The sucrose non-fermenting 1-related protein kinase SAPK2 enhances rice yield under drought conditions

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Original article

Abstract

Background: Drought stress is an important factor limiting crop productivity worldwide. Rice is critical for food security because it is consumed by more than half of the global population. Thus, enhancing the drought tolerance of rice is crucial for ensuring the production of this important crop can satisfy the demands of future generations.

Results: Compared with wild-type plants, the *sapk2* rice mutant lines were shorter and produced fewer grains per panicle and smaller grains. Subsequent analyses suggested that SAPK2 considerably influences the nitrate, phosphorus, and potassium contents of rice grains. The examination of rice seedling growth and development under nutrient-deprived conditions (−K, −N, and −P) proved that SAPK2 can significantly affect rice seedling growth and root development in hydroponic cultures lacking N and K^+. Moreover, the NO_3^- influx rate and nitrate concentration of the analyzed plant materials indicated that SAPK2 promotes nitrate uptake and assimilation and influences the number of tillers and the number of grains per panicle by regulating nitrate-related transporters.

Conclusion: These results suggest that *SAPK2* is a key target gene for rice breeders aiming to increase yield.

Background

The sustainability of crop production has been challenged by climate changes, an insufficient freshwater supply, and an increasing global population (Comas et al. 2013; Gray and Brady 2016). Additionally, drought stress, which is an important abiotic factor limiting crop productivity worldwide, is responsible for extensive crop losses and will likely worsen in the near future. Thus, there is increasing international interest in developing drought-tolerant crops. In rice, plant architecture and grain yield are affected by environmental conditions and genetics. Enhancing the drought tolerance of rice is challenging because of the complexity of this trait and the incomplete characterization of the physiological and molecular mechanisms associated with drought responses. Accordingly, considerable work is required to address the adverse effects of drought stress and ensure the food security of future generations.

Drought stress can affect plants at all growth stages, and the extent of the changes to productivity depend on the plant species and its genotype, age, and size as well as the duration and severity of the stress (Gall et al. 2015). Plant growth is highly sensitive to water deficit, largely because of the resulting inhibition of cell elongation. Water-stressed plants are shorter than normal and have a smaller leaf area, which decreases the amount of photosynthetically active radiation absorbed by leaves, the photosynthetic rate, and ultimately yield. Water deficit induces stomatal closure, which leads to limited CO_2 uptake by leaves and decreased net photosynthesis (Yang et al. 2017). It also affects carbohydrates, the ATP content, respiration, and abscisic acid (ABA). Moreover, it leads to the excessive accumulation of reactive oxygen species, resulting in oxidative stress that seriously damages cellular functions (Deeba et al. 2012). In addition to its direct impact on plants, drought stress also has various indirect effects on

crop growth and yield. Nutrients, especially macronutrients, are very important for plant growth and yield, and their uptake is restricted under drought stress conditions (Aroca 2012). The uptake of nitrogen (N) and potassium (K) reportedly decreases in cotton plants exposed to drought stress (McWilliams 2003). The concentration of growth-retarding substances usually increases under stress conditions to adjust plant water levels for various processes (Farooq et al. 2009).

Plant responses to drought stress include stress signal perception, signal transduction and amplification, and adaptations at the morphological, physiological, and molecular levels. Phytohormones are the key mediators of plant responses to drought stress (Sah et al. 2016). Water deficit also alters the endogenous synthesis of various phytohormones, including jasmonic acid (JA), ABA, salicylic acid (SA), ethylene (ET), auxin, gibberellins (GAs), and cytokinins (CKs). Abscisic acid is the major stress-responsive hormone produced after the drought signal is perceived by plants. Osmotic stress promotes ABA synthesis, which activates gene expression and adaptive physiological changes (Yamaguchi-Shinozaki and Shinozaki 2006). Additionally, ABA remains the best-studied hormone for plant stress responses, and the interaction between ABA and other classical stress-related hormones enables plants to rapidly respond and properly adapt to drought stress (Brodribb and McAdam 2017). The ABA-dependent signaling pathways are critical for the expression of genes responsive to various stresses, especially osmotic stress. The SnRK2s are protein kinases that promote ABA responses (Hrabak et al. 2003). To date, 10 plant-specific SnRK2s have been identified in Arabidopsis thaliana (SnRK2.1–2.10) and rice (SAPK1–10) (Kobayashi et al., 2004). In A. thaliana, SnRK2.2, SnRK2.3, and SnRK2.6 are involved in ABA responses (Fujii and Zhu 2009). Additionally, in rice, members of SnRK2 subclass III (SAPK8–10), as well as SAPK2, help regulate ABA-dependent gene expression via the ABA signal transduction pathway mediated by OsPYL/RCARs and PP2Cs (Kim et al. 2012).

A recent study confirmed that the overexpression of SAPK9 may significantly enhance drought tolerance, while also increasing the grain yield under drought conditions (Dey et al. 2016). Previously, SAPK2, a subclass II member, was confirmed as a positive regulator of drought resistance in rice (Lou et al. 2017). These reports indicate that SAPK2 may be useful for improving crop yields under drought conditions. However, the effect of SAPK2 on the productivity of drought-stressed plants remains unclear.

In this study, we characterized a rice sapk2 mutant, which produced fewer grains per panicle and smaller grains than the wild-type (WT) control plants. To confirm that the SAPK2 function influences rice yield, we examined knock-out mutant lines (sapk2; sapk2-1 and sapk2-7), which we previously developed with the CRISPR/Cas9 system, and SAPK2-overexpressing lines (OE; OES2-1 and OES2-2). Our analyses revealed that SAPK2 positively regulates rice grain number and size.

Results

SAPK2 considerably affects plant height and grain number

Under drought conditions, the two sapk2 lines were shorter than the WT control plants (Fig. 1a–c). To further investigate the SAPK2 roles influencing rice yield, we analyzed OE lines (OES2-1 and OES2-2) and sapk2 knock-out mutant lines (sapk2-1 and sapk2-7). An analysis of plants exposed to reproductive stage drought stress (RS) indicated that the two sapk2 lines had substantially more tillers than the WT plants (Fig. 1a, d), whereas there were no significant differences in the two OE lines (Fig. 1b–d). However, although they had more tillers, the sapk2 mutant plants produced fewer grains per plant than the WT controls under drought conditions (Fig. 1f). A subsequent investigation of the regulatory effect of SAPK2 on the number of effective tillers in rice revealed that the sapk2 mutant lines had considerably fewer effective tillers than the WT plants, but there were no significant differences in the two OE lines (Fig. 1e). This result further confirmed that the sapk2 mutant produces fewer grains than WT plants under drought conditions.

Overall, our observations indicated that a lack of SAPK2 expression significantly decreases rice plant height and the number of grains per plant under drought conditions. Additionally, overexpressing SAPK2 does not appear to enhance rice plant growth or grain production.

