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ORIGINAL PAPER

GbMPK3, a mitogen-activated protein kinase from cotton, enhances drought and oxidative stress tolerance in tobacco

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Abstract Mitogen-activated protein kinase (MAPK) cascades are highly conserved signaling modules found in all eukaryotes, and play significant roles in developmental and environmental signal transduction. In this study, a MAPK gene (*GbMPK3*), which showed homologous to *AtMPK3* and *NtWIPK*, was isolated from sea-island cotton (*Gossypium barbadense*) and induced during multiple abiotic stress treatments including salt, cold, heat, dehydration and oxidative stress. Transgenic tobacco (*Nicotiana benthamiana*) with constitutively higher expression of *GbMPK3* was conferred with enhanced drought tolerance, reduced water loss during drought treatment and improved plant height and survival rates after re-watering. Additionally, the gene expression levels and enzymatic activity of antioxidant enzymes were more strongly induced with depressed hydrogen peroxide accumulation in *GbMPK3*-overexpressing tobacco compared with wild-type under drought condition. Furthermore, observation of seed germination and leaf morphology showed that tolerance of transgenic plants to methyl viologen was improved due to increased antioxidant enzyme expression, suggesting that *GbMPK3* may positively regulate drought tolerance through enhanced reactive oxygen species scavenging ability.

Keywords *Gossypium barbadense* · *Nicotiana benthamiana* · *GbMPK3* · Drought tolerance · Oxidative stress tolerance

Introduction

In nature, plants are continually challenged by different biotic and abiotic stresses during growth and development. To cope with unfavorable environmental conditions, plants must efficiently transduce extracellular stimuli into appropriate intracellular responses (Pitzschke et al. 2009; Zhang and Klessig 2001). Mitogen-activated protein kinase (MAPK) cascades are highly conserved signaling modules found in all eukaryotes, including fungi, plants and animals, and they play essential roles in developmental and environmental signal transduction (Rodriguez et al. 2010). A MAPK cascade generally consists of three components: a MAPKKK (MAPKK kinase); a MAPKK (MAPK kinase); and a MAPK. Through serial phosphorylation, MAPKKKs, MAPKKs and MAPKs are able to transduce signals received from upstream factors to a variety of downstream substrates including transcription factors and other kinases (Chang and Karin 2001; Colcombet and Hirt 2008).

Since the identification of MsERK1 as the first MAPK found in plant in 1993, genomic analysis had revealed the existence of more than 20 MAPKs in *Arabidopsis thaliana* and 17 MAPKs in rice that might be involved in the regulation of growth, development and responses to environmental stimuli (Asai et al. 2002; Duerr et al. 1993; Reyna and Yang 2006). In Arabidopsis, AtMPK18 specifically interacted with PHS1 and mediated cortical microtubule functions in directional cell expansion in plant roots (Walia et al. 2009). AtMPK12 was demonstrated to be a physiological

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substrate of MAPK phosphatases IBR5 and a negative regulator of auxin signaling (Lee et al. 2009). Meanwhile, *AtMPK3*, *AtMPK4* and *AtMPK6* from Arabidopsis and *OsMPK1, OsMPK5* and *OsMPK12* from rice were shown to participate in plant innate systems through regulation of salicylic acid- or jasmonic-acid/ethylene-dependent responses (Cheong et al. 2003; Han et al. 2010; Li et al. 2012; Ren et al. 2008; Song and Goodman 2002; Xiong and Yang 2003).

Reactive oxygen species (ROS) are central signaling molecules in multiple stress responses. A series of MAPK cascades were shown to be involved in ROS signaling (Kovtun et al. 2000). *AtMPK9* and *AtMPK12* were preferentially expressed in guard cells and acted downstream of ROS and cytosolic Ca^{2+} to positively regulate ROS-mediated abscisic acid (ABA) signaling (Jammes et al. 2009). *AtMPK8* had been hypothesized to be involved in monitoring or maintaining the essential part of ROS homeostasis by integrating ROS, Ca^{2+} and protein phosphorylation in the wound signaling pathway (Takahashi et al. 2011). Of note, *AtMPK3*, *AtMPK4* and *AtMPK6* could be rapidly and transiently activated upon oxygen deprivation and reoxygenation in Arabidopsis seedlings (Chang et al. 2012).

Cotton (*Gossypium* spp.) is one of the most important commercial crops, producing both fiber and oil. Biotic and abiotic stresses result in severe annual cotton yield losses. Study of MAPKs may contribute to uncovering the mechanism of cotton adaptability to environmental challenges. In the last decade, researchers had identified and characterized several genes encoding MAPKs in cotton. *GhMPK2* and *GhMPK16* had been proved to play important roles in osmotic stress tolerance, disease resistance and controlling the production of ROS (Zhang et al. 2011a, b; Shi et al. 2011). *GhMPK7* was implicated in plant development and SA-regulated broad-spectrum resistance (Shi et al. 2010). Moreover, *GhMPK6* was involved in ABA-induced *CAT1* expression and H_2O_2 production (Luo et al. 2011). Despite the demonstrations of cotton MAPKs participating in multiple signaling pathways and stress responses, the complex regulation mechanisms of cotton MAPKs are still unclear. In our previous work, an EST putatively encoded a MAPK, *GbMPK3*, was isolated from sea-island cotton (*Gossypium barbadense*) (Xu et al. 2011). In the present study, *GbMPK3* was shown to be induced by diverse abiotic stresses at the transcriptional level. The gene was introduced into tobacco (*Nicotiana benthamiana*) and the homozygous lines overexpressing *GbMPK3* were employed to determine its functional role in response to different abiotic stress conditions. The results showed that transgenic tobacco plants could enhance tolerance to drought and oxidative stress with elevated gene expression and enzymatic activity of antioxidant enzymes, which provided evidence for the involvement of *GbMPK3* in positively regulating tolerance to abiotic stress and controlling the production of ROS.

