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Overexpression of the Poplar *WRKY51* Transcription Factor Enhances Salt Tolerance in *Arabidopsis thaliana*

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Abstract: Salt is a severe environmental stressor that affects growth and development in plants. It is significant to enhance the salt tolerance in plants. In this study, a salt-responsive WRKY transcription factor *PtrWRKY51* was isolated from *Populus trichocarpa* (clone 'Nisqually-1'). *PtrWRKY51* was highly expressed in mature leaves and root and induced by salt stress. The *PtrWRKY51* was overexpressed in *Arabidopsis* to investigate its biological functions. Compared with Col-0 lines, Overexpressed lines had an increase in germination rate of seed, root length, higher photosynthetic rate, instantaneous leaf WUE, chlorophyll content to improve salt tolerance under salt stress conditions. In contrast, compared to overexpressed and Col-0 lines, the mutant *wrky51* was more sensitive to salt stress with lower photosynthetic rate and WUE. Additionally, it was found that the complementary lines (*wrky51*/ *PtrWRKY51*) had almost the same salt response as Col-0. In conclusion, *PtrWRKY51* is a potential target in the enhancement of poplar tolerance by genetic engineering strategies.

Keywords: poplar; transcription factor; *PtrWRKY51*; salt tolerance; photosynthetic rate; water-use efficiency

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1. Introduction

As one of the most widely distributed and adaptable forest species around the world, poplar is one of the most promising tree species for traditional afforestation and dealing with wood shortages. With the gradual aggravation of soil salinization, salt stress has become an essential factor restricting tree growth. Salt is one of the key environmental stress factors, which affects plant growth and development [1]. It is reported that more than 800 million hectares of land are affected by salinization in world, which across all continents including Africa, Asia, Australasia, Americas, and China, occupying an estimated 37.4 million hectares of salinized soil [2,3]. Moreover, soil salinization is becoming more serious due to environmental degradation, improper irrigation, climate change, growing population and industrial pollution [4,5]. Excessive salinity in plants will lead to excessive accumulation of intracellular metal ions, destroy ion balance, damage plant cell membrane structure, and thus affect the physiological and biochemical metabolic processes in plants [6,7]. Many studies have shown that the water potential of the soil in the saline-alkali soil was decreased, and the absorption of water by seed was inhibited, which seriously affected the germination rate, vigor index and germination index of seeds, which have a reduction in seed emergence rate and strong seedling rate [8,9]. Photosynthesis is the sum of a series of complex metabolic reactions which are essential in plant growth. Photosynthesis provides material and energy to maintain normal growth and development in plants, and chloroplasts in leaves are the place in which photosynthesis is carried out in higher plants. Salt stress affects plant absorb water and inorganic ions, inhibit the normal synthesis of

chlorophyll, and reduce the content of chlorophyll, which affect the normal function of the pigment protein complexes, reduce the rate of plant convert light energy into chemical energy, which eventually make plant to a serious shortage of energy supply, inhibit the growth and development in plants [10,11]. At the same time, salt stress can also cause damage to chloroplast structure, and then affect its photosynthetic performance. It has been reported that under salt stress, the net photosynthetic rate, chlorophyll fluorescence and stomatal conductance of leaves are greatly reduced in citrus, which inhibit growth [12]. Similarly, it has been confirmed that salt stress seriously inhibits the photosynthetic rate and electron transport rate in photosynthesis, which affected plant growth in mustard [13]. Therefore, it is important to improve the salt tolerance in plants. During evolution, plants have developed various strategies to adapt to salt stress [14]. Signal sensing and signal transduction are key ways for plants to cope with challenges in environment, which involve many regulatory genes and proteins. Among these, the transcription factors, such as WRKY, NAC, bZIP, MYB, HSFs, AP2/EREBP have been identified as key regulators in controlling intrinsic development processes and regulating stress resistance by stimulating the expression of downstream targets in improving salt resistance in plants [15–19].

WRKY transcription factor is a key regulator in plants, and significant study has been made in WRKY transcription factor over the past 20 years [20]. *SPF1* is the first WRKY gene, cloned from sweet potato [21]. Subsequently, WRKY genes were cloned and reported in *Arabidopsis thaliana*, wild oats and parsley, which were found that the proteins were encoded by WRKY genes that could specifically bind to DNA sequence element (T) (T) TGAC (C/T) [22]. The specific DNA sequence element (T) (T) TGAC (C/T) is also known as the W-box [22–24]. It is well known that WRKY transcription factor contain a highly conserved WRKY domains with WRKYGQK sequence and a zinc-finger-like motif CX4–7-CX23–28-HX1–2-(H/C) (C2H2 or C2HC) at N and C termini [25]. In general, WRKY proteins can be categorized into three groups based on the number of WRKY domains and the pattern of the zinc finger motif [25]. Group I WRKY transcription contains two WDs and C2H2 zinc finger, and group II and III WRKY transcription factors contain one WD and C2H2 zinc finger motif [25].