Mutations to SAPK2 decrease grain yield

The number of grains per panicle is one of the three key factors determining rice grain yield (Xing and Zhang 2010). Thus, we investigated the SAPK2 roles related to panicle and grain development by analyzing the number of grains per panicle, the seed setting rate, the grain length and width, and the 1,000-grain weight in response to RS. The panicles and grains of the sapk2 mutant lines were smaller than those of the WT controls (Fig. 2a). Additionally, the average number of grains per panicle of the sapk2 mutant lines was 75% and 60.8% of that of the WT (Fig. 1e) and OE (Fig. 2b) plants. The seed setting rate of the OE lines did not differ from that of the WT plants, whereas the seed setting rate of the sapk2 mutant lines decreased significantly (76.9% of that of the WT plants) (Fig. 2c). Compared with the WT grains, the grains of the OE lines were significantly longer, whereas there was no significant difference in the grain length of the sapk2 mutant lines (Fig. 2d). In contrast, the grains of the sapk2 mutant lines were significantly thinner than the WT grains, whereas the grain width of the OE lines was not significantly different (Fig. 2e). Moreover, the 1,000-grain weight was much lower for the sapk2 mutant lines than for the WT and OE plants (Fig. 2f) (31.6% lower than that of WT). These results implied that SAPK2 influences panicle and grain sizes in rice.

Mutations to SAPK2 decrease nitrate, phosphorus, and potassium contents in rice grains under reproductive-stage drought

Umezawa et al. (2004) reported that the expression of SnRK2.8, which is a homolog of rice SAPK2, is down-regulated by potassium deprivation. This down-regulation is associated with a substantial

decrease in the growth of A. thaliana under nutrient-deprived conditions. As mentioned earlier, SAPK2 influences rice panicle and grain sizes. To clarify the mechanisms by which SAPK2 influences panicle and grain sizes in rice, we measured the nitrate, phosphorus, and potassium contents of seeds. Under RS conditions, the seeds of the sapk2 mutant lines had lower nitrate, phosphorus, and potassium concentrations than the WT controls, with the biggest difference observed for the nitrate concentration (Fig. 3a–c). However, the nitrate, phosphorus, and potassium concentrations were relatively consistent between the OE and WT plants (Fig. 3a–c).

Next, we investigated the SAPK2 expression profiles under control conditions (i.e., sufficient nutrients) and nutrient-deficient conditions [i.e., lacking K^+ (− K), N (− N), and P (− P)]. The qRT-PCR analyses revealed that the SAPK2 transcript levels in the roots decreased in the absence of N, P, and K^+ (Fig. 3d–f). These findings confirmed that in rice, the seed nitrate, phosphorus, and potassium contents are largely affected by SAPK2. Therefore, we hypothesized that SAPK2 influences panicle and grain sizes by modulating metabolic processes involving N, P, and K^+.

SAPK2 affects seedling growth and root development in response to N and K^+ deprivation

To support our hypothesis, we investigated the effects of knocking out and overexpressing SAPK2 on rice seedling growth and development in hydroponic cultures under different nutrient-deprived (− K, −N, and −P) conditions.

The sapk2 mutant seedlings under N-deprived conditions produced weaker culms than the WT plants (Fig. 4a, f), whereas the OE lines were phenotypically similar to the WT controls (Fig. 4a; the OE phenotype is not presented). A previous study proved that the root morphology influences plant interactions with soil nitrates, making it important for N absorption (Hachiya and Sakakibara 2017). Accordingly, we examined the root development of the SAPK2 transgenic lines. In the sapk2 mutant lines, root growth was inhibited, resulting in roots that were shorter than those of WT seedlings (Fig. 4b). In contrast, the root phenotypes of the OE and WT plants were similar (Fig. 4b; OE phenotype is not presented). The root and shoot dry weights of the sapk2 mutant lines were significantly lower than the corresponding WT weights, but there were no significant differences in the OE lines (Fig. 4c, d). Similarly, compared with the WT plants, the sapk2 mutant lines had fewer roots, whereas there were no significant differences in the number of roots in the OE lines (Fig. 4e).

The effects of the K^+-deprived conditions were similar to those of the N-deprived conditions. For example, the sapk2 mutant seedlings produced weaker culms and had lower root and shoot dry weights than the WT plants (Fig. 5a–f). In contrast, the exposure to P-deprived conditions did not result in any significant differences in the seedling growth and root development of the WT, OE, and sapk2 seedlings (40 days after germination; Additional file 1, Fig. S1a–f).

These findings suggested that SAPK2 can significantly influence rice seedling growth and root development in hydroponic cultures under N- and K$^+$-deprived conditions.

SAPK2 influences the NO$_3^-$ influx rate and nitrate concentration under drought stress conditions

To explore the potential mechanism underlying the effects of SAPK2 on rice seedling growth and root development under N-deprived conditions, we investigated the NO$_3^-$ influx rate and nitrate concentration of the WT, OE, and sapk2 plants under control and drought conditions. Under control conditions, there were no significant differences among the WT, OE, and sapk2 plants (Fig. 6a, c). However, in response to drought stress, the NO$_3^-$ influx rate and nitrate concentration significantly decreased (relative to the values under control conditions) in the WT, OE, and sapk2 plants (Fig. 6a–d). Additionally, the rate of NO$_3^-$ influx into the roots was lower for the sapk2 mutant lines than for the WT plants (Fig. 6b), suggesting that silencing SAPK2 expression weakens the nitrate uptake by the roots. Regarding the sapk2 mutant lines, we also detected a lower rate of NO$_3^-$ influx into the leaf sheath and leaf blade, implying that SAPK2 promotes the translocation of NO$_3^-$ from the roots to the leaf sheath (Fig. 6b). Moreover, the root, leaf sheath, and leaf blade nitrate concentrations were consistent with the NO$_3^-$ influx rates in the different lines (Fig. 6d). These results demonstrated that SAPK2 enhances nitrate influx and increases the nitrate concentration by promoting the translocation of nitrate from the roots to the leaf sheath.

Nitrogen use efficiency is an important trait for the development of sustainable agricultural production (Xu et al. 2012). Plants have diverse transporters facilitating N uptake and internal distribution (Rentsch et al. 2007). In higher plants, members of the NPF family (previously called the PTR/NRT1 family) can take up and translocate nitrate or small peptides. Of the rice NPF family members, only a few have been studied. For example, OsNPF7.2, which encodes a positive regulator of nitrate influx and concentration, helps control the allocation of nitrate between the roots and shoots (Wang et al. 2018). In rice, the peptide transporter OsNPF7.3 (OsPTR6) mediates the transport of organic N from the leaves to the grains and increases the grain yield (Fang et al., 2017). Additionally, OsNPF6.5 (OsNRT1.1B) is predominantly expressed in the root hairs, epidermis, and stellar cells adjacent to the xylem in roots. The osnrt1.1b mutant is reportedly defective in both nitrate uptake and root-to-shoot nitrate transport, suggesting that OsNRT1.1B is involved in nitrate uptake and transport (Hu et al., 2015). Down-regulating OsNRT2.3a expression impairs the loading of nitrate into the xylem and inhibits plant growth under low-nitrate conditions, implying OsNRT2.3a contributes to the long-distance transport of nitrate from the roots to the shoots (Tang et al., 2012). The silencing of OsNPF2.4 diminishes the low-affinity nitrate acquisition by roots, disrupts the K-coupled root-to-shoot nitrate transport, and inhibits the redistribution of nitrate from old leaves to N-starved roots or young leaves (Xia et al., 2015).

To further validate our hypothesis that SAPK2 influences panicle and grain sizes by modulating metabolic processes involving N, we determined the expression levels of genes crucial for the absorption, transport, and assimilation of nitrate among the WT, OE, and sapk2 plants cultured under control and drought conditions. The OsNPF7.2, OsNPF7.3, OsNPF5.6, OsNPF2.2, OsNRT2.3a, and OsNPF2.4 expression levels were significantly lower in the sapk2 mutant lines than in the WT plants under drought conditions (Fig. 7a–f). However, the opposite expression patterns were detected for the OE lines (Fig. 7a–f). These results implied that SAPK2 promotes nitrate uptake and assimilation by regulating nitrate-related transporters.