Materials and methods

Plant materials and abiotic stress treatments

Cotton seedlings (*G. barbadense* L. cv 7124) were cultured in Hoagland's solution at 22/25 °C (night/day) for 15 days and subjected to different abiotic treatments. For dehydration, seedlings were placed on dry filter papers. For cold and heat treatments, plants were removed into 4 and 42 °C incubators, respectively. For other abiotic stress treatments, seedlings were treated with 200 mM NaCl and 10 μM methyl viologen (MV, Invitrogen) containing Hoagland's solution, respectively. Tobacco (*N. benthamiana*) seeds were surface-sterilized and planted in 1/2 MS medium for germination. Three-week old seedlings were then transferred into soil in a climate chamber at 22/25 °C (night/day).

Cloning and sequence analysis of GbMPK3

Total RNA was extracted from '7124' roots using the guanidine thiocyanate method (Zhu et al. 2005). First strand cDNA was synthesized using an M-MLV reverse transcript system (Promega, USA), and DNA was extracted using a plant genomics DNA kit (Tiangen, China). The expressed sequence tag (EST) sequence of *GbMPK3* was isolated from a defense-related cDNA library of '7124' (Xu et al. 2011). The full-length sequence was obtained through the 5′- and 3′-rapid amplification of cDNA end (5′- and 3′-RACE) according to the GeneRacer Kit user manual (Invitrogen, USA) with '7124' cDNA as the PCR template. The open reading frame (ORF) was predicted with ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html); the molecular weight (Mw) and isoelectric point (pI) were predicted with the Compute pI/Mw tool (http://web.expasy.org/compute_pi/). Sequence similarity analysis was performed with DNAMAN software, and sequence alignments were completed by ClustalX and MEGA4 software with the neighbour-joining method. Plant MAPK proteins used for alignments were listed in Supplemental Table 1.

The genomic organization of *GbMPK3* was checked through southern blotting. The DNA (15 μg/sample) from three cotton species, including *G. arboretum* (AA genome), *G. trilobum* (DD genome) and *G. barbadense* (AADD genome), was digested with *Hin*dIII, *Xba*I and *Eco*RV for 48 h, respectively. The digested samples were then separated on 0.8 % agarose gels by electrophoresis and transferred onto a positively charged nylon membrane (MILLIPORE, USA). A probe of 367 bp in length from a

3′-terminal specific section of *GbMPK3* sequence was labeled with dCTP-[a-^{32}P] according to the manufacturer's instructions (Promega, USA). The Southern blotting was completed according to the protocol described previously (Liu et al. 2012).

Vector construction and plant transformation

The ORF fragment of *GbMPK3* was inserted into the vector pK2GW7.0 and introduced into *Agrobacterium tumefaciens* strain EHA105 for tobacco leaf disc transformation (Horsch et al. 1985). The seeds of regenerated plants were harvested and screened on 1/2 MS medium containing 50 μg/mL kanamycin. The positive seedlings from single insertion lines were selected for further study.

RT-PCR, QRT-PCR analysis and northern blotting

To determine the expression of *GbMPK3* in transgenic tobacco, 20 μg total RNA was transferred onto nylon membranes. The blots were hybridized with the 367 bp probe described above. To analyze related gene expression in transgenic tobacco, 2 μg total RNA was used for first-strand cDNA synthesis, and RT-PCR were performed at 95 °C for 3 min, followed by 28-32 cycles of amplification (95 °C for 20 s, 55–60 °C for 20 s, 72 °C for 20 s). The tobacco *Actin2* gene (*ACT*) was used as a control and PCR products were separated on 1 % agarose gel and stained with ethidium bromide (EB). QRT-PCR was performed with three replicates on an ABI 7500 Real Time PCR system (Applied Biosystems, USA) using SYBR green (Bio-Rad, USA). PCR cycles were as follows: 95 °C for 1 min, followed by 40 cycles of amplification (95 °C for 15 s, 60 °C for 50 s). The cotton *Ubiquitin7* gene (*UB7*) was used as the reference gene to normalize the amount of cDNA in each reaction. Relative changes were calculated with $2^{-\Delta Ct}$ using the SDS software from the 7500 Real Time PCR System (Livak and Schmittgen 2001; Schmittgen and Livak 2008). Primers used for PCR amplification were listed in Supplemental Table 2.

Subcellular localization

The *GbMPK3* gene was inserted into a GFP-fusion expression vector pGWB452, and introduced into tobacco leaves to determine the subcellular localization of GbMPK3 protein (Kumar et al. 2009). The vector 35S::GFP was used as a positive control. The 4,6-Diamino-2-phenyl indole (DAPI) was used for cell nuclear staining, and GFP expression was observed using a Microsystems TCS SP2 AOBS confocal microscope (Leica, Japan).