Many studies have found that WRKY transcription factors is a key role in plant response to abiotic stresses such as drought and salt stress. Northern blot hybridization showed that 10 WRKY genes in rice had different responses to salt, osmotic stress, cold and heat treatments [26]. In wheat, eight of the 15 WRKY genes also showed response to PEG, low temperature, high temperature and NaCl stress treatments [27]. In *Arabidopsis*, overexpression of *OSWRKY45* and *AtWRKY46* can regulate stomatal movement in response to drought and salt stresses [28,29]. Heterologous expression of *GmWRKY54* in *Arabidopsis* increased drought and salt tolerance [30]. In addition, WRKY genes such as *TaWRKY2* and *TaWRKY19*, *TaWRKY146*, *TaWRKY1* and *TaWRKY33*, *VaWRKY*14, *ZmWRKY17* and *ZmWRKY40*, and *PeWRKY83* were overexpressed in *Arabidopsis* can improve plant tolerance to drought and/or salt stresses, respectively [31–37]. Previous study has shown that *PtrWRKY51* responds to salt stress [38], but functional characterization of *PtrWRKY51* in salt stress response remains unclear.

In this study, *PtrWRKY51* was cloned from the *Populus trichocarpa* to investigate its function in salt stress in *Arabidopsis*. We analyzed its expression pattern in root, stem and leaf tissues and in response to salt stress. Moreover, the functional characterization of *PtrWRKY51* in salt response was studied in *Arabidopsis*. Our results demonstrate that overexpression of *PtrWRKY51* enhanced salt tolerance in *Arabidopsis*.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

The solid Lloyd and McCown's Woody Plant Basal Salts (WPM) medium was used for cultivation of the *Populus trichocarpa* in vitro [39]. After plantlet regeneration, they were individually transplanted and grown in pots (20.5 × 17 × 14.5 cm; top diameter/height/bottom diameter) containing a mixture of soil and vermiculite (2:1) and incubated in the green-

house at 22 °C under a 16 h/8 h (light/dark) photoperiod (150 μmol m^{-2} s^{-1}) and 70% relative humidity.

Arabidopsis Col-0 was selected as the wild-type control, and the mutant *wrky51* (stock name SALK_022198.56.00.x) were obtained from Arabidopsis Biological Resource Center (ABRC; https://abrc.osu.edu/). *Arabidopsis* seeds were washed two times with water, then with 75% alcohol for 1 min followed by 1% NaClO within 10 min and five washes in distilled water. Seeds were sown on 1/2MS medium plates containing 30 g/L sucrose and 6 g/L agar. The seeded plates were vernalized at 4 °C in the dark for 2 days and then moved to 22 °C under 16/8 light/dark cycle. When *Arabidopsis* sprouted, it was transplanted at a density of four plants per pot (7 × 7 × 6.5 cm; top diameter/height/bottom diameter) with a mixture of soil and vermiculite (2:1) at 22 °C under 16/8 light/dark cycle and 70% relative humidity.

2.2. PtrWRKY51 Cloning and Transformation

Total RNA was extracted from the young leaves of *Populus trichocarpa* by using the RN38 EASYspin Plus Plant RNA Kit (Aidlab Biotech, Beijing, China). The first strand cDNA synthesis was reverse-transcribed using the Tiangen FastQuant RT Kit (Tiangen) according to the manufacturer's instructions. The resultant cDNA was used as a template, and the *PtrWRKY51* (Accession number: *Potri.005G085200*) sequence was amplified by PCR with gene-specific primers (Supplementary Table S1). The gene-specific primers were designed by using Primer Premier 5 software.

For overexpression of *PtrWRKY51* and complementation of the *Arabidopsis wrky51* mutant with *PtrWRKY51*, the *PtrWRKY51* cDNA was cloned into the pCAMBIA-1301 binary vector under the control of the Cauliflower mosaic virus (CaMV) 35S promoter, and the plasmids were introduced into *Agrobacterium tumefaciens* strain GV3101 by heat shock. Then, the 35S: PtrWRKY51: GFP plasmid transformed into the *Arabidopsis* Col-0 and *wrky51* mutant lines respectively by the floral dip method [40]. The transgenic lines were identified using half-strength MS plates containing 100 mg l^{-1} hygromycin. The third (T_3) generation of seeds were used in the experiments to make sure hereditary stability in transgenic lines.

2.3. Molecular Verification of Transgenic Plants

Genomic DNA of Col-0 and transgenic *Arabidopsis* lines was extracted from leaves by using the CTAB method [41]. Transformants were identified by PCR amplification using the gene-specific primers PtrWRKY51-F and GFP-R (Supplementary Table S1). The transcript levels of *PtrWRKY51* were quantified by Quantitative real-time PCR (RT-qPCR) in transgenic lines.

2.4. Quantitative Real-Time PCR Analysis

The ABI StepOnePlus™ Real-Time PCR System (Applied Biosystems, Inc., Carlsbad, CA, USA) is used to perform Quantitative real-time PCR (RT-qPCR) experiments according to the manufacturer's instructions. The $2^{-\Delta\Delta CT}$ method [42] was used to calculate the relative expression level of *PtrWRKY51*, with poplar *Actin* employed as the internal control (Supplementary Table S1).

2.5. Physiological Experiments

To determine germination rates of overexpressed *PtrWRKY51*(*oePtrWRKY51*), Col-0, *wrky51* and *wrky51/ PtrWRKY51* seeds, 100 seeds of different genotypes were seeded separately on the same 1/2MS medium with or without 200 mM NaCl stress and germination rates were recorded daily. When the seeds germinated, 10 plants of each genotype were selected and placed vertically on the medium with or without 200 mM NaCl stress to grow for 10 days, and the root length was recorded.

Net photosynthetic rate and transpiration rate were measured of *oePtrWRKY51*, Col-0, *wrky51* and *wrky51/ PtrWRKY51* plants by using an infrared gas analysis system

(LI-COR 6400, Lincoln, NE, USA) as previously described [43,44]. Instantaneous leaf WUE was defined as the ratio of the rate of photosynthetic rate/transpiration rate [45].

Leaves of *oePtrWRKY51*, Col-0, *wrky51* and *wrky51/ PtrWRKY51* plants grown for 4 weeks were used for chlorophyll content determination with 80% acetone. A UV/visible spectrophotometer (YHB-061; GE Healthcare, Little Chalfont, Buckinghamshire, UK) was used to measure absorbance at 663 for chlorophyll a and 645 nm for chlorophyll b, and then the chlorophyll contents were calculated according to Lichtenthaler's method [46].

2.6. Salt Experiments

For the salt treatments of *Populus trichocarpa*, 4-week-old seedlings were removed from the soil carefully and then placed in a salt solution containing 200 mM NaCl for 24 h. Subsequently, leaves were harvested at 0 h, 1 h, 3 h, 6 h, 8 h, 12 h and 24 h for RT-qPCR analysis.

Arabidopsis seeds of *oePtrWRKY51*, Col-0, *wrky51* and *wrky51/ PtrWRKY51* were seeded on 1/2MS medium and transplanted at a density of four plants per pot (7 × 7 × 6.5 cm; top diameter/height/bottom diameter) with a mixture of soil and vermiculite (2:1) at 22 °C under 16/8 light/dark cycle and 70% relative humidity after two true leaves had grown. It was cultured in the greenhouse with normal watered for two weeks, and then it was watered with 200 mM NaCl solution every 3 days for 15 days to observe its phenotype. The photosynthetic rates and instantaneous leaf WUE were measured at each stage as previously.

3. Results

3.1. The Analysis of Expression Pattern in PtrWRKY51

The tissue-specific expression of *PtrWRKY51* transcripts in young leaf, mature leaf, senescent leaf, stem and root of *P. trichocarpa* was detected by RT-qPCR. The result showed that *PtrWRKY51* transcript levels in mature leaves and root was higher than that in young leaf, senescent leaf and stem (Figure 1A). To study the response of *PtrWRKY51* to salt stress, *P. trichocarpa* plants were subjected to salt stress and the *PtrWRKY51* transcript levels were quantified by RT-qPCR. The results showed that the *PtrWRKY51* transcript level gradually increased and peaked at 6 h of the NaCl treatments, and thereafter decreased (Figure 1B). These results indicated that *PtrWRKY51* was mainly expressed in mature leaves and roots and up-regulated in response to salt stress.

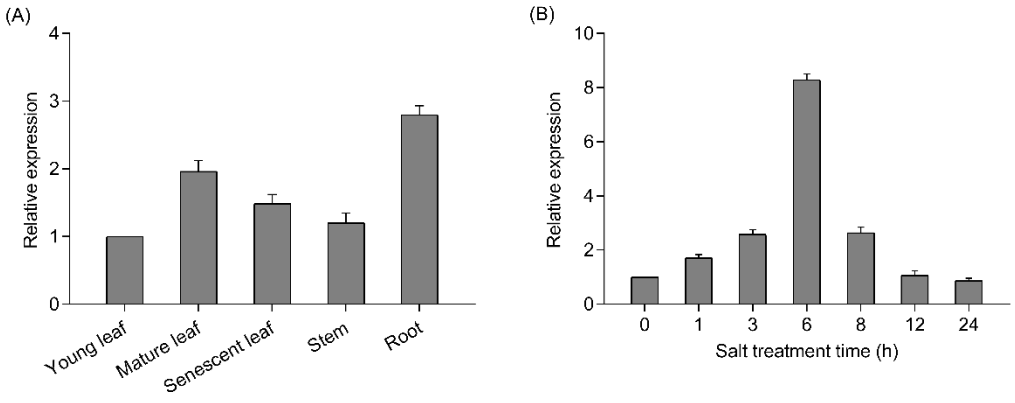

Figure 1. Expression patterns of *PtrWRKY51*. (**A**)Transcript level of *PtrWRKY51* in various organs including young leaf, mature leaf, senescent leaf, stem and root of *P. trichocarpa*. (**B**) RT-qPCR analysis of *PtrWRKY51* transcript levels under salt conditions. Data are shown as mean ± SE (n = 6).