Discussion

Mutations to SAPK2 decrease grain yield

Drought is one of the most important environmental stresses affecting the productivity of most field crops (Gray and Brady 2016). Elucidating the complex mechanisms underlying the drought resistance of crops will accelerate the development of new varieties with enhanced drought resistance. Improving the grain yield is the primary aim for most rice breeders. The rice yield potential is determined by the biomass and harvest index.

To identify additional rice grain yield-related genes affected by drought stress, we functionally characterized SAPK2 by examining sapk2 mutant lines exposed to drought stress. Under RS conditions, the sapk2 mutant plants produced fewer effective tillers and grains per plant than the WT and OE plants. Rice grain yield is determined by the following three traits: number of panicles, number of grains per panicle, and grain weight (Xing and Zhang 2010). Consequently, we were interested in whether SAPK2 can be used to improve rice yields under RS conditions.

We also investigated the SAPK2 roles associated with panicle and grain development in response to RS conditions. Specifically, the panicle size, number of grains per panicle, grain size, seed setting rate, and 1,000-grain weight were significantly lower for the sapk2 mutant lines than for the WT plants (Fig. 2a–f). These results indicated that SAPK2 increases rice yields under RS conditions by influencing panicle and grain sizes.

SAPK2 affects seedling growth and root development in response to N and K $^+$ deprivation

A previous study revealed that SRK2C/SnRK2.8 may mediate the phosphorylation of enzymes involved in metabolic processes (Umezawa et al., 2004). To investigate whether the decrease in the grain yield of the sapk2 mutant lines under RS conditions is related to nutrient metabolism, we measured the seed nitrate, phosphorus, and potassium concentrations. The data indicated the sapk2 mutant seeds had lower nitrate, phosphorus, and potassium concentrations than the WT seeds, with the difference especially

pronounced for the nitrate concentration (Fig. 3a−c). Moreover, SAPK2 expression in the roots was down-regulated in response to N, P, and K^+ deprivation (Fig. 3d−f). These findings indicate that SAPK2 influences panicle and grain sizes via its effects on metabolic processes involving N, P, and K^+.

The roots, which are responsible for the uptake of water and nutrients from the soil, are vital for plant growth, development, and fitness. Additionally, ABA controls several key steps associated with lateral root initiation as well as meristem activation and elongation (De Smet et al. 2003; Ding and De Smet 2013). Root development is directly affected by environmental factors. Furthermore, the plant root system architecture is plastic and dynamic, enabling plants to respond to environmental changes, which then promotes root growth and development to avoid water deficit stress in the early stages of drought stress. Nitrogen availability is a major determinant of plant growth and crop productivity (Hachiya and Sakakibara 2017; Li et al. 2017). Plants use inorganic forms in natural soils, including nitrates, nitrites, and ammonium, and nitrate is the major form of N in most aerated soils. Of these available N sources, researchers have mainly focused on nitrate and ammonium because these are often present in natural and cropland soils at much higher levels than the other N sources (Miller and Cramer, 2004).

To further validate the relationship between SAPK2 and N metabolism, we investigated the effects of silencing and overexpressing SAPK2 on rice seedling growth and development in hydroponic cultures under nitrate-deprived conditions. The sapk2 mutant seedlings under N-deprived conditions had weaker culms than the WT plants, with inhibited root growth, significantly lower root and shoot dry weights, and fewer roots (Fig. 4a−f). However, we previously observed that seedling root development does not significantly differ between the sapk2 mutant lines and WT plants under drought conditions (lou et al. 2017).

Earlier investigations confirmed the importance of K^+ for enzyme activities and ionic homeostasis in plants (Shabala and Pottosin 2014; Ahmad and Maathuis 2014). Additionally, we previously determined that SAPK2 regulates the expression of genes related to Na^+ and K^+ homeostasis, including OsSOS1, OsNHX1, OsHKT1;1, and OsHKT1;5 (lou et al. 2018). In the current study, the sapk2 mutant seedlings deprived of K^+ produced weaker culms and had lower root and shoot dry weights than the WT controls (Fig. 5a−f).

Therefore, the data presented herein further confirm that SAPK2 is important for increasing the rice grain yield through its effects on the metabolic processes related to N and K^+.

Sapk2 Influences The No Influx Rate And Nitrate Concentration

Nitrogen uptake, assimilation, and recycling in plant roots reportedly determine plant development and productivity (Yamaya and Kusano, 2014). Plant growth under natural conditions is often limited by N availability. Therefore, plants have developed transport and signaling mechanisms for their specific N

sources (Kiba and Krapp, 2016). To further validate the relationship between SAPK2 and nitrogen absorption and utilization by investigating the effects of silencing and overexpressing SAPK2 on rice. Under drought stress conditions, in sapk2 mutant lines, we detected a lower rate of NO_3^- influx into roots and also into the leaf sheath and leaf blade (Fig. 6b). Besides, the detected nitrate concentration was consistent with the rate of NO_3^- influx in different lines (Fig. 6d). These results demonstrated that SAPK2 enhanced nitrate influx and concentration by promoting nitrate uptake by roots and translocation of nitrate from roots to leaf sheath.

Although there are more than 80 NRT1/PTR, 4 NRT2, and 2 NAR2 members in rice, only a few NRT1/PTR family members have been characterized (Araki and Hasegawa, 2006; Cai et al. 2008; Feng et al. 2011). Additionally, only a few nitrate transporters (OsNPF7.2, OsNPF7.3, OsNPF6.5, OsNPF2.2, OsNRT2.3a, and OsNPF2.4) have been characterized in rice (Wang et al. 2018; Fang et al. 2017; Hu et al. 2015; Li et al. 2015; Tang et al. 2012; Xia et al. 2015).

In this study, we analyzed the expression of several nitrate transporter genes (OsNPF7.2, OsNPF7.3, OsNPF5.6, OsNPF2.2, OsNRT2.3a, and OsNPF2.4). Under drought conditions, the expression levels of these six genes were significantly lower in the sapk2 mutant lines than in the WT plants in response to drought stress (Fig. 7a–f). Furthermore, OsNPF6.5 regulates the number of rice tillers and promotes grain production (Hu et al. 2015). The overexpression of OsNPF7.2 and OsNPF7.3 increases the production of tillers, panicles per plant, and filled grains per panicle, while also increasing the grain N content (Wang et al., 2018; Fang et al., 2017). The osnpf2.2 (OsPTR2) mutants are defective in long-distance nitrate transport, with repressed nitrate unloading from the xylem, resulting in plant growth retardation and abnormal grain filling (Li et al., 2015). Our study revealed that the sapk2 mutant lines produced significantly fewer tillers than the WT plants (Fig. 1d). Moreover, the sapk2 mutant seeds had a lower nitrate concentration than the WT seeds under RS conditions (Fig. 3a).

On the basis of our results, we conclude that under drought conditions, SAPK2 promotes nitrate uptake and assimilation and influences the number of tillers and the number of grains per panicle by regulating nitrate-related transporters.

Conclusions

We analyzed transgenic rice lines that differed regarding their SAPK2 expression levels and determined that SAPK2 positively affects the number of tillers, the number of grains per panicle, the seed setting rate, and grain size under drought conditions by regulating the expression of nitrate transporter genes to enhance the N use efficiency, ultimately leading to an increase in the rice grain yield.