Drought and oxidative stress tolerance assay in transgenic tobacco plants

Wild-type (WT) and homozygous (T_2) transgenic tobacco seedlings were grown for 5 weeks in a culture room at 22/25 °C (night/day); after the cessation of watering for 14 days, plants were re-watered for a recovery period. Survival rate, plant height and number of wilting leaves were measured. Leaves at an early drought stage and 2 h after MV inoculation were used to assay the gene expression and enzymatic activity of ROS-scavenging enzymes. Hydrogen peroxide content was analyzed using an H_2O_2 Quantitative Assay Kit (Sangon Biotech, China). Diaminobenzidine (DAB) staining and ascorbic acid peroxidase (APX) activity assays were performed as described previously (Sun et al. 2010; Pan et al. 2012). To measure MV sensitivity, tobacco seeds were surface-sterilized and sown on 1/2 MS medium containing 0, 0.5, 1 and 2 μM MV, respectively. Seeds were considered germinated when radicles completely penetrated the seed coat, and germination rate was measured at the indicated time. In addition, sensitivity analysis to MV was carried out at vegetative stage. Leaf disks (1 cm in diameter) were incubated in solutions of various concentrations of MV (0, 1 and 3 μM) for 3 days, and chlorophyll content was measured (Sun et al. 2012).

Results

Cloning and characterization of *GbMPK3*

The full-length *GbMPK3* cDNA sequence is 1,609 bp in length, including a 61 bp 5′-untranslated region (UTR), a 420 bp 3′-UTR and a 1,128 bp ORF which encode a predicted protein of 375 amino acids with an pI of 5.50 and a putative molecular mass of 43.02 kDa. This protein has a TEY motif at the phosphorylation site between subdomains VII and VIII, a common docking (CD) domain at the C-terminal end and 11 highly conserved subdomains with moderate sequence similarity to AtMPK3 (*A. thaliana*), NtWIPK (*Nicotiana tabacum*), AhMPK3 (*Arachis hypogaea*) and PtMPK3-1 (*Populus trichocarpa*) (Fig. 1). Phylogenetic analysis was performed and GbMPK3 was classified as a group A MAPK (Fig. 2a). A total of 3,009 bp of *GbMPK3* genomic sequence was isolated from '7124'. Sequence comparison between *GbMPK3* in the genome and the actual transcript revealed that the *GbMPK3* gene contains six exons and five introns, which is consistent with homologous genes from other plant species. In spite of the similarity in exon lengths and intron phases, the length of introns has large discrepancies between different plant species (Fig. 2b). The genomic constitution of

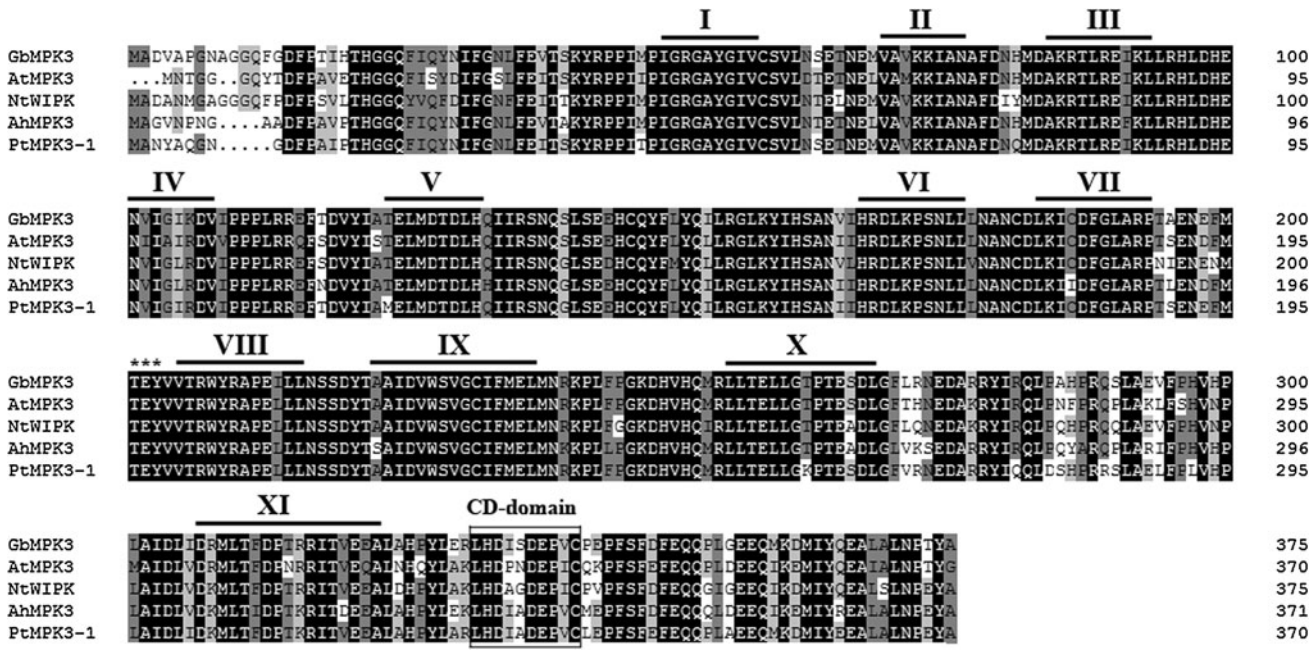

Fig. 1 Alignment of GbMPK3. Alignment of deduced GbMPK3 amino acids with AtMPK3 (*A. thaliana*, NP_190150), NtWIPK (*N. tabacum*, AB052964), AhMPK3 (*A. hypogaea*, DQ068453) and PtMPK3-1 (*P. trichocarpa*, XM_002313981). Identical amino acids are shaded in black, the 11 subdomains are indicated by Roman numerals, the TxY motif is marked with an asterisk, and the CD-domain is boxed

*GbMPK*3 in different cotton species was explored by southern blot. The results indicated that at least one locus was detected in diploid cotton *G. arboretum* and *G. trilobum*, but two loci were identified in the allotetraploid cotton *G. barbadense* (Fig. 2c).

