

## AtWRKY40参与拟南芥干旱胁迫响应过程

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**摘要:** 以拟南芥(*Arabidopsis thaliana*)野生型、*AtWRKY40*缺失突变体和过表达株系为材料, 研究*AtWRKY40*在植物干旱胁迫响应过程中的作用及其生理和分子机制。结果显示, *AtWRKY40*受干旱胁迫诱导; *AtWRKY40*缺失导致干旱胁迫下种子萌发率降低, 叶片失水加剧, 而*AtWRKY40*过表达植株呈现出相反的特征; 干旱胁迫下, *AtWRKY40*缺失突变体植株叶片过氧化氢(H<sub>2</sub>O<sub>2</sub>)、超氧阴离子(O<sub>2</sub><sup>-</sup>)及丙二醛(MDA)含量显著高于野生型及其过表达株系, 超氧化物歧化酶(SOD)和过氧化氢酶(CAT)活性、脯氨酸(Pro)和可溶性糖含量以及相关基因*AtCu/ZnSOD*、*AtCAT1*、*AtP5C1S*、*AtG6PD5*和*AtBAM4*表达量显著低于野生型, 同时*AtWRKY40*过表达株系的渗透物质含量和保护酶活性及其基因表达量则高于野生型。由此说明, *AtWRKY40*通过调节植株抗氧化能力及渗透调节能力参与拟南芥干旱胁迫响应过程。

**关键词:** *AtWRKY40*; 干旱; 渗透调节物质; 保护酶; 拟南芥

干旱是世界范围内限制植物生长发育和作物产量的主要非生物逆境, 是影响区域最广、发生最频繁的气象灾害之一。解析植物对干旱的适应机制是科研工作者长期致力研究的重要课题。

WRKY、AP2/EREBP、bZIP、MYB/MYC以及NAC等家族转录因子业已证明参与植物对干旱胁迫的响应过程(Gahlaut等2016), 其中, WRKY转录因子是高等植物中最大的转录因子家族之一, 含有高度保守的WRKY结构域和锌指结构, 根据其保守结构域的数目及锌指结构的特征, WRKY转录因子分为I、II和III三个亚族。研究发现, WRKY家族的许多成员参与干旱胁迫响应过程, 对植物抗旱性起正或负调控作用。抗旱性强的小麦(*Triticum aestivum*)品种‘Sivas111/33’根部*TaWRKY16*、*TaWRKY16-A*及*TaWRKY17*等受干旱诱导(Okay等2014); 过表达小麦*TaWRKY33*、金桔(*Fortunella crassifolia*)*FcWRKY70*、棉花(*Gossypium hirsutum*)*GhWRKY41*和拟南芥(*Arabidopsis thaliana*)*AtWRKY57*使植株抗旱性增强(He等2016; Gong等2015; Chu等2015; Jiang等2012)。而拟南芥WRKY46、WRKY54和WRKY70抑制干旱响应基因表达, 降低植株的抗旱性(Chen等2017)。由此可见, 在干旱应答过程中WRKY的作用是复杂的。

拟南芥*AtWRKY40*属于IIa类WRKY转录因子, 有报道其与植物对多种生物和非生物逆境的响应有关(Xu等2006; Wenke等2012; Schön等2013; Birkenbihl等2017; Liu等2015)。E-2-己烯醛(E-2-

hexenal)是叶片受到机械损伤、昆虫取食或病原菌侵染产生的挥发性信号物质, *WRKY40*受E-2-己烯醛诱导, 参与E-2-己烯醛信号途径(Mirabella等2015); WRKY40、WRKY18和WRKY60通过调节H<sub>2</sub>S产生参与镉胁迫应答过程(Liu等2015); WRKY40亦可与茉莉酸(jasmonic acid, JA)信号途径抑制因子*JAZ*基因以及*ABI5*等脱落酸(abscisic acid, ABA)响应基因启动子区域的W-box元件结合, 抑制其表达, 参与JA和ABA信号途径(Pandey等2010; Shang等2010; Liu等2012, 2013); Chen (2010)等研究发现*WRKY40*受渗透胁迫诱导, 暗示WRKY40可能参与植物干旱胁迫响应过程, 但目前尚未见WRKY40在植物干旱胁迫响应过程中的作用及机制的研究报道。因此本文拟以拟南芥野生型、*AtWRKY40*缺失突变体及过表达株系为材料, 探究*AtWRKY40*在拟南芥响应干旱胁迫过程的作用及其生理和分子机制。