Methods

Generation of transgenic rice lines

We employed knock-out mutant lines (sapk2, sapk2-1 and sapk2-7) which we built previously by the CRISPR/Cas9 system and over-expression lines (OE, OES2-1 and OES2-2) (Lou et al. 2018). Concisely, the third coding exons of SAPK2 were selected for guide RNA design. Double-strand DNA generated by annealing the oligo pairs, and then was cloned into the pYLCRISPR/Cas9Pubi-H vector. For mutation detection, genomic DNA extracted from mutant seedlings (all plant) were used for PCR. In T_0 generation, we collected 20 hygromycin-resistant plants for each gene. Based on mutation detection results, we identified two independent homozygous mutant lines in the T_1 generation, which we named sapk2-1, sapk2-7. To generate SAPK2 overexpression transgenic plants, the full-length cDNA of SAPK2 was cloned into the p1301 vector in the sense orientation behind the CaMV 35S promoter. Rice (Oryza sativa L. japonica.) was used for transformation. The primers used in this study were listed in the Additional file 2: Table S1.

Plant Cultivation And Agronomic Traits Analysis

For basic agronomic traits analysis, rice plants were grown in the paddy field from March to August at the rice experimental station of Xishuangbanna Tropical Botanical Garden. Ten plants at a spacing of 16.5 cm × 26.5 cm were planted in a row and 5 rows of each line were planted. At reproductive stage, 20 plants of each line were randomly chosen to detect agronomic traits. The grain number per panicle was measured as the total number of grains per plant divided by the number of panicles per plant. The 1000-grain weight was calculated as the weight of the total grains per plant and divided by the grain number, then converted to 1000-grain weight. Grain yield was measured as the weight of total grains per plant.

For reproductive-stage drought stress, transplanted experiments were maintained as described by Sandhu et al (2016). The drought stress was initiated at 32 days after transplanting. After the inception of the stress, the soil water potential was measured using tensiometers (30 cm depth). The plots in the reproductive stage drought stress treatments were rewatered when the soil water potential dropped to − 50 to − 70 kPa (tensiometer). The decline in water table depth was measured on a daily basis with a meter scale inserted into a 1.1-m polyvinyl chloride pipe in the experimental fields at regular intervals in all RS treatments. The pipes were placed at 1.0-m depth with 10 cm of pipe remaining above the soil surface. The plots were rewatered when water table level reached 100 cm below the soil surface and most lines were wilted and exhibited severe leaf drying.

To analyze SAPK2 function in seedling growth and development under different nutrient-deprived conditions, seedlings at 7 DAG were cultured in basic nutrient solution (pH = 5.8) for a week. Then seedlings at 14 DAG were transferred to nutrient-deprived solutions. Each nutrient solution was renewed every three days. Daytime conditions in the greenhouse were 32 °C, with light from a sodium lamp (400 W) for 14 h; night-time conditions were 25 °C, and dark for 10 h. At 34 DAG, root length, shoot length, root number and dry weight of each lines were measured.

Measurement Of Nitrate Influx And Nitrate Concentration

To analyze the nitrate influx and nitrate concentration under drought stress conditions, seedlings were transferred into half-strength MS liquid

medium supplemented with 25% PEG6000 (m/v) for 24hours. Free NO_3^- content was determined from a standard curve of KNO_3 (Cai et al. 2009). Concisely, free NO_3^- content analysis was carried out by homogenizing plant tissues in cold extraction buffer [50 mM Tris-HCl (pH 7.0), 10 mM imidazole, and 0.5% (w/v) β-mercaptoethanol]. The suspension was centrifuged at 12,000 rpm for 30 min and the supernatant was collected. NO_3^- influx was calculated as the difference in NO_3^- content between the plants under control conditions and drought stress conditions in an hour. At least three independent biological experiments were conducted. One representative result was displayed here.

Rna Extraction And Qrt-pcr Analysis

To detect the transcript level of SAPK2 under different nutrient-deprived conditions, 14 DAG seedlings were transferred to different nutrient-deprived solutions for 20 days. To detect the transcript level of target genes under drought stress, seedlings were transferred into half-strength liquid medium supplemented with 25% PEG6000 (m/v) for 24 hours.

For the qRT-PCR analysis, we used the same method as described (Jiang et al., 2016). Total RNA was isolated from whole seedlings using the TriZol reagent (Invitrogen). The cDNAs were obtained by using Superscript II in accordance with manufacturer's instructions (Invitrogen). The qRT-PCR analysis was performed using SYBR Premix Ex Taq kit (Takara).

At least three independent biological experiments were conducted (three independent samples were conducted for each experiment and three technological replications in every independent experiment). One representative result was displayed here. Gene-specific primers used in qRT-PCR analysis were listed in Additional file 2: Table S1.

Statistical analysis

The experiments were arranged in a completely randomized design with at least three replicates for each treatment. Excel 2010 was used for making charts. Two-tailed Student's t tests were performed using the SPSS 10 software (IBM, Inc.). "* and **" indicate significance at $P < 0.05$ and $P < 0.01$, respectively. The data represent mean ± standard error (SE) of three independent experiments.

Declarations

Abbreviations

SAPK2: osmotic stress/ABA–activated protein kinase 2; *S2-1*:*sapk2-1*; *S2-7*:*sapk2-7*; *OE*: Overexpression; WT: Wild type; ROS: reactive oxygen species; JA: jasmonic acid; ABA: abscisic acid; SA: salicylic acid; ET: ethylene; Gas: gibberellins; CKs: cytokinins; RS: reproductive-stage drought; qRT-PCR: Quantitative Reverse Transcription Polymerase Chain Reaction; DAG: Days after germination

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its additional files.

Authors' contributions

D. J. L., X. Y. Y. planned and designed the research; D. J. L., and Z. C. performed the experiments; D. J. L. wrote the manuscript; X. Y. Y. analyzed the data and edited the article. All authors read and approved the final article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Supplementary Files Legend

Additional file 1 S Fig. 1 *SAPK2* affects seedling growth and root development under P deprivation. **a-e** Statistical analysis of shoot length **(a)**,root length **(b)**, dry weight of shoot **(c)**, dry weight of root **(d)** and root number **(e)** among WT, *sapk2* mutant lines and *OE* lines under P-deprived conditions. **f** Phenotypic analysis of seedlings at 31 DAG among WT, *sapk2* mutant lines and *OE* lines under P-deprived conditions. Data in **a-e** are shown as means ± SD (n = 20) from three replicates. A student's t-test was used to generate P values; "**" indicate significance at $P < 0.01$.

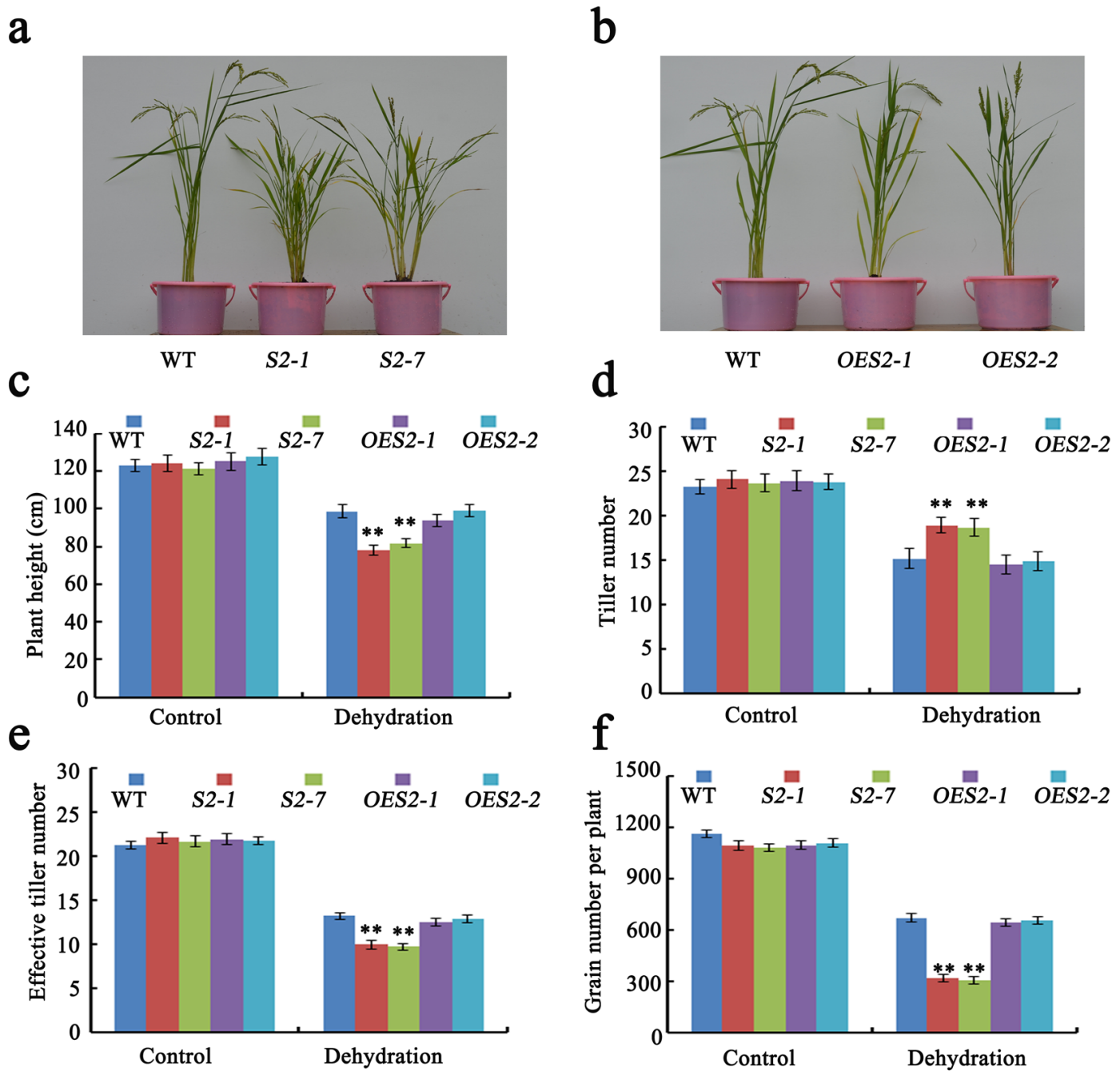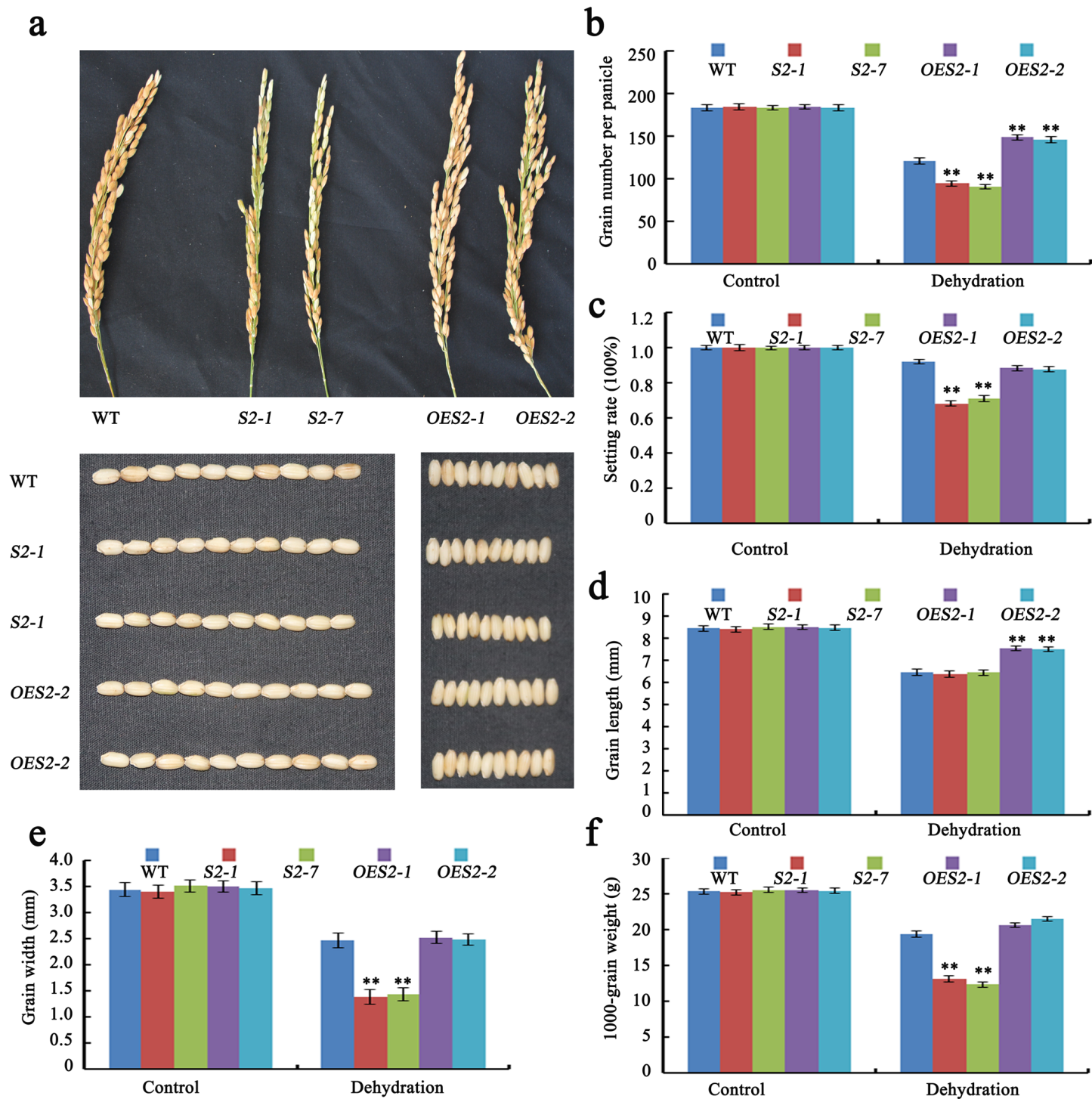**Additional file 2 Table S1.** Primers used in this study.