The homologues of GbMPK3 from other plant species have been shown to be phosphorylated and targeted to both nucleus and cytoplasm in previous studies (Yap et al. 2005; Kumar et al. 2009). To investigate subcellular localization of GbMPK3 protein, the *35S-GFP::GbMPK3* and *35S-GFP* constructs were completed (Fig. 3a) and introduced into tobacco leaves. As shown in Fig. 3b, the positive control GFP distributed throughout the whole cell, whereas the GFP-GbMPK3 fusion protein mainly accumulated in the nucleus.

GbMPK3 responds to multiple abiotic stresses

The expression pattern of *GbMPK3* in cotton tissues under normal conditions was evaluated by RT-PCR, and the results showed that *GbMPK3* was preferentially expressed in roots, petals, anthers and fibers. Moderate expression was observed in the hypocotyls, cotyledons and ovules. A low level of expression was detected in the leaves (Fig. 4a). Total RNA was extracted from cotton seedlings after exposure to NaCl, MV, dehydration, cold and heat. QRT-PCR was employed to determine whether the expression of *GbMPK3* was affected by abiotic stress, and *GbMPK3* was up-regulated upon all abiotic stresses tested in our study. A similar expression pattern of *GbMPK3* was found between cold (4 °C), heat (42 °C) and 10 μM MV treatments, while more extreme change in expression levels were identified under the 200 mM NaCl and dehydration treatments (Fig. 4b). The expression was strongly induced by NaCl in the 30 min following treatment, while a continuous increase in transcript level was found upon dehydration. These results suggested that *GbMPK3* was involved in the responses of cotton to multiple abiotic stresses.

Overexpression of *GbMPK3* in tobacco enhances drought tolerance

To investigate its role in abiotic stress responses, *GbMPK3* under the control of the CaMV 35S promoter was introduced into tobacco. Two independent homozygous T_2 lines (OE27 and OE28) with relatively high expression levels of *GbMPK3,* which were confirmed by northern blotting, were selected for further study (Fig. 5a).

Because *GbMPK3* could be strongly and continually induced by dehydration, *GbMPK3*-overexpressing tobacco