3.2. Identification of PtrWRKY51-Overexpressing Transgenic Plant

To further explore the function of *PtrWRKY51* in salt stress conditions, the 35S: PtrWRKY51: GFP is transformed into Col-0 *Arabidopsis*. Eight transgenic lines (*oePtrWRKY51 #1-7*) were obtained and confirmed by PCR analyses using the combination of the gene-

specific primers PtrWRKY51-F and GFP-R. The expected band was amplified in all transgenic plants but not in Col-0 plants (Figure 2A). In addition, RT-qPCR analysis of the above transgenic plants showed that *PtrWRKY51* was overexpressed in all selected transgenic lines (Figure 2B). Then three of *PtrWRKY51*-overexpressing lines (*oePtrWRKY51#1*, *#2*, and *#3*) with higher expression level than other lines were selected for further analysis in salt tolerance.

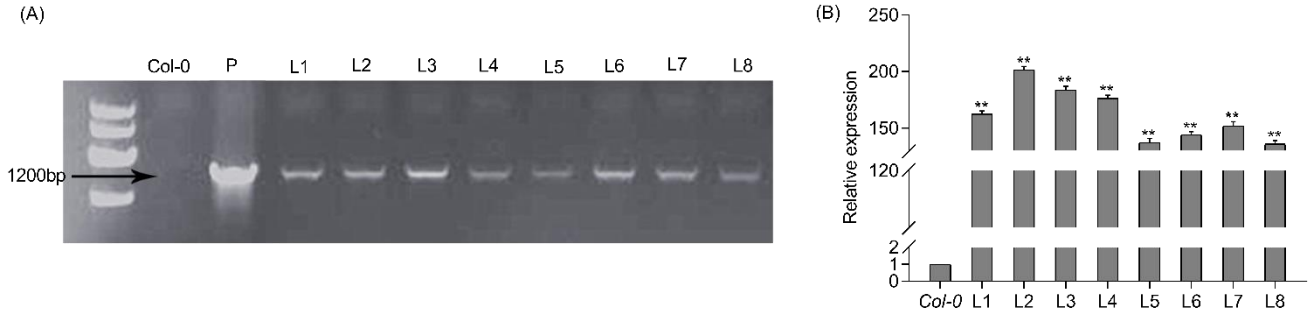

Figure 2. Analysis of overexpressing *PtrWRKY51* in transgenic plants. (**A**) Eight putatively transgenic plants of *PtrWRKY51* were identified by PCR. (**B**) RT-qPCR was used to detect the transcription level of *PtrWRKY51* in different lines. The data are represented as means ± SE (n = 6). The asterisks (**) represent a significant differences (** $p < 0.01$) compared to the control.

3.3. Plant Phenotype Analysis under Well-Watered Conditions

To explore the function of *PtrWRKY51*, the growth phenotype of *oePtrWRKY51*, Col-0, *wrky51* and *wrky51/ PtrWRKY51* plants were observed under normal conditions. The result showed that the primary root length of overexpressed plants was significantly longer than that of Col-0 (1.17-fold) and *wrky51* (1.61-fold) after 10 days of vertical growth on 1/2 MS medium (Figure 3A,B). These results indicated that overexpression of *PtrWRKY51* could promote root growth.

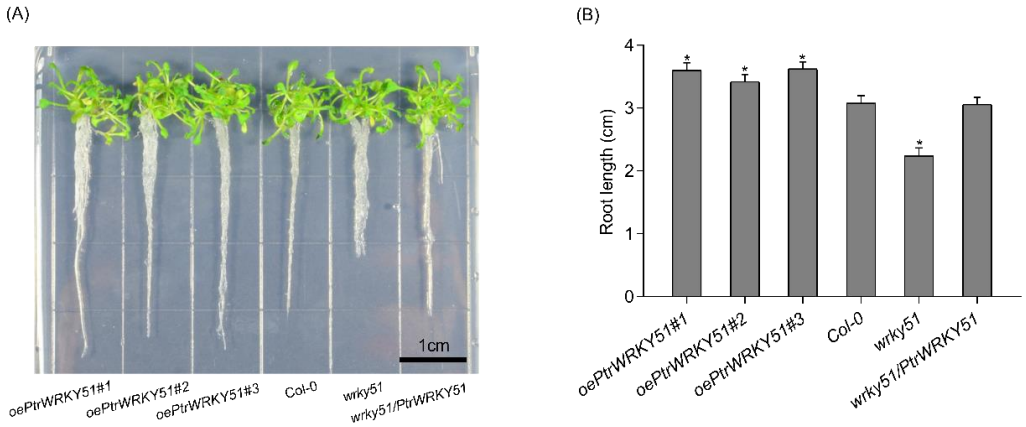

Figure 3. Root length analysis of overexpressing *PtrWRKY51* in plants under normal conditions. (**A**) Phenotypic analysis of the primary root length for 10 days on 1/2MS medium. Bars, 1 cm. (**B**) Difference in the primary root length after 10 days of vertical growth on 1/2MS medium. The data are represented as means ± SE (n = 12). The asterisks (*) represent significant differences (* $p < 0.05$) compared to the control.

3.4. Overexpression of PtrWRKY51 Enhanced WUE in Arabidopsis

Photosynthetic physiological analysis that the net photosynthetic rates of *oePtrWRKY51* lines was higher than that of Col-0 and *wrky51* under normal conditions, and the net photosynthetic rate of *wrky51/ PtrWRKY51* lines was equal to that of Col-0 (Figure 4A). In addition, the transpiration rate of each genotype was found that the *oePtrWRKY51* lines

showed slower rates than the Col-0 and *wrky51* mutant (Figure 4B). As a result, based on the higher photosynthetic capability and lower transpiration level, the instantaneous WUE values of the *oePtrWRKY51* lines were observably higher than that of Col-0 and *wrky51* mutant (Figure 4C). In general, overexpression of *PtrWRKY51* enhanced the WUE in *Arabidopsis*.

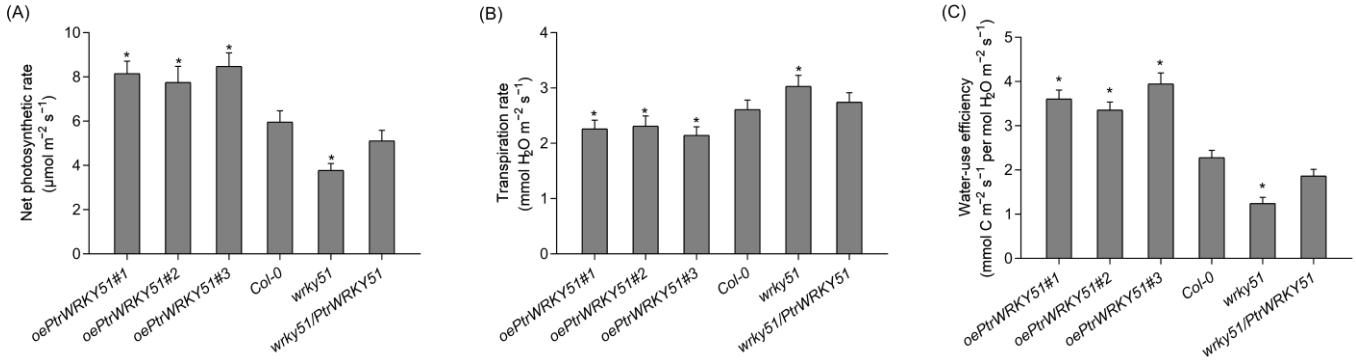

Figure 4. Gas exchange analysis of overexpressing *PtrWRKY51* lines showing higher instantaneous WUE in *Arabidopsis***.** The Net photosynthesis rate (**A**), transpiration rate (**B**), instantaneous leaf WUE (**C**) of 3-week-old seedlings. The data are represented as means ± SE (n = 12). The asterisks (*) represent significant differences (* $p < 0.05$) compared to the control.

3.5. Overexpression of PtrWRKY51 Increases Salt Tolerance under Salt Stress Conditions

A series of experiments were conducted to explore the differences in salt tolerance of *oePtrWRKY51*, Col-0, *wrky51* and *wrky51/ PtrWRKY51* plants. The seeds of *oePtrWRKY51*, Col-0, *wrky51* and *wrky51/ PtrWRKY51* were sown on 1/2 MS culture containing 200 mM NaCl to observe germination rates. After 4 days, the *oePtrWRKY51* plants had a higher germination rate (73.4%) than the Col-0 (42.8%) and mutant (20.2%) plants (Figure 5A). In addition, the primary root length of the seedlings was also different after 10 days of vertical growth under salt stress conditions. The primary root length of *oePtrWRKY51* plants was significantly longer (1.48- and 1.85- fold, respectively) than that of Col-0 and mutant (Figure 5B,C). After the seedlings were transplanted to soil, the salt stress was imposed for 15 days. As expected, the Col-0 and *wrky51* mutant plants showed more severe wilting than over-expressing plants, especially the *wrky51* mutants, whereas those of the *oePtrWRKY51* lines continued to show development and growth (Figure 5D).

The net photosynthesis rate and WUE of *oePtrWRKY51*, Col-0, *wrky51* and *wrky51/ PtrWRKY51* plants were measured and calculated under the salt treatments conditions (Figure 6A,B). The result showed that compared with the Col-0 and *wrky51* mutant plants, the *oePtrWRKY51* lines could maintain a higher photosynthetic rate and have a higher instantaneous leaf WUE under salt stress conditions.

To further assess the potential biological functions of *PtrWRKY51* in regulation of salt tolerance, the chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents of *oePtrWRKY51*, Col-0, *wrky51* and *wrky51/ PtrWRKY51* plants were also measured under normal and salt stress conditions. The result showed that there was no significant difference in chlorophyll content between the *oePtrWRKY51* and the Col-0 plants, but there was a difference between them and the *wrky51* mutant under normal conditions. However, under salt stress conditions, the chlorophyll content of *oePtrWRKY51* plants was markedly higher than that of Col-0 and *wrky51* mutant (Figure 7), indicating that the *oePtrWRKY51* plants showed a better ability to absorb light energy compared with Col-0 and *wrky51* mutant, thus the *oePtrWRKY51* plants maintained a higher photosynthetic rate under salt stress conditions. Therefore, the expression of *PtrWRKY51* was beneficial for plant growth under salt stress conditions.

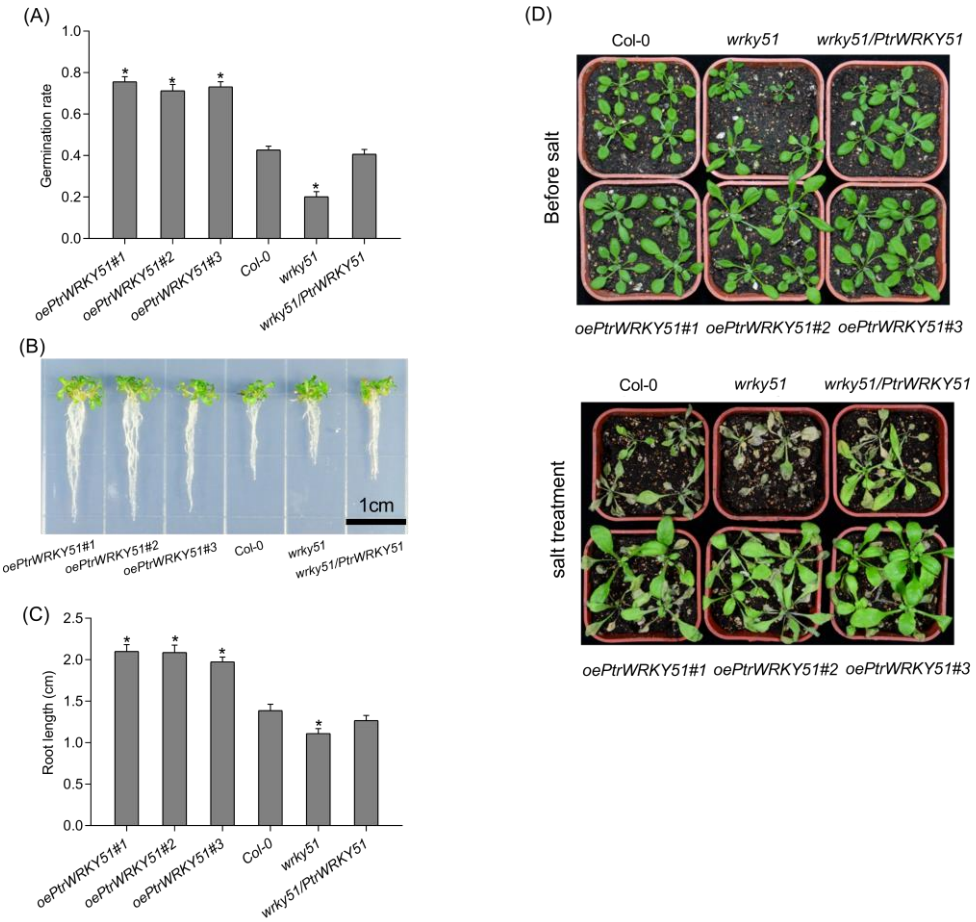

Figure 5. Overexpression of *PtWRKY51* confers salt tolerance in *Arabidopsis*. (**A**) Difference in germination ratio among oe*PtWRKY51*, Col-0, *wrky51*, and *wrky51/ PtWRKY51* plants grown on 1/2 MS medium with 200 mM NaCl. (**B**) Morphological comparisons of the primary root length for 10 d under 1/2 MS medium with 200 mM NaCl conditions. (**C**) Difference in the primary root length after 10 days of vertical growth on 1/2 MS medium with 200 mM NaCl conditions. (**D**) Morphological differences in salt treatments experiments. The data are represented as means ± SE (n = 12). The asterisks (*) represent significant differences (* $p < 0.05$) compared to the control.