## 1 材料与方法

### 1.1 实验材料与培养

拟南芥(*Arabidopsis thaliana*)生态型为Columbia-0, *AtWRKY40*缺失突变体(*wrky40*)及过表达株系(OE4和OE15)购置于美国生物资源中心。将野

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生型(WT)拟南芥、*AtWRKY40*缺失突变体及过表达株系的种子用0.1% NaClO消毒10 min, 点种于无菌1/2MS固体培养基, 4°C条件下处理2~4 d打破种子休眠, 然后转入光照培养箱于22°C、16 h/8 h光/暗周期培养一周, 再移栽到培养土(市售花卉营养土):蛭石为1:1 (V/V)的培养介质中, 于光/暗周期16 h/8 h、温度22~25°C、光强120  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 、相对湿度70%环境条件下培养。

取野生型拟南芥叶片, 用200  $\text{mmol}\cdot\text{L}^{-1}$ 甘露醇处理, 分别于0、0.5、1.0、1.5 h取样测定*AtWRKY40*相对表达量。

将野生型拟南芥、*AtWRKY40*缺失突变体及过表达株系自然干旱10 d, 测定*AtCu/ZnSOD*、*AtCAT1*、*AtP5CS1*、*AtG6PD5*和*AtBAM4*表达量以及可溶性糖、丙二醛(malonaldehyde, MDA)、脯氨酸(proline, Pro)、 $\text{H}_2\text{O}_2$ 、超氧阴离子(superoxide anion,  $\text{O}_2^-$ )含量等生理指标。

## 1.2 实验方法

### 1.2.1 种子萌发率测定

将野生型拟南芥、*AtWRKY40*缺失突变体及过表达株系的种子播种到含200  $\text{mmol}\cdot\text{L}^{-1}$ 甘露醇的1/2MS培养基上, 每5 h统计一次种子萌发数, 连续统计7 d。以露白长度达到种子长度的1/2作为萌发标准。

### 1.2.2 植株生长表型观测

拟南芥种子春化并播种在培养土(市售花卉营养土):蛭石为1:1 (V/V)的培养介质中, 正常浇水, 生长2周后自然干旱处理12 d, 观察表型变化并统计莲座叶的萎蔫率, 以莲座叶中叶片发黄或出现萎蔫死亡现象为标准, 统计每种处理材料在干旱胁迫后的莲座叶总数以及出现萎蔫的叶片数。

### 1.2.3 基因相对表达量测定

溴化十六烷三甲基铵(CTAB)法提取叶片总RNA, 反转录得到cDNA, 以cDNA为模板, *ACTIN*为内参, 实时荧光定量PCR (quantitative real-time PCR, qPCR)法测定基因表达量。qPCR程序为: 95°C 5 min; 95°C 30 s, 56°C 30 s, 72°C 30s, 40个循环; 每个处理重复3次。qPCR引物如表1中所示。

### 1.2.4 生理指标测定

$\text{H}_2\text{O}_2$ 、 $\text{O}_2^-$ 、MDA和Pro含量测定参照Wang等(2017); 二氨基联苯胺(DAB)染色法测定 $\text{H}_2\text{O}_2$ 含量参照Zhang等(2012); 氮蓝四唑(NBT)染色法测定

表1 qPCR引物序列

Table 1 qPCR primer sequences

引物名称	引物序列(5'→3')
<i>AtWRKY40-FP</i>	TTGTGTAAGAAGCAGAGAGAAGAG
<i>AtWRKY40-RP</i>	CATCTTTCACAACGAGGGTAG
<i>AtACTIN-FP</i>	GGTAACATTGTGCTCAGTGG
<i>AtACTIN-RP</i>	CACGACCTTAATCTTCATGC
<i>AtCu/ZnSOD-FP</i>	CTGCATCTCTACTGGACCTC
<i>AtCu/ZnSOD-RP</i>	CCACATCCAACCTCTCGAGC
<i>AtCAT1-FP</i>	GTCTCTGGGATTACAGACAGGC
<i>AtCAT1-RP</i>	GGCCTCACGTTAAGACGAGT
<i>AtP5CS1-FP</i>	AGGGAAAGTTCAGAAAG
<i>AtP5CS1-RP</i>	CATAACTAAGCGAGCCAC
<i>AtBAM4-FP</i>	GCTTCCGTCTGTCTCGTCA
<i>AtBAM4-RP</i>	TGCCTTAGTCCCATCTCATC

$\text{O}_2^-$ 含量参照Qiao等(2016); 超氧化物歧化酶(super-oxide dismutase, SOD)和过氧化氢酶(catalase, CAT)活性测定参照He等(2017); 使用蒽酮法测定可溶性糖含量(Fu等2016)。

## 1.3 数据的统计分析

测定结果用数据处理系统(data processing system, DPS)作方差分析, 所有实验均重复3次。

## 2 实验结果

### 2.1 *AtWRKY40*参与拟南芥干旱胁迫响应过程

#### 2.1.1 干旱胁迫对*AtWRKY40*表达的影响

以200  $\text{mmol}\cdot\text{L}^{-1}$ 甘露醇处理模拟干旱胁迫, 发现*AtWRKY40*受干旱胁迫诱导。正常情况下, *AtWRKY40*表达量无显著变化, 干旱处理60 min后, *AtWRKY40*基因表达量显著升高, 随后降低(图1), 推测*AtWRKY40*可能参与拟南芥干旱胁迫响应过程。

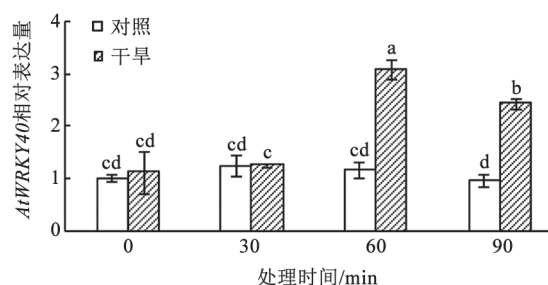


图1 干旱胁迫对拟南芥*AtWRKY40*相对表达量的影响  
Fig.1 Effect of drought stress on relative expression level of *AtWRKY40* in *A. thaliana*

各柱形上用不同小写字母标识表示数据间差异显著( $P < 0.05$ ), 下同。

### 2.1.2 干旱胁迫对*AtWRKY40*缺失突变体及过表达株系萌发率的影响

图2结果显示,正常条件下,拟南芥野生型、*AtWRKY40*缺失突变体*wrky40*及过表达株系OE4和OE15种子萌发无显著差异,在干旱胁迫下,各株系萌发率均降低,且*wrky40*种子萌发率显著低于野生型及过表达株系。

### 2.1.3 干旱胁迫对*AtWRKY40*缺失突变体及过表达株系生长的影响

将拟南芥野生型、*AtWRKY40*缺失突变体及过表达株系幼苗自然干旱处理两周,各株系均出现了不同程度的萎蔫,叶片出现暗绿色失去光泽;*AtWRKY40*缺失突变体受胁迫程度明显高于野生型和过表达株系,再经一定时间干旱胁迫后,*AtWRKY40*缺失突变体植株的萎蔫率高于野生型及过表达株系,而地上部分鲜重则显著低于野生型及过表达株系,这进一步说明*AtWRKY40*能够提高植物抵抗干旱胁迫的能力(图3)。

## 2.2 *AtWRKY40*响应干旱胁迫的生理及分子机制

以上实验结果表明*AtWRKY40*参与干旱响应过程。*AtWRKY40*提高抗旱性的生理和分子机制如何?为此,本文进一步研究了干旱胁迫下拟南芥各株系渗透调节物质积累和抗氧化酶活性及相关基因表达的变化。

### 2.2.1 干旱胁迫对*AtWRKY40*缺失突变体及过表达株系Pro和可溶性糖含量及相关基因表达的影响

正常情况下,拟南芥野生型、*AtWRKY40*缺失突变体及过表达株系Pro和可溶性糖含量无显著差

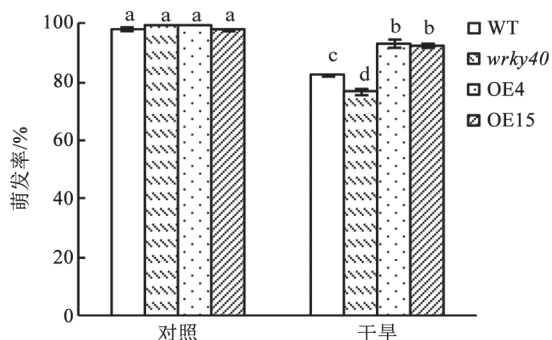


图2 干旱胁迫对*AtWRKY40*缺失突变体和过表达株系种子萌发率的影响

Fig.2 Effect of drought stress on germination rates of *AtWRKY40*-depleted mutant and overexpression lines

异(图4);干旱处理后,各株系Pro含量均显著升高,其中过表达株系升高幅度最大,其次是野生型,缺失突变体植株增加幅度最小;干旱胁迫对缺失突变体植株可溶性糖含量无显著影响,野生型和过表达株系干旱胁迫下可溶性糖含量均显著升高,其中过表达株系升高幅度大于野生型。qPCR结果显示,Pro合成关键酶 $\Delta'$ -吡咯啉-5-羧酸合成酶( $\Delta'$ -pyrroline-5-carboxylate synthetase, P5CS)基因*AtP5CS1*、糖代谢相关基因 $\beta$ -淀粉酶基因*AtBAM4*、葡萄糖-6-磷酸脱氢酶(glucose-6-phosphate dehydrogenase, GD6P)基因*AtG6PD5*表达呈相似变化趋势。