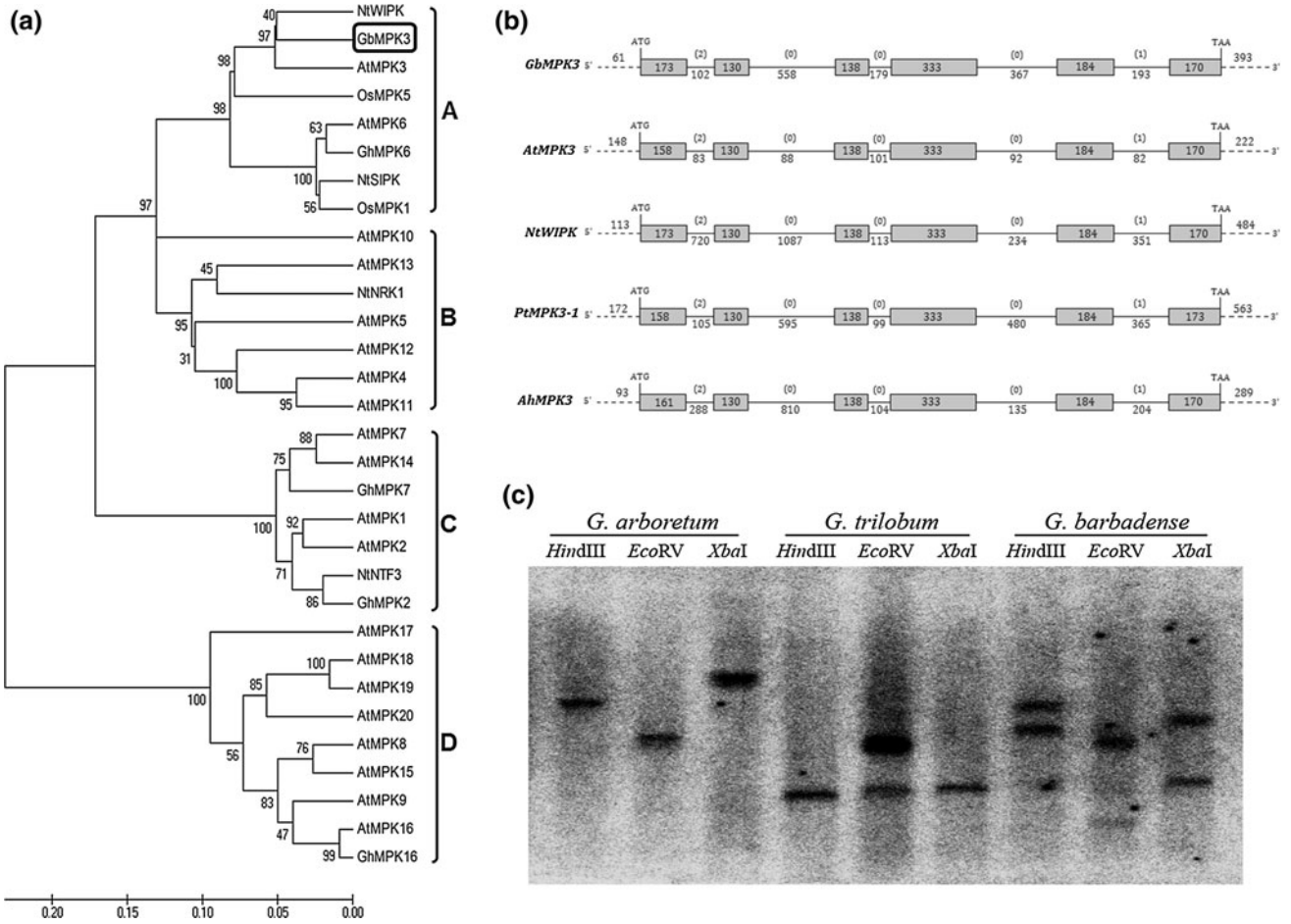

Fig. 2 Sequence analysis of *GbMPK3*. **a** The phylogenetic relationship of GbMPK3 with MAPK family members from other plant species; numbers above or below the branches indicate bootstrap values (>50 %) from 1,000 replicates. **b** Schematic representation of *GbMPK3* gene structure and its comparison with *AtMPK3*, *NtWIPK*, *AhMPK3* and *PtMPK3-1*. Introns are represented by *lines* and exons by *boxes*. The lengths of individual exons, introns and UTRs are given in base pairs. *Numbers* between *brackets* correspond to the intron phase. Drawings are not to scale. **c** Genomic organization of *GbMPK3* in different cotton varieties

and the WT plants were grown under the cessation of watering for 14 days to explore the effect of *GbMPK3* overexpression on drought tolerance. Significant differences were found in leaf wilting between the WT and the transgenic plants from 10 to 13 days after water deprivation (Fig. 5b, c). The WT plants showed a reduced water-retaining ability and an increased rate of leaf wilting compared with OE27 and OE28. Meanwhile, after 2 days of recovery by re-watering, obvious differences in plant height were also observed between the transgenic and WT plants (Fig. 5d). The overexpression of *GbMPK3* in tobacco helped to maintain the growth of the plants under drought conditions. As a result, the survival rate of the transgenic plants was twice that of WT (Fig. 5e), which suggested that plant growth was seriously influenced by drought stress and that the overexpression of *GbMPK3* improved drought tolerance.

Overexpression of *GbMPK3* enhances ROS scavenging ability of tobacco under drought stress

Antioxidant enzymes maintain cellular ROS homeostasis during abiotic stress. Therefore, the expression patterns of genes involved in ROS scavenging under drought treatment were analyzed in WT and transgenic plants. The transcriptional levels of glutathione S-transferase (*NbGST*) and catalase (*NbCAT*) were slightly increased, while *NbAPX* was more significantly up-regulated in transgenic seedlings compared with WT following drought treatment (Fig. 6a). This result was further confirmed by enzymatic activity analysis of APX (Fig. 6b). Interestingly, the concentration of H_2O_2 was a little lower in WT than in transgenic plants under normal conditions (Fig. 6c). However, the content of H_2O_2 was significantly higher in WT plants compared with the transgenic plants under drought stress, which was also

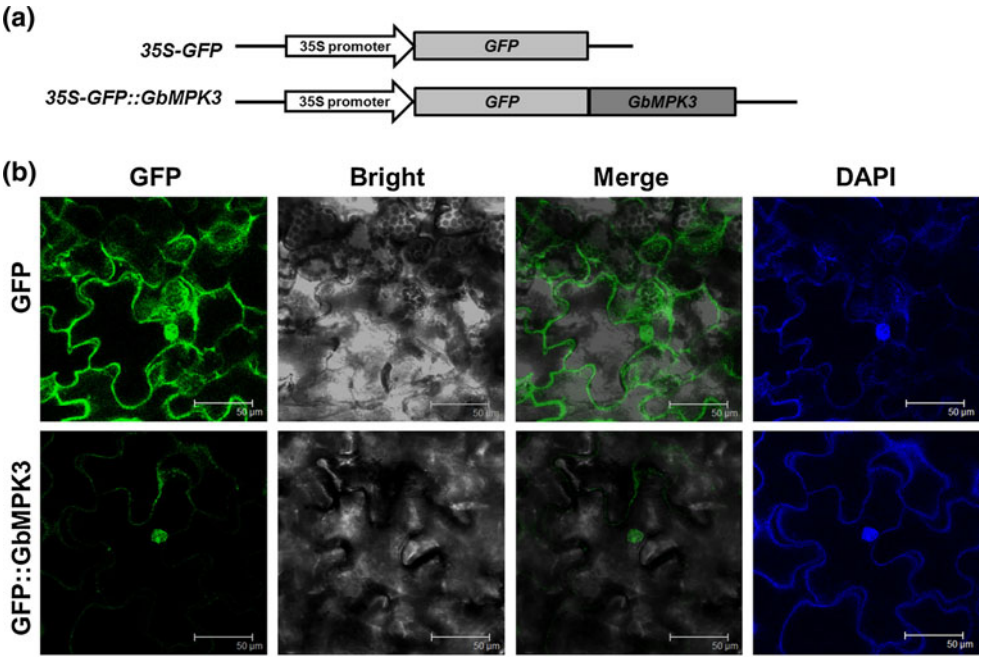

Fig. 3 Subcellular localization of GbMPK3. **a** Schematic diagram of *GFP* and *GFP::GbMPK3* fusion constructs. **b** Transient expression of GFP and GFP::GbMPK3 fusion proteins were visualized 48 h post agroinfiltration in tobacco leaves, DAPI was used for nuclear staining