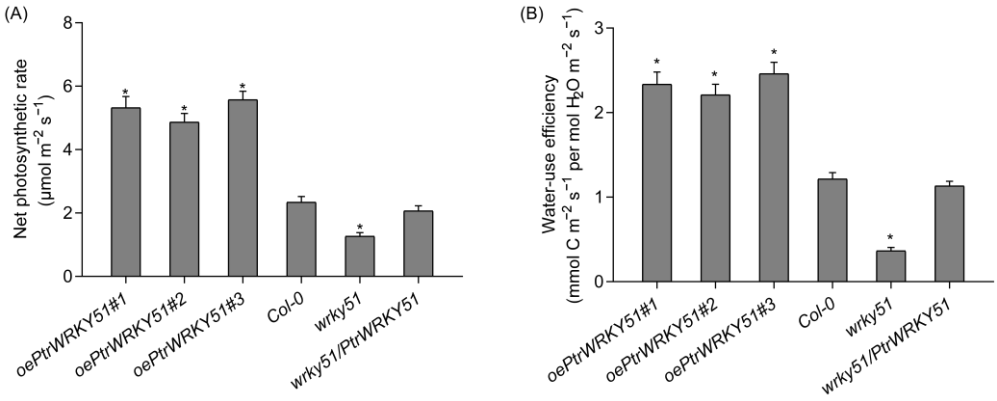

Figure 6. Physiological analysis of over-expressing *PtWRKY51* lines under salt stress conditions. Difference in net photosynthetic rate (**A**) and instantaneous leaf WUE (**B**) among oe*PtWRKY51*, Col-0, *wrky51*, and *wrky51/ PtWRKY51* plants under salt stress conditions. The data are represented as means ± SE (n = 12). The asterisks (*) represent significant differences (* $p < 0.05$) compared to the control.

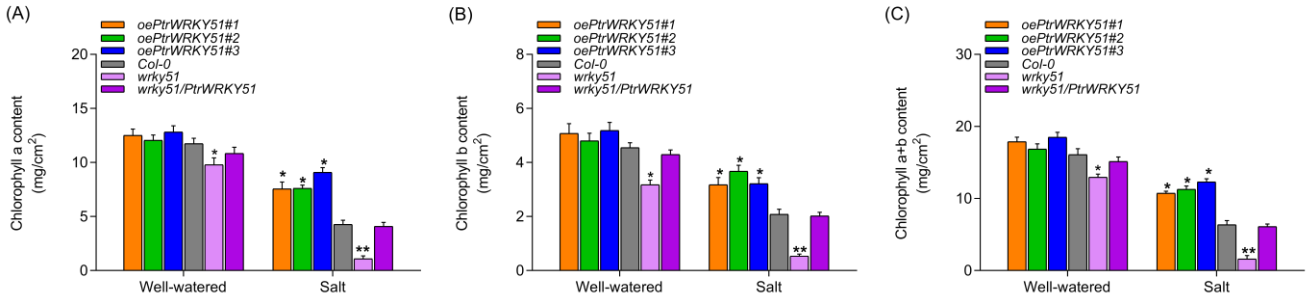

Figure 7. The chlorophyll content analysis of over-expressing *PtWRKY51* plants under salt treatment conditions. Determine of the content of chlorophyll a (**A**), chlorophyll b (**B**) and chlorophyll a + b (**C**) among oe*PtWRKY51*, Col-0, *wrky51*, and *wrky51*/ *PtWRKY51* plants under salt stress conditions. The data are represented as means ± SE (n = 12). The asterisks (* and **) represent significant differences (* $p < 0.05$ and ** $p < 0.01$) compared to the control.

4. Discussion

Salt stress has seriously affected and inhibited plant growth and development. Stress-induced transcription factors (TFs) are important pivotal regulators in response to stress. They can bind membrane protein acting elements and promote related genes expression in response to stress to reduce damage of the stress. WRKY is a plant-specific zinc finger type transcription factor. It is involved in a wide variety of biotic and abiotic stress responses [20,38,47–51]. Studies have shown that *OsWRKY51* and *OsWRKY71* regulate GA and ABA signal transduction in seed germination [52]. In addition, *AtWRKY51* was involved in JA-induced defense response [53]. Nevertheless, little was known about the function of *WRKY51* in abiotic, especially in salt stress. Previous studies have shown that *PtrWRKY51* responds to salt [38]. However, the function of *PtrWRKY51* in salt stress remains unclear. In the study, *PtrWRKY51* was cloned from poplar and genetically modified in *Arabidopsis* to determine its potential role resistance to salt stress.

