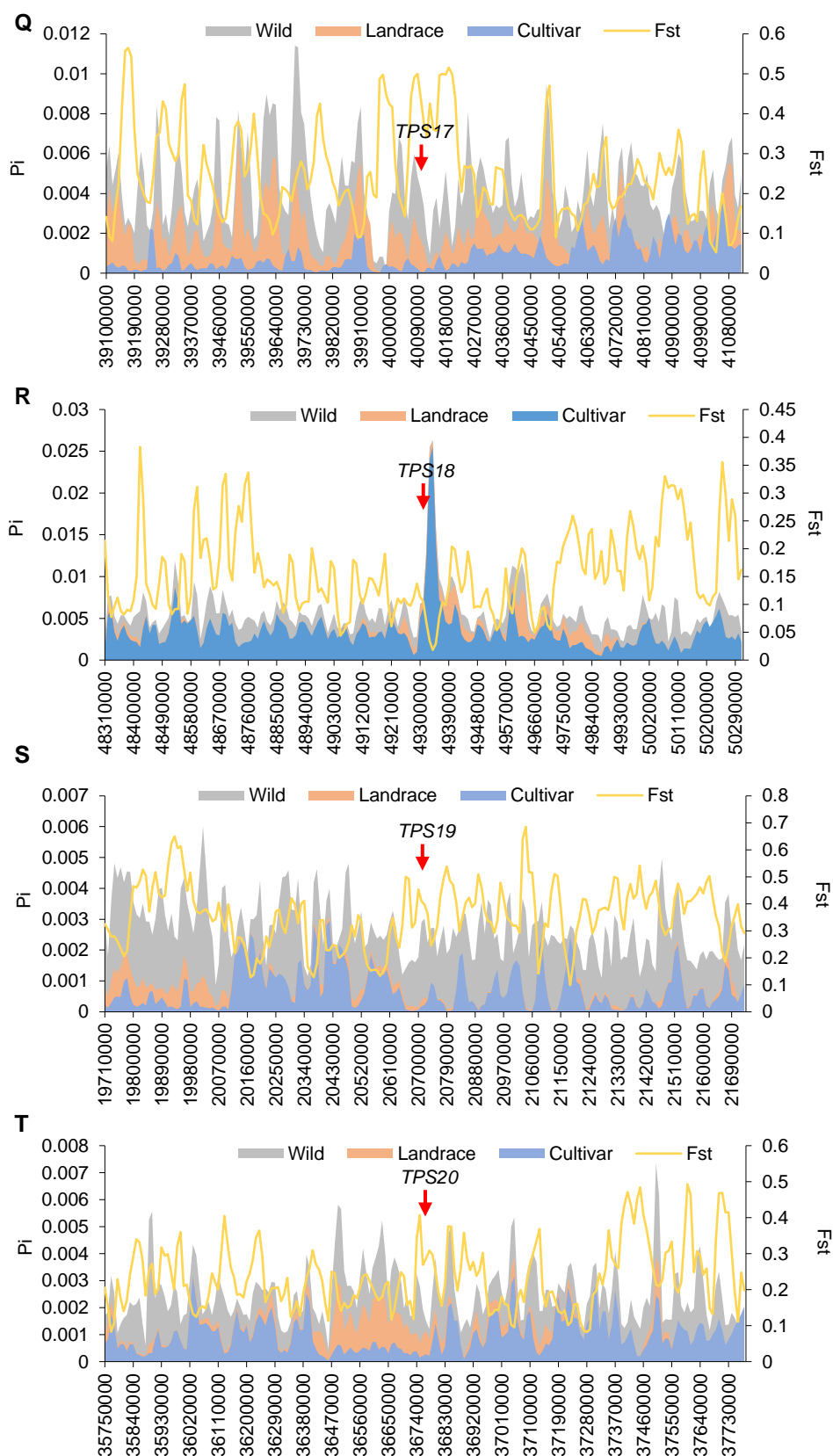
A~T Fst and Pi of 20 soybean *TPS* genes. The red arrow represented the location of *TPS*. Data are combined from 559 sequenced accessions (121 wild soybeans, 207 landraces and 231 improved cultivars).



附图 4 在 TPS 基因周围 2-Mb 基因组区域内的野生豆、农家品种和栽培品种的 F_{st} 和 P_i A~T 20 个大豆 TPS 基因的 F_{st} 和 P_i 值。红色箭头表示 TPS 基因位置。数据来自 559 份已测序的材料 (121 份野生大豆、207 份农家品种和 231 份栽培品种)。

Appendix figure 4 F_{st} and P_i in wild soybeans, landraces and improved cultivars across the 2-Mb genomic region surrounding TPS

A~T F_{st} and P_i of 20 soybean TPS genes. The red arrow represented the location of TPS . Data are combined from 559 sequenced accessions (121 wild soybeans, 207 landraces and 231 improved cultivars).



附图 4 在 *TPS* 基因周围 2-Mb 基因组区域内的野生豆、农家品种和栽培品种的 Fst 和 Pi
A~T 20 个大豆 *TPS* 基因的 Fst 和 Pi 值。红色箭头表示 *TPS* 基因位置。数据来自 559 份已测序的材料 (121 份野生大豆、207 份农家品种和 231 份栽培品种)。

Appendix figure 4 Fst and Pi in wild soybeans, landraces and improved cultivars across the 2-Mb genomic region surrounding *TPS*

A~T Fst and Pi of 20 soybean *TPS* genes. The red arrow represented the location of *TPS*. Data are combined from 559 sequenced accessions (121 wild soybeans, 207 landraces and 231 improved cultivars).