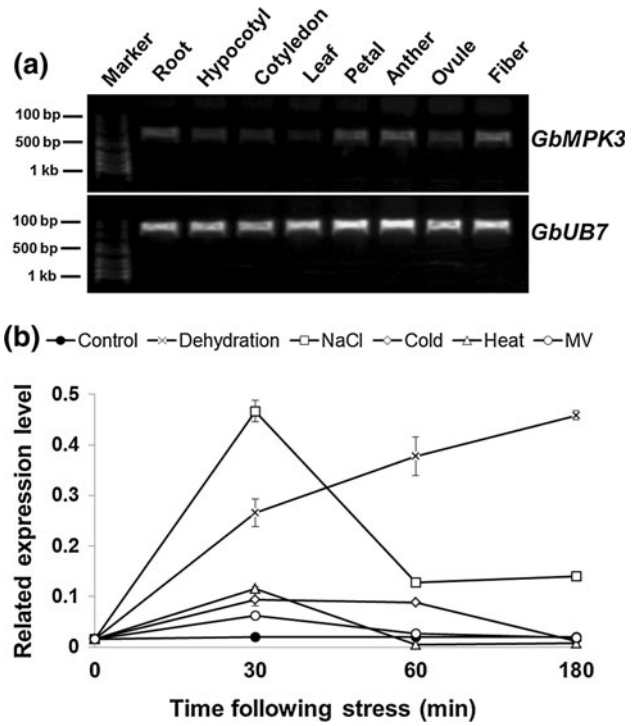

Fig. 4 Expression profiles of *GbMPK3*. **a** Relative expression level of *GbMPK3* in different cotton tissues are shown by RT-PCR. **b** Expression profiles of *GbMPK3* in cotton seedlings under dehydration, cold (4 °C), heat (42 °C), 200 mM NaCl and 10 μM MV treatments by QRT-PCR, The cotton *UB7* gene was used as the reference gene

confirmed by DAB staining (Fig. 6c, 6d). These results suggested that the overexpression of *GbMPK3* may enhance the ROS scavenging capacity under water shortages.

Enhanced oxidative stress tolerance in *GbMPK3*-overexpressing plants

As an inducer of oxidative stress, MV could inhibit electron transport during photosynthesis, thereby generating H_2O_2 in plants (Ning et al. 2010). Because *GbMPK3* could be induced by MV and transgenic tobacco showed increased ROS scavenging capacity under drought stress, the response to MV was also investigated in transgenic plants. No obvious difference was found in the germination rates of OE27, OE28 and WT seeds under normal conditions or upon 0.5 μM MV treatment on day 3 after sowing. All transgenic and WT seeds suffered severely in the presence of 1 μM or more MV. Approximately 60 % of the seeds of both transgenic lines germinated in the presence of 2 μM MV, while only 25 % of the WT seeds germinated under this condition (Fig. 7a, 7b). Similar results were observed in 2-month-old transgenic and WT plants when they were subjected to MV treatment. The leaves from the transgenic lines remained green under 1 μM MV treatment for 3 days. Meanwhile, obvious chlorosis occurred in WT leaves (Fig. 7c), which was also observed in the measurements of chlorophyll content (Fig. 7d). Furthermore, the genes involved in ROS scavenging showed similar expression patterns after MV treatment compared to drought treatment (Fig. 6a, 7e).

Discussion

Plants have developed complex and efficient signal transduction networks to cope with the continual challenges of