To explore the role of genes in response to biological or abiotic stress, tissue-specific gene expression patterns play a crucial role in evaluating the potential function of genes [54–56]. Therefore, a novel idea was provided to investigate the potential functions of *PtrWRKY51* by studying the tissue-specific expression. Analysis of transcription patterns showed that *PtrWRKY51* was predominantly expressed in mature leaves and root (Figure 1A). In addition, the result showed that the primary root length of *oePtrWRKY51* plants was significantly longer than that of Col-0 and *wrky51* on 1/2 MS medium (Figure 3A,B). Moreover, *PtrWRKY51* were up-regulated by salt stress (Figure 1B), and *oePtrWRKY51* plants have a prominently longer root that of Col-0 and *wrky51* on 1/2 MS medium with 200 mM NaCl (Figure 5B,C). These findings suggested that *PtrWRKY51* plays a key role in the regulation of growth and salt tolerance in plants.

In generally, the growth status and external morphology of a plant can reflect the stress degree in the plant. Seed germination is a critically and extremely sensitive period in plant growth, and most plants in salt environment have a negative impact on their growth rate. In the germination stage, dry seed germination is promoted through water expansion, soil water potential is decreased in salt soil, and the absorption of water by seeds is inhibited, which seriously affects the germination rate, vigor index and germination index of seeds, which leading to the reduction of seed emergence rate [8,9]. The result showed that the *oePtrWRKY51* plants had a higher germination rate than the Col-0 and mutant plants under salt treatments (Figure 5A). Therefore, overexpression of *PtrWRKY51* is beneficial to plant in growth under salt stress conditions. Salt stress not only inhibited seed germination, but also significantly inhibited the growth of seedlings. Under salt stress conditions, the root structure of the plant was significantly damaged, and the root length was dramatically reduced, which affected the transport of water and nutrients from the root system to the aboveground part, which led to the obstruction of organic synthesis, and ultimately affected the growth and development of plants [57]. In this study, the

primary root length of *oePtrWRKY51* lines was significantly longer than that of Col-0 and *wrky51* mutant under salt stress conditions, which was conducive to plant growth and development (Figure 5B, C). In summary, these results further supported the conclusion that overexpression of PtrWRKY51 enhances salt tolerance in salt stress conditions.

Photosynthesis is one of the most sensitive physiological processes in plant response to stress [58]. Under normal growth conditions, the photosynthesizing organic matter of plants is enough to supply their needs, but under some special stress, the photosynthesis of plants will be affected, and they cannot synthesize enough organic matter to ensure the growth and breeding in plants. These stresses include cold, drought, high temperature, salt and so on. Among them, the damage area of salt stress is wider, and the damage degree of salt stress is deeper. The net photosynthetic rate analysis showed that photosynthesis was higher in the *oePtrWRKY51* lines compared to Col-0 and *wrky51* mutant under normal conditions, while the transpiration rate was lower than that of Col-0 and *wrky51* (Figure 4A,B). Therefore, the instantaneous WUE of the *oePtrWRKY51* lines were significantly higher than those of Col-0 and *wrky51* mutant lines (Figure 4C). WUE has been considered as one of the key factors which affect plant growth, and high WUE can promote plant growth [36]. WUE is also an important physiological index to assess plant stress resistance [59,60]. Under salt stress, the net photosynthetic rate and WUE of plants decreased, but *oePtrWRKY51* lines still had a higher net photosynthetic rate and WUE (Figure 6). Therefore, overexpression of *PtrWRKY51* improve plant growth and salt tolerance.

The chlorophyll content of leaves is closely related to photosynthesis in plants [61]. Salt stress can damage the ultrastructure of chloroplasts and affect the normal function of cells, which affecting the growth and development of plants [62]. We observed that under normal growth conditions, there was no significant difference in chlorophyll content between *oePtrWRKY51* and Col-0 plants, but both were higher than that of *wrky51* mutant plants. Under salt stress, chlorophyll content of *oePtrWRKY51* plants was significantly higher than that of Col-0 and *wrky51* mutant, which suggested *oePtrWRKY51* lines have a growth advantage under salt stress conditions. Taken together, these data indicate that *PtrWRKY51* is a promising gene target in increasing the tolerance of plants under salt stress conditions.

5. Conclusions

In this study, we illustrate the functional characterization of the poplar WRKY transcription factor PtrWRKY51 in the salt response. A PtrWRKY51 was isolated from *Populus trichocarpa*. RT-qPCR analysis revealed that PtrWRKY51 was mainly expressed in mature leaves and root. In addition, PtrWRKY51 is induced by salt stress. Overexpression of PtrWRKY51 in *Arabidopsis* improved salt tolerance. Consistently, overexpression of PtrWRKY51 exhibit an increase in seed germination rate, root length, photosynthetic rate, instantaneous leaf WUE, chlorophyll content under salt stress conditions. Taken together, our data indicate that PtrWRKY51 is a potential candidate gene in the improvement of salt tolerance in poplar by biotechnological strategies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14020191/s1, Table S1: Primer sequences used for cloning of PtrWRKY51 cDNA and RT-PCR.

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