### 2.2.2 干旱胁迫对*AtWRKY40*缺失突变体及过表达株系活性氧和MDA含量的影响

活性氧代谢失调是干旱胁迫诱导的主要次生伤害,为此检测了*AtWRKY40*缺失及过表达对干旱胁迫下拟南芥幼苗叶片活性氧和MDA含量的影响。结果显示,正常情况下,*AtWRKY40*缺失突变体及过表达株系中 $H_2O_2$ 、 $O_2^-$ 及MDA含量无显著差别;干旱胁迫诱导各株系 $H_2O_2$ 、 $O_2^-$ 及MDA含量增加,其中*AtWRKY40*缺失突变体植株各指标增加幅度高于野生型拟南芥和过表达株系(图5),表明*AtWRKY40*缺失导致膜脂过氧化加剧。

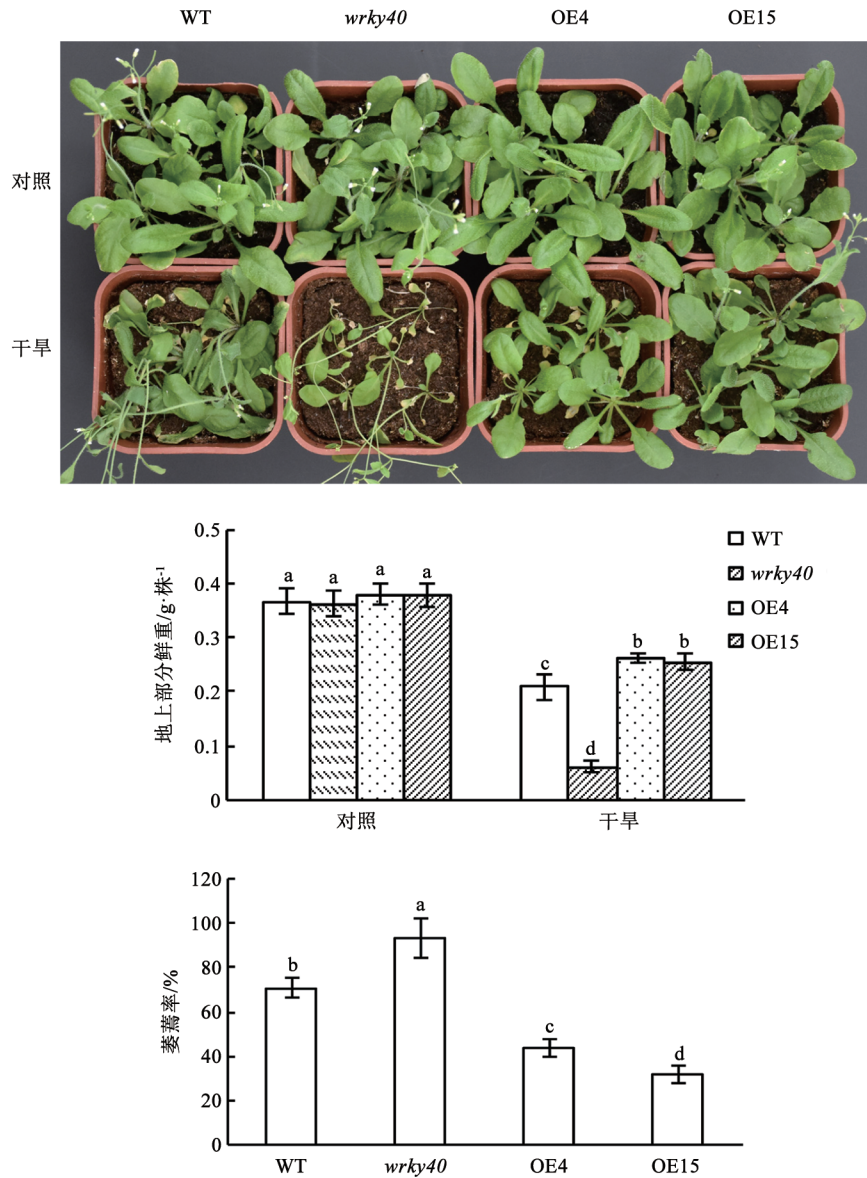
### 2.2.3 干旱胁迫对*AtWRKY40*缺失突变体及过表达株系SOD和CAT活性及相关基因表达的影响

由图6可知,正常条件下野生型拟南芥、*AtWRKY40*缺失突变体及过表达株系中的CAT活性无显著差异,干旱胁迫下,野生型及过表达株系CAT活性显著升高,但*AtWRKY40*缺失突变体植株变化不显著。qPCR结果显示,干旱条件下野生型及缺失突变体植株*AtCu/ZnSOD*表达水平较正常条件下显著降低,而过表达株系无显著变化;干旱处理后,各株系*AtCAT1*表达均升高,但*AtWRKY40*缺失突变体胁迫和非胁迫条件下*AtCAT1*表达量均低于野生型和过表达株系。

综合图4~6结果,推测干旱胁迫下*AtWRKY40*通过促进植株渗透调节物质积累,提高植株抗氧化酶活性,降低干旱对植株的损伤。

## 3 讨论

干旱是限制植物生长发育和作物产量的重要非生物逆境。抗旱相关基因表达在植物对干旱的

图3 干旱对*AtWRKY40*缺失突变体和过表达株系生长状况的影响Fig.3 Growth phenotypes of *AtWRKY40*-depleted mutant and overexpression lines under drought stress

适应过程中起重要作用,许多WRKY家族转录因子参与抗旱基因表达调节,本实验(图1)和Chen等(2010)的研究发现*AtWRKY40*受干旱胁迫诱导,暗示*AtWRKY40*可能参与干旱胁迫响应过程。进一步研究发现,干旱胁迫下,*AtWRKY40*缺失突变体种子萌发率降低(图2),叶片萎蔫率加剧,地上部分生长受抑制程度显著大于野生型植株(图3),而*AtWRKY40*过表达表现相反作用(图2和3),表明*AtWRKY40*在拟南芥干旱响应过程中起正调节作用。

干旱首先对植物造成渗透胁迫,进而引发氧

代谢失调等次生伤害(Sofa等2015; Jaffar等2016)。渗透胁迫下,植物通常通过积累Pro和可溶性糖等有机物进行渗透调节以维持水分平衡(Sperdouli和Moustakas 2012; 赵超等2017; 孙聪聪等2017)。进一步研究表明,*AtWRKY40*缺失突变体植株Pro和可溶性糖含量以及Pro合成和糖代谢相关基因*AtP5CS1*、*AtG6PD5*和*AtBAM4*表达量显著低于野生型,而*AtWRKY40*过表达株系高于野生型(图4)。SOD和CAT是植物体内重要的抗氧化酶,*AtWRKY40*缺失导致拟南芥叶片SOD和CAT活性以及*AtCu/*

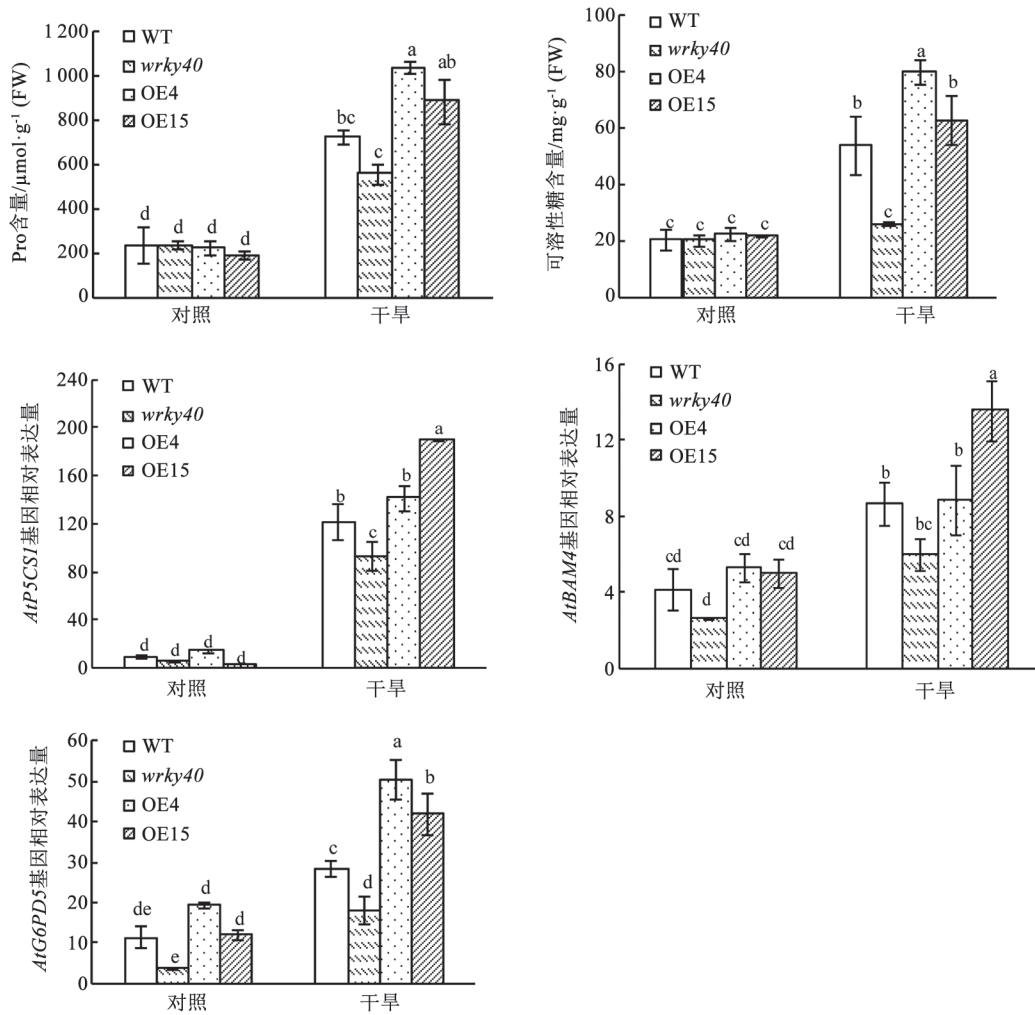


图4 干旱胁迫对*AtWRKY40*缺失突变体和过表达株系中Pro和可溶性糖含量以及*AtP5CS1*、*AtG6PD5*和*AtBAM4*基因相对表达量的影响

Fig.4 Effect of drought stress on contents of proline and soluble sugar as well as relative expression levels of *AtP5CS1*, *AtG6PD5* and *AtBAM4* in *AtWRKY40*-depleted mutant and overexpression lines

*ZnSOD*和*AtCAT1*表达量显著低于野生型,  $\text{H}_2\text{O}_2$ 、 $\text{O}_2^-$ 水平和丙二醛含量显著升高, 膜脂过氧化加剧, 而*AtWRKY40*过表达则提高SOD和CAT活性, 使*AtCu/ZnSOD*和*AtCAT1*表达量升高(图5和6)。综合以上结果可以推测, 干旱胁迫下, *AtWRKY40*通过增强植株渗透调节能力和抗氧化能力维持细胞吸水, 防止氧化损伤, 提高植株对于干旱胁迫的抵抗能力。

有报道表明, *WRKY40*参与ABA信号途径, *WRKY40*可以与ABA响应基因*ABI4*和*ABI5*启动子区域的W-box特异结合, 抑制其表达, 在ABA抑制种子萌发和萌发后生长过程中起负调节作用(Shang等2010; Liu等2012; Chen等2010)。关于ABA与

*WRKY40*的关系还存在不同研究结果, Shang等(2010)和Yan等(2013)研究表明ABA抑制*WRKY40*表达, 解除其对ABA响应基因表达的抑制作用, 而Chen等(2010)研究显示*WRKY40*受ABA诱导。干旱胁迫下, *WRKY40*对种子萌发的调节是否与ABA信号途径有关还需进一步研究。本研究初步证明*AtWRKY40*可能位于*AtP5CS1*、*AtG6PD5*、*AtBAM4*、*AtCu/ZnSOD*和*AtCAT1*上游参与植物对于干旱胁迫的响应, 还需要进一步分析*Cu/ZnSOD*和*CAT1*等基因启动子区域是否存在W-box, 结合酵母单杂交等分子生物学技术可以进一步研究*AtWRKY40*对*AtCu/ZnSOD*和*AtCAT1*等基因表达的调节机制。在许多