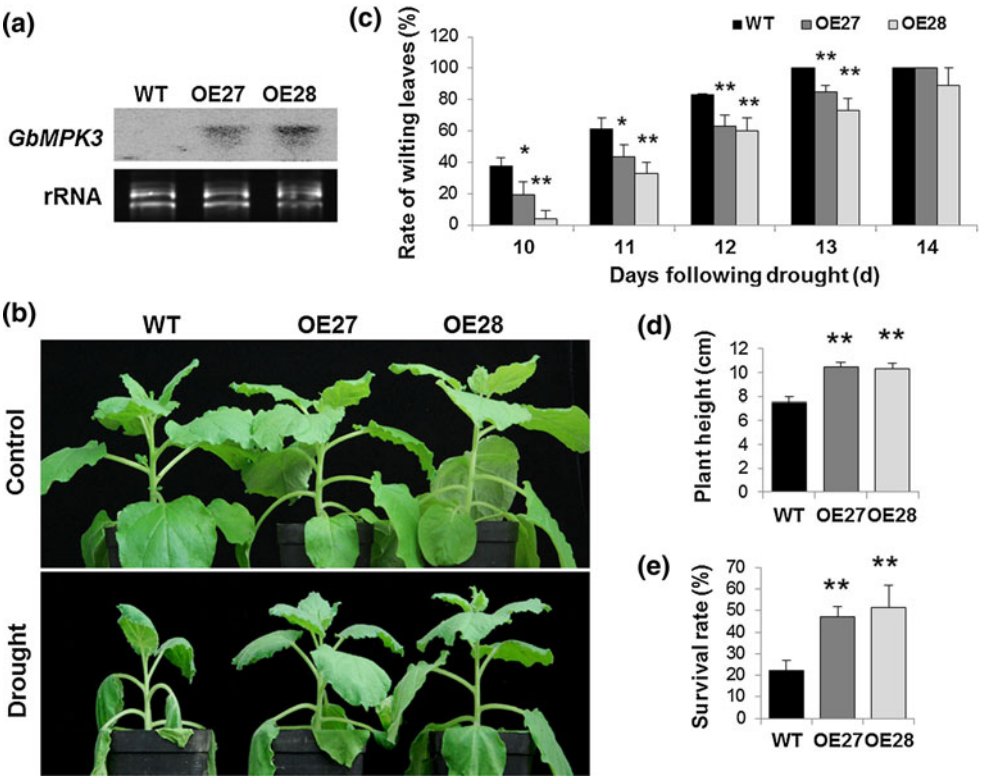

Fig. 5 Enhanced drought tolerance of *GbMP3*-overexpressing tobacco lines. **a** Expression analysis of *GbMPK3* in transgenic tobacco through Northern blot. **b** Transgenic tobacco under normal and drought conditions for 10 days. **c** Measurement of plants wilting leaves during drought treatment. Comparison of height (**d**) and survival rate (**e**) of the plants from 14 days drought after re-watering; standard deviations were calculated from the results of three independent experiments (*$0.01 \leq P < 0.05$, **$P < 0.01$)

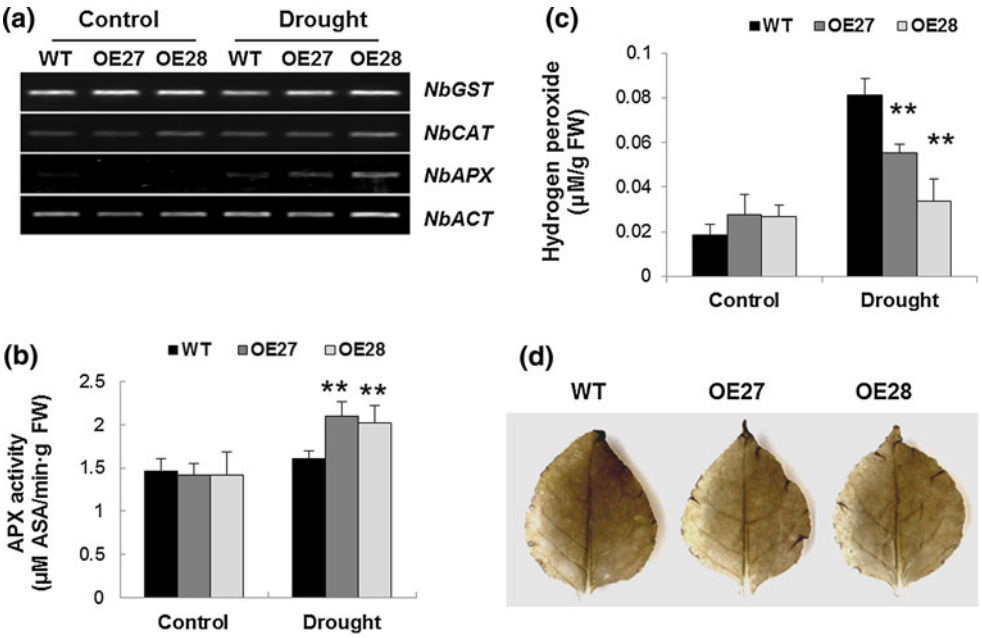

Fig. 6 ROS accumulation, antioxidant enzymes expression and activity in WT and transgenic tobacco under normal and drought conditions. **a** Relative expression of ROS-scavenging enzymes, the tobacco *Actin2* gene was employed as the internal control. **b** APX activity in WT and transgenic plants under normal and drought conditions. **c** Analysis of H_2O_2 accumulation in WT and transgenic plant leaves on day 8 during drought treatment. **d** H_2O_2 accumulation visualized by DAB on day 8 during drought treatment. Standard deviations were calculated from the results of three independent experiments (*$0.01 \leq P < 0.05$, **$P < 0.01$)

an unfavorable environment. MAPK cascades are a vital link between extracellular stimuli and intracellular responses (Pitzschke et al. 2009; Zhang and Klessig 2001). However, the involvement and significance of MAPK cascades in plants responsive to biotic and abiotic stresses are still not fully elucidated. In this work, a MAPK gene, *GbMPK3*, which could be induced at the transcriptional level upon abiotic stresses, was cloned and overexpressed in tobacco. The putative roles of *GbMPK3* in salt, cold and drought were then investigated. To our surprise, phenotypic differences between WT and transgenic plants were only observed under drought treatment.

The mechanisms of drought tolerance in plants are complex. ABA is considered a key signaling molecule that