Figures

Figure 1

SAPK2 has large effects on plant height and grain number. a Phenotypes analysis of WT and sapk2 mutant lines at mature stage. b Phenotypes analysis of WT and OE lines at mature stage. c-f Comparison of agronomic traits including plant height (c), tiller number(d), effective tiller number(e), grain number per plant (f) among WT, sapk2 mutant lines and OE lines. Data in c-f are shown as means ± SD (n = 20) from three replicates. A student's t-test was used to generate P values; "**" indicate significance at P < 0.01.
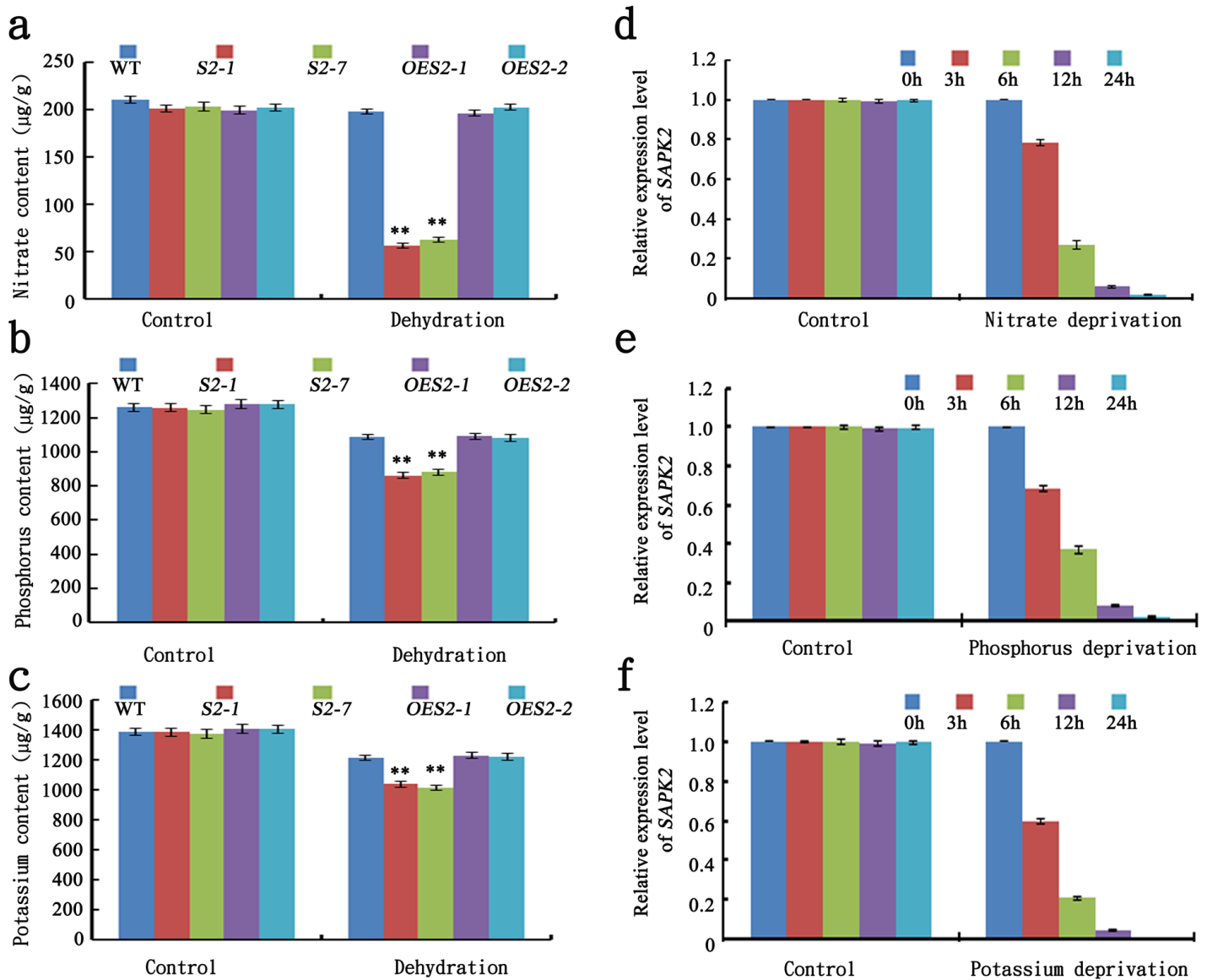
Figure 2

SAPK2 has large effects on grain size and weight. a Panicle and grain phenotypes of WT, sapk2 mutant lines and OE lines. b-f Comparison of agronomic traits including grain number per panicle(b), setting rate (c), grain length (d), grain width (e), 1000-grain weight (f) among WT, sapk2 mutant lines and OE lines. Data in b-f are shown as means ± SD (n = 20) from three replicates. A student's t-test was used to generate P values; "**" indicate significance at P < 0.01.
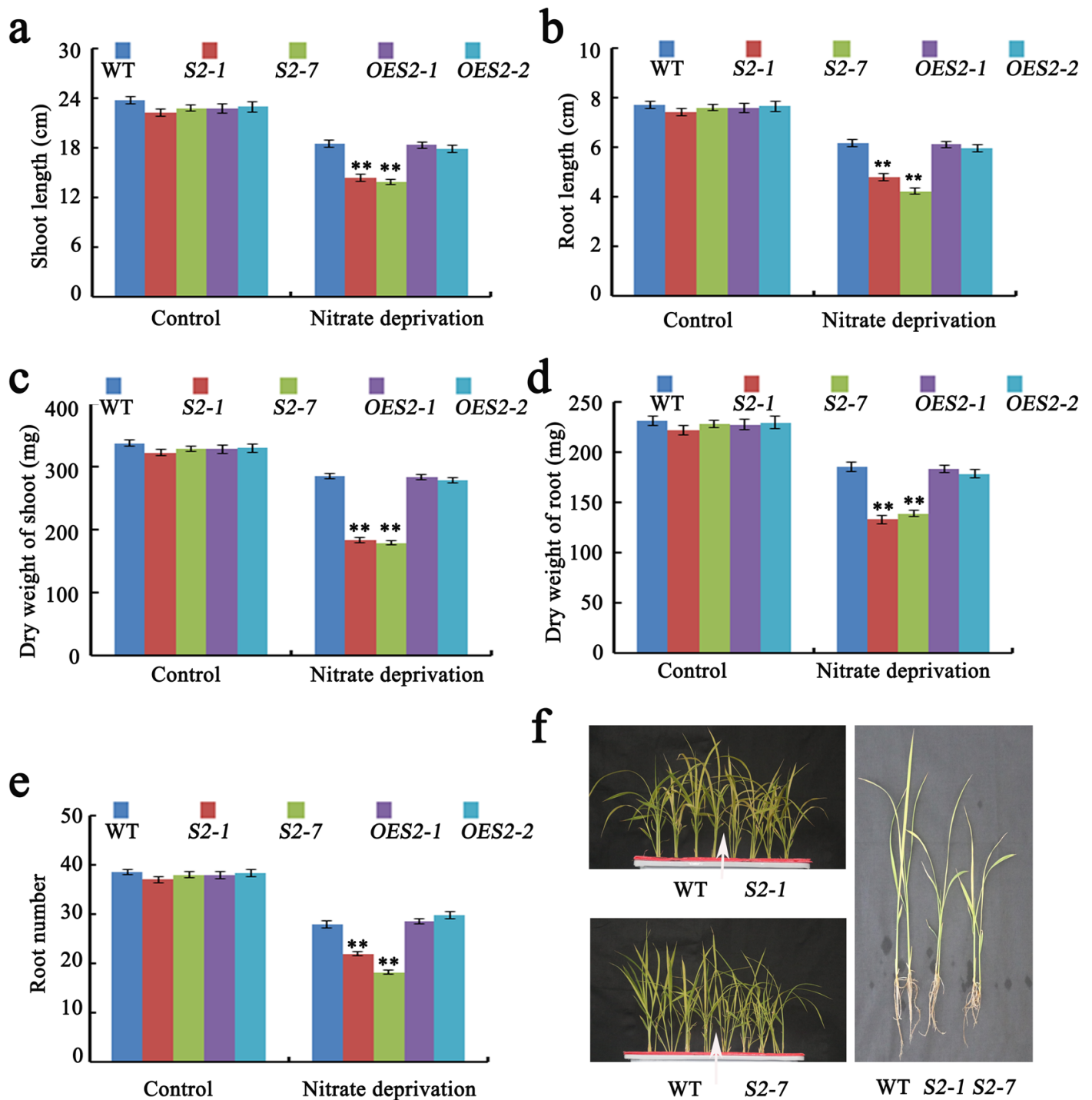
Figure 3

SAPK2 influenced Nitrate, phosphorus, potassium content in rice grains under reproductive-stage drought. a-c Comparison of nutrient content including nitrate (a), phosphorus (b), potassium (c) among WT, sapk2 mutant lines and OE lines in rice seeds under reproductive-stage drought. d-f The expression analysis of SAPK2 under N(d), P(e), and K+(f) deprivation. Transcript accumulation was assessed by qRT-PCR. Data are shown as means ± SD (n = 20) from three replicates. A student's t-test was used to generate P values; "**" indicate significance at P < 0.01.
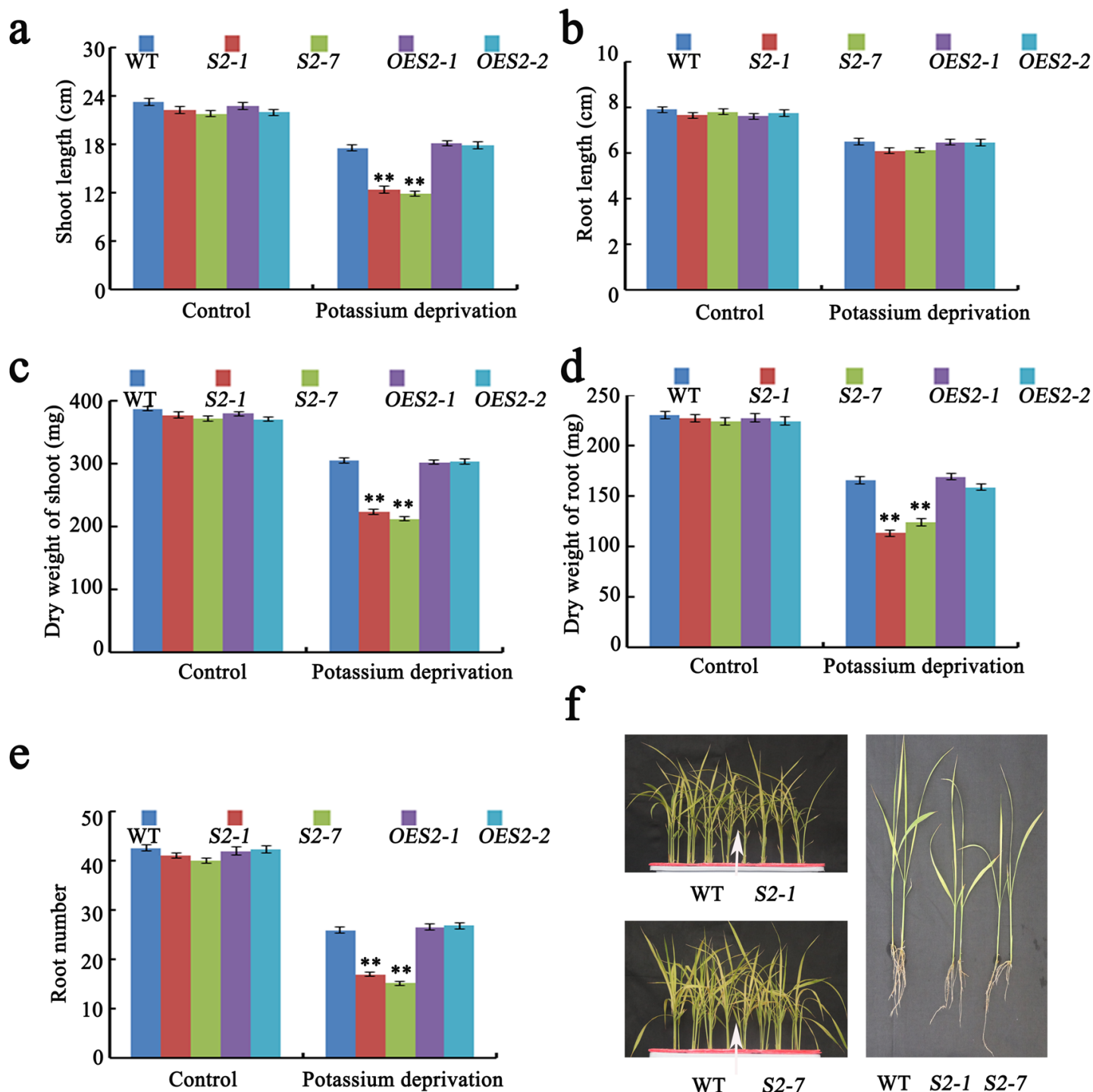
Figure 4

SAPK2 affects seedling growth and root development under N deprivation. a-e Statistical analysis of shoot length (a),root length (b), dry weight of shoot (c), dry weight of root (d) and root number (e) among WT, sapk2 mutant lines and OE lines under N-deprived conditions. f Phenotypic analysis of seedlings at 31 DAG among WT, sapk2 mutant lines and OE lines under N-deprived conditions. Data in a-e are shown as means ± SD (n = 20) from three replicates. A student's t-test was used to generate P values; "**" indicate significance at P < 0.01.

Figure 5

SAPK2 affects seedling growth and root development under K+ deprivation. a-e Statistical analysis of shoot length (a),root length (b), dry weight of shoot (c), dry weight of root (d) and root number (e) among WT, sapk2 mutant lines and OE lines under K+-deprived conditions. f Phenotypic analysis of seedlings at 31 DAG among WT, sapk2 mutant lines and OE lines under K+-deprived conditions. Data in a-e are shown as means ± SD (n = 20) from three replicates. A student's t-test was used to generate P values; "**" indicate significance at P < 0.01.
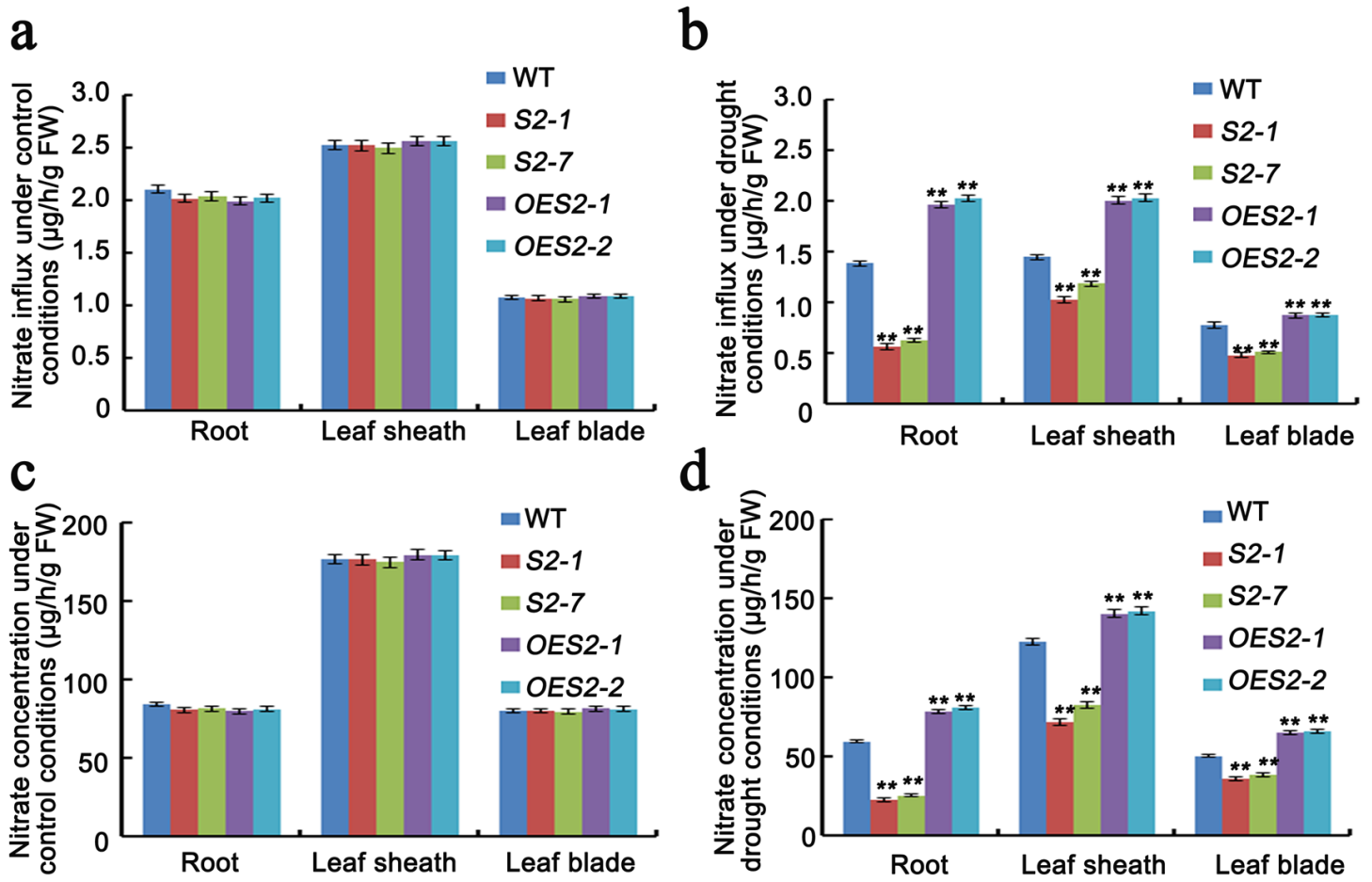
Figure 6

SAPK2 influenced the rate of NO3− influx rate and nitrate concentration. a-b Analysis of NO3− influx rate among WT, sapk2 mutant lines and OE lines under control conditions (a) and drought stress conditions (b). c-d Analysis of nitrate concentration among WT, sapk2 mutant lines and OE lines under control conditions (c) and drought stress conditions (d). Data are shown as means ± SD (n = 20) from three replicates. A student's t-test was used to generate P values; "**" indicate significance at $P < 0.01$.
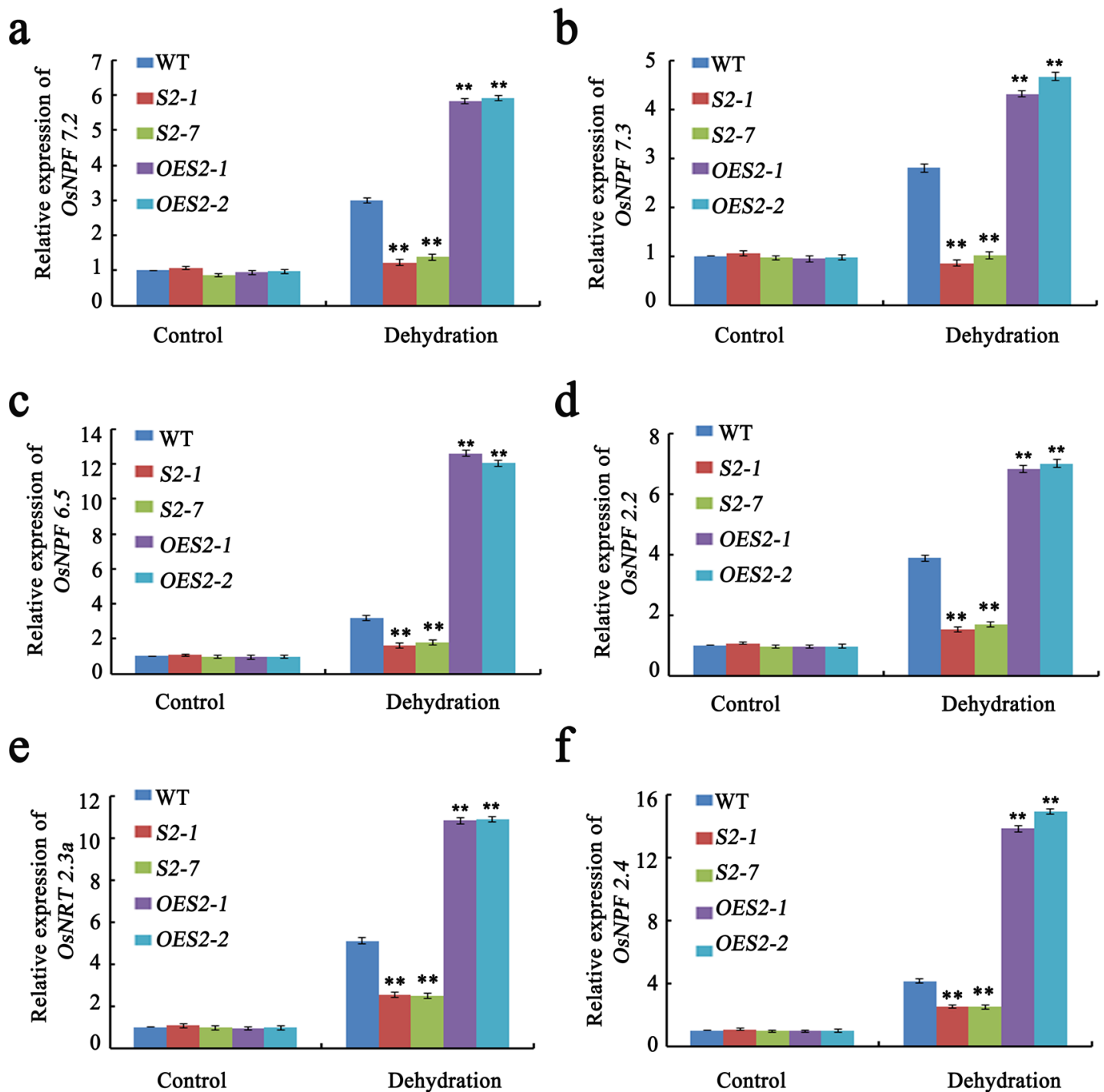
Figure 7

Expression of genes involved in absorbing, transporting and assimilation of nitrate among WT, OE lines and sapk2 mutant lines. a-f Relative expression analysis of OsNPF7.2 (a), OsNPF7.3 (b), OsNPF5.6 (c), OsNPF2.2 (d), OsNRT2.3a (e) and OsNPF2.4 (f) among WT, sapk2 mutant lines and OE lines control conditions and drought stress conditions. Data are shown as means ± SD (n = 20) from three replicates. A student's t-test was used to generate P values; "**" indicate significance at $P < 0.01$.

Supplementary Files

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