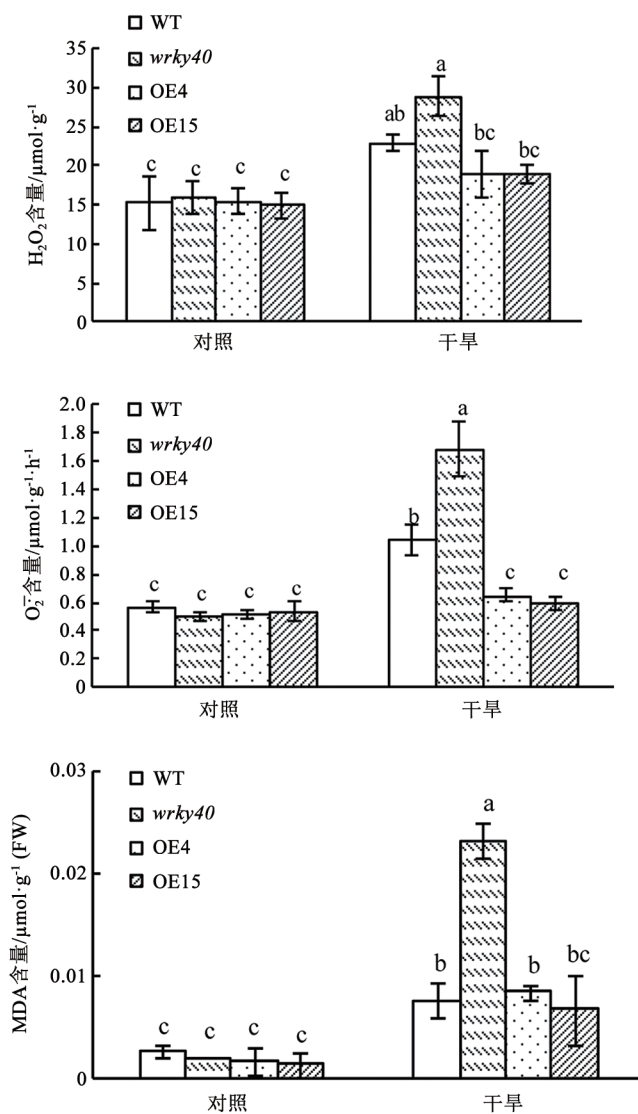
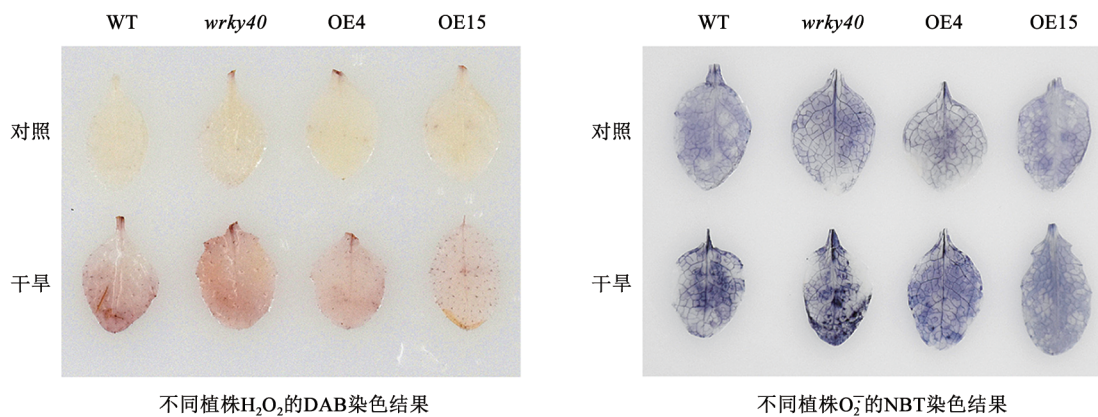


图5 干旱胁迫对*AtWRKY40*缺失突变体和过表达株系H<sub>2</sub>O<sub>2</sub>、O<sub>2</sub><sup>-</sup>和MDA含量的影响

Fig.5 Effect of drought stress on contents of H<sub>2</sub>O<sub>2</sub>, O<sub>2</sub><sup>-</sup> and MDA in *AtWRKY40*-depleted mutant and overexpression lines

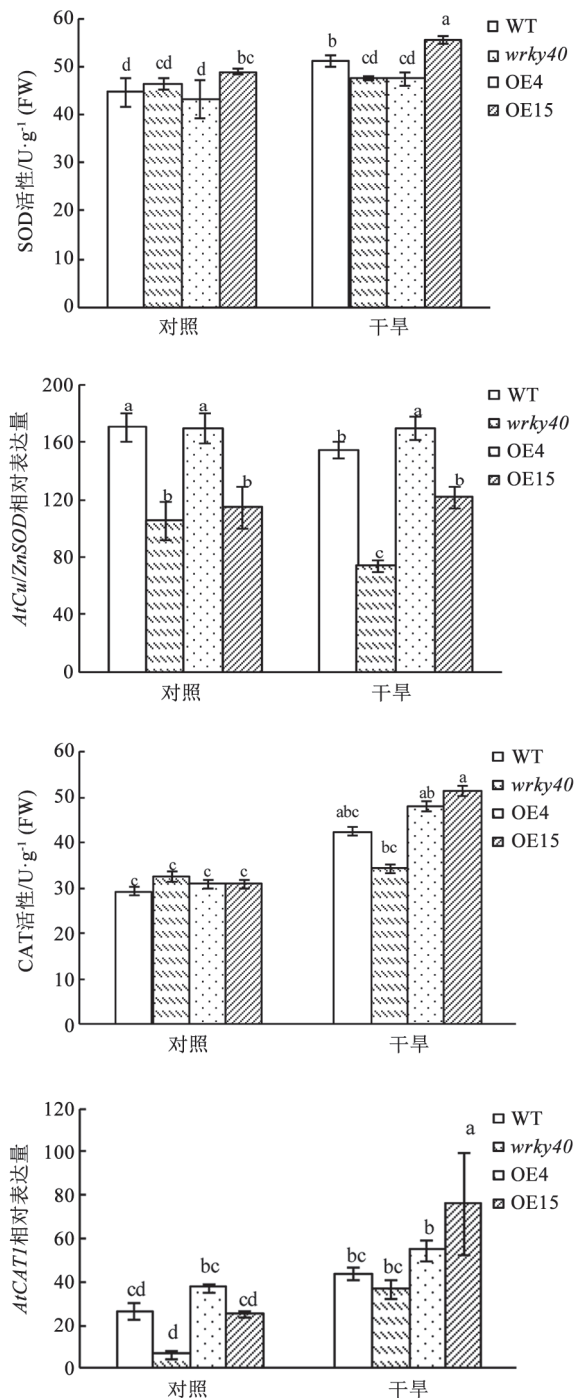


图6 干旱胁迫对*AtWRKY40*缺失突变体和过表达株系SOD和CAT活性及*AtCu/ZnSOD*和*AtCAT1*表达量的影响  
Fig.6 Effect of drought stress on activities of SOD and CAT as well as relative expression levels of *AtCu/ZnSOD* and *AtCAT1* in *AtWRKY40*-depleted mutant and overexpression lines

生理过程中WRKY40与WRKY18和WRKY60存在复杂的相互作用, 如, WRKY40与WRKY18和WRK-

Y60参与拟南芥对丁香假单胞菌(*Pseudomonas syringae*)和灰霉菌(*Botrytis cinerea*)的抗性过程以及ABA信号转导过程, WRKY40与WRKY18和WRKY60之间的互作影响其对靶基因的结合活性(Xu等2006; Liu等2015)。WRKY40与WRKY18和WRKY60之间存在自抑制和交叉抑制作用(Yan等2013)。WRKY18和WRKY60是否参与拟南芥干旱响应过程? 其与WRKY40之间是否存在相互作用, 其作用机制如何? 利用各种突变体和过表达株系结合酵母双杂交、双分子荧光互补等分子生物学技术对这些问题深入研究, 有助于全面解析WRKY转录因子的作用机制。

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## AtWRKY40 functions in drought stress response in *Arabidopsis thaliana*

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**Abstract:** WRKY transcription factors are involved in diversely biotic and abiotic stresses in plants. In the current study, we investigated the role of AtWRKY40 in plant drought stress responses using wild type and transgenic *Arabidopsis thaliana* plants. Under drought stress, the expression of *AtWRKY40* was strongly induced in the wild-type plants. Loss-of-function mutants had seed germination rate decreased and leaf water loss rate increased under drought, while the trends were opposite in the overexpression lines. In addition, *Atwrky40* mutant plants accumulated more malondialdehyde (MDA) than the wild type and overexpression lines, indicating elevated lipid peroxidation in the mutants. To further elucidate the underlying mechanisms, we measured the activities of two reactive oxygen species (ROS)-scavenging enzymes, osmolyte accumulation, as well as the relative expression levels of relevant genes under drought stress. In the mutant lines, the activities of superoxide dismutase (SOD) and catalase (CAT) as well as the contents of proline and water-soluble sugars were all lower than those in the wild type plants. In consistent, the expression levels of *Cu/ZnSOD*, *CAT1*, *Δ'-pyrroline-5-carboxylate synthetase 1 (P5CS1)*, *glucose-6-phosphate dehydrogenase 5 (G6PDH5)* and *β-amylase 4 (BAM4)* were significantly lower in the mutant lines compared to the wild type plants. Again, we observed an opposite trend of regulation by drought stress of these enzymes, osmolytes, and genes in the overexpression lines. Putting together, our results strongly suggest a positive role of AtWRKY40 in plant drought stress responses, presumably by interfering with the ROS-scavenging pathway and osmolyte accumulation process.

**Key words:** AtWRKY40; drought; osmotic substance; antioxidant enzyme; *Arabidopsis thaliana*

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