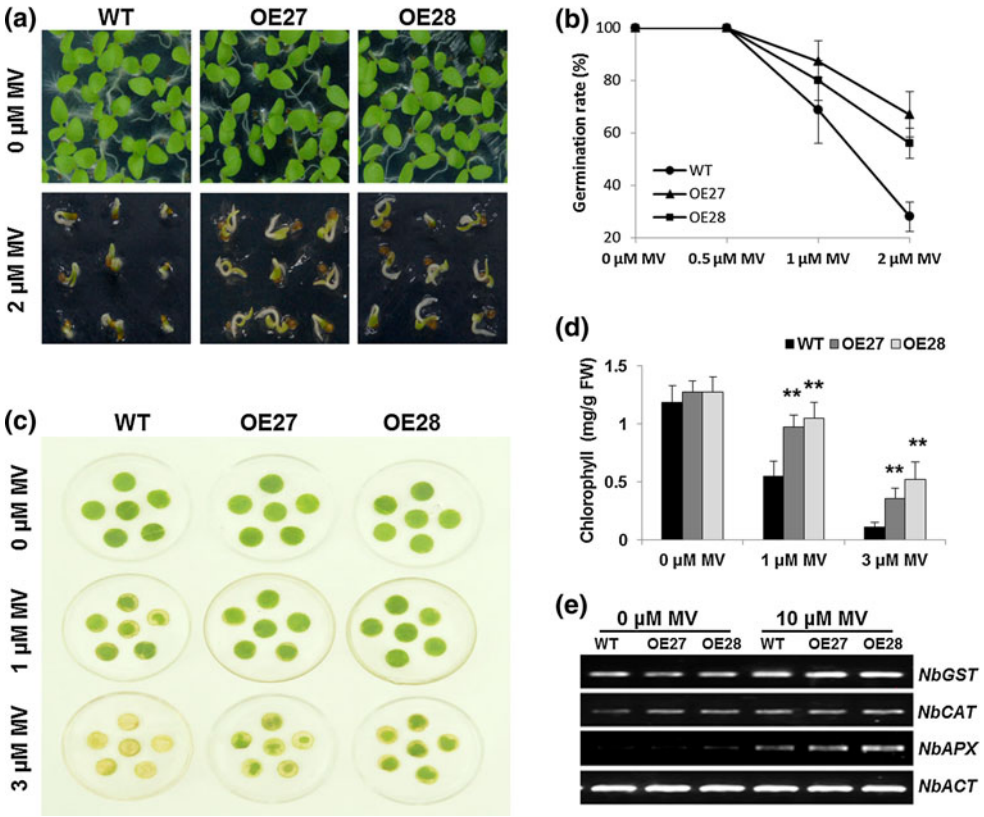

Fig. 7 Oxidative stress tolerance in transgenic tobacco plants. **a** Photographs of seedlings grown on media containing 0 and 2 μM MV for 6 days. **b** Germination rate of the seeds grown on media containing 0, 0.5, 1 and 2 μM MV for 3 days. **c** Leaf disks of WT and transgenic plants incubated in water supplemented with or without different concentrations of MV. **d** Chlorophyll content of leaf disks while leaf disks floating in water serve as a control. **e** Relative expression of ROS-scavenging enzymes in leaves treated with 10 μM MV, the tobacco *Actin2* gene was employed as the internal control. Standard deviations were calculated from the results of three independent experiments (*$0.01 \leq P < 0.05$, **$P < 0.01$)

controls stomatal movement to protect against drought stress (Sirichandra et al. 2009; Wang and Song 2008). Recent studies suggested that a variety of MAPKs may be involved in ABA-dependent drought tolerance, including the newly identified cotton MAPKs, *GhMPK2* and *GhMPK6a* (Zhang et al. 2011a; Li et al. 2013). The overexpression of *GbMPK3* in tobacco resulted in enhanced drought tolerance. However, ABA sensitivity of the seeds and seedlings and ABA accumulation under water deprivation were not enhanced by overexpression of *GbMPK3* in tobacco in our research (data not shown), which suggests that *GbMPK3* may mediate tobacco drought tolerance in an ABA-independent manner.

ROS are also involved in plant drought tolerance, and their functions in plant responses to biotic and abiotic stresses are dose-dependent. Within the adaptive concentration range of the plant, ROS could act as signaling molecules for the initiation of cellular responses through ROS-responsive signaling pathways. High concentrations of ROS would ultimately result in oxidative stress and cell damage in the absence of rapid scavenging by antioxidant enzymes (Huang et al. 2012; Jaspers and Kangasjarvi 2010; Mullineaux and Baker 2010). The significant involvement of MAPKs in ROS-related signaling pathways had been described previously (Chang et al. 2012; Kovtun et al. 2000; Luo et al. 2011). In addition, upstream genes of MAPKs were also identified to be implicated in plant abiotic stress resistance. *AtMKK4*-overexpressing plants exhibited lower water-loss rates and accumulated fewer ROS under dehydration conditions (Kim et al. 2011). MKK5 was a known cognate MAPKK that activates MPK3 and MPK6. When *MKK5* was suppressed in *Arabidopsis*, the activation of MPK3 and MPK6 was markedly reduced. Furthermore, *MKK5*-suppressed plants were hypersensitive to ozone and showed visible leaf damage and elevated concentrations of leaf-localized H_2O_2 upon ozone treatment (Miles et al. 2009). Despite the increasing evidence showing that MAPK cascades contribute to drought and oxidative stress tolerance in plants (Shou et al. 2004), more research is still needed.

In this study, we showed that the constitutive expression of *GbMPK3* increased plant tolerance to drought. Furthermore, the improved tolerance was associated with increased gene expression and enzymatic activity of APX in transgenic tobacco under drought stress. APX is a major antioxidant in higher plants that protects plant cells from oxidative damage when water is depleted (Verslues et al. 2006; Shigeoka et al. 2002). It had been reported that MAPKs were responsible for the activation of antioxidant enzymes in plants (Samuel 2002; Zong et al. 2009), which was also demonstrated in this study: the up-regulation of *GbMPK3* was responsible for the increased *APX* transcripts under the oxidative stress generated by MV treatment. It was interesting that the expression patterns of ROS scavenging-related genes of plants under drought stress were

similar to those of plants under MV treatment, suggesting a common signaling pathway between the drought and the oxidative stress responses mediated by *GbMPK3*. Thus, we speculated that the increased drought tolerance in *GbMPK3*-overexpressing tobacco might be attributed to enhanced APX activity that protects the transgenic plants from ROS damage.

In conclusion, this study showed that *GbMPK3* was up-regulated by diverse abiotic stresses and likely played a role in drought and oxidative stress tolerance in tobacco by regulating gene expression and the activities of ROS-scavenging enzymes.

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