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In vivo assessment of salinity stress tolerance in transgenic *Arabidopsis* plants expressing *Solanum tuberosum D200* gene

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Abstract

Transgenic *Arabidopsis* plants expressing a potato *D200* gene encoding a hypothetical protein were subjected to salinity stress and assessed for their tolerance. The D200 *Arabidopsis* lines exhibited increased chlorophyll content, improved stomatal conductance, less electrolyte leakage, lower accumulation of malondialdehyde (MDA), and a higher amount of proline compared to the wild type (WT) plants under salinity stress. The gene expression analysis revealed that D200 plants accumulated a significantly higher amount of mRNA transcripts of genes encoding three major antioxidant enzymes ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD). Chlorophyll *a* fluorescence kinetics analyses showed the D200 plants were more efficient in terms of primary photochemistry of photosystem II and performance indices. Furthermore, the quantum yields and efficiencies that represent the critical steps of photosynthetic light reactions were analyzed and it was found that D200 plants were photosynthetically more active than the WT plants under salt stress conditions. Overall, these findings suggest that the *D200* gene is a potential candidate gene for developing stress-resilient crops in future.

Keywords: Arabidopsis, chlorophyll, fluorescence, salinity, transgenic plants.

Introduction

Developing abiotic stress-resilient crops has been one of the major objectives of plant scientists across the globe. Given the magnitude of economic losses the farmers are bearing due to abiotic stress factors, it becomes important to develop stress-tolerant crops rapidly. Novel candidate genes must be characterized and used in crop improvement programs. An estimated 20 - 40 % of the entire eukaryotic genomes known so far are of unknown functions (Gollery *et al.* 2006). Several previous reports have demonstrated the remarkable response of proteins of unknown function, against oxidative stress in *Arabidopsis* (Davletova 2005, Luhua *et al.* 2008). Since oxidative stress is well known to act as secondary stress to various abiotic factors, it is reasonable to characterize these proteins under other environmental factors such as salinity, drought, and metal toxicity.

Photosynthesis is the most important metabolic process that drives the existence of life on earth. This two-part metabolic reaction is comprised of light reactions and the Calvin cycle that leads to the production of sugars. The light reactions are performed by complex photosynthetic machinery that is comprised of two photosystem (PS) complexes, PS II and PS I. Adverse effects of various abiotic stress factors on PS II have been extensively studied and documented (Gururani *et al.* 2015c, Sasi *et al.* 2018). Salt stress has been one of the major threats to crop productivity, and according to an estimate, the salt-stressed irrigated land is expected to expand by 50 % by the year 2050 (Hussain *et al.* 2017). Excessive salinity leads to the reduction of water potential in the soil, which in turn reduces the water absorption by the roots (Ibrahimova *et al.* 2021a). The primary effect that all the abiotic stresses bring about, is the production of toxic reactive oxygen species (ROS) that induce massive damage to the photosynthetic

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Abbreviations: APX - ascorbate peroxidase; CAT - catalase; Chl *a* - chlorophyll *a*; MDA - malondialdehyde; PS - photosystem; SOD - superoxide dismutase; WT - wild type.
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machinery at various levels (Nath *et al.* 2013, Choudhury *et al.* 2017). The disturbances created by the ROS molecules in the photosynthesis process consequently lead to further disturbances in the metabolism of various stress-responsive phytohormones (Gururani *et al.* 2015a, Kurepin *et al.* 2015).

Chlorophyll *a* (Chl *a*) fluorescence analysis is an excellent, non-invasive tool that provides relevant details of the damage to photosynthetic components in plants under different abiotic stress conditions (Strasser and Tsimilli-Michael 2001, Baker 2008). The so-called JIP-test (where J, I, and P are the steps of a transient curve) has been widely used over the years in various plants exposed to various types of stress conditions (Kalaji *et al.* 2014, Zivcak *et al.* 2014, Gururani *et al.* 2018, Akilan *et al.* 2019).

In a previous report, 69 potential drought-responsive genes were identified in potato using a yeast-based functional screening approach (Kappachery *et al.* 2013). The authors further reported that one of the identified genes (*D200;* Genbank acc. No. JX951423) with unknown function, showed a high response toward multiple stresses. The *D200* gene is located in the 8th chromosome and encodes a hypothetical protein that has not been characterized in any plant. In our previous study, the potato *D200* gene was cloned and characterized in *Arabidopsis* plants under polyethylene (PEG)-induced osmotic stress (Akilan *et al.* 2019). In this study, transgenic *Arabidopsis* lines expressing the potato *D200* gene were subjected to salinity stress and their stress tolerances were assessed using physiological, biochemical, and molecular approaches.

Material and methods

Plants, growth conditions, and stress treatments: Wild type (WT) and T3 generation D200 transgenic *Arabidopsis* lines (D200-81 and D200-82) were used for the study. Transgenic lines were developed as described earlier (Akilan *et al.* 2019). Seeds were surface sterilized with 5 % (m/v) NaClO for 10 min and rinsed 5 times with distilled water before transferring to half-strength Murashige and Skoog medium supplemented with 7 g dm^{-3} of phytoagar. Plants were grown in plant growth chambers at a photon flux density of 150 µmol m^{-2} s^{-1}, a 16-h photoperiod, relative humidity of 60 %, and a temperature of 22 ± 2 °C.

For salinity stress treatments, WT and D200 plants grown on MS medium were transferred to pots containing sterile peat (*Van Egmond*, Potgrond, The Netherlands). Four-week-old plants grown under normal conditions were soaked with NaCl solutions. Salinity stress was induced gradually from 50 mM NaCl to 200 mM NaCl with each increment at 50 mM for 2 d. The treatments were divided into two groups for each *Arabidopsis* line. The plants grown under no stress (NS) conditions were labelled as WT-NS, D200-81-NS and D200-82-NS while plants exposed to NaCl stress were labelled as WT-NaCl, D200-81-NaCl and D200-82-NaCl. All the analyses were made after two weeks from the beginning of NaCl treatment.

Estimated parameters: Chlorophyll estimation in WT, D200-81, and D200-82 plants grown under stressed and normal conditions was performed as described earlier (Ábrahám *et al.* 2010).

The morphological and growth parameters were recorded for the WT and D200 transgenic plants exposed to NaCl-induced stress and no-stress control conditions. Plant height, number of leaves per plant, fresh mass, and root length were measured to estimate the effect of salinity stress on WT and D200 plants.

Stomatal conductance in WT and D200 plants under NS and NaCl conditions was recorded on the adaxial surfaces of fully developed leaves using a leaf porometer (*SC-1*, *Decagon Devices*, Pullman, WA, USA) at a temperature of 25 ± 1 °C, and 55 ± 5 % relative humidity. Calibration of the porometer was done as per the manufacturer's instructions each day before the measurements.

Electrolyte leakage was measured following a protocol described earlier (Sullivan and Ross 1979) by using an equal number of leaf discs from each treatment. The leaf discs were first incubated in 10 cm^3 boiling water and the filtrate was collected. The electrical conductivity (EC) of the filtrate was measured and labelled as ECa. The filtrate was then heated to 55 °C for 30 min and the EC was measured again and labelled as ECb. Finally, the filtrate was again kept in boiling water for 10 min and EC was measured which was labelled as ECc. Electrolyte leakage [%] was calculated as (ECb - ECa/ECc) × 100.

For estimation of MDA content, 0.5 g of leaf tissue was homogenized in 5 cm^3 of 50 mM buffer (0.07 % m/v, NaH_2PO_4 . 2 H_2O and 1.6 % Na_2HPO_4·. 12 H_2O), and centrifuged at 20 000 *g* and 4 °C for 30 min. Then, 4 cm^3 of 20 % trichloroacetic acid containing 0.5 % thiobarbituric acid was added to 1 cm^3 of the supernatant. The reaction mixture was incubated at 95 °C for 30 min followed by incubation on ice for 10 min. The mixture was centrifuged at 10 000 *g* for 10 min, and absorbance was measured at 532 nm in a spectrophotometer (*Shimadzu UV-3600*, Kyoto, Japan). The values for non-specific absorption at 600 nm were subtracted from the absorbance values at 532 nm. The content of MDA was determined using an MDA coefficient of absorbance of 155 mM^{-1} cm^{-1} as described earlier (Fu and Huang 2001). Proline content in WT and D200 plants was estimated as described previously (Varghese *et al.* 2019) by homogenizing 0.5 g leaf samples in 10 cm^3 of 3 % (m/v) sulfosalicylic acid. The homogenate was filtered, and 2 cm^3 of the homogenate was mixed with 2 cm^3 of acid-ninhydrin, and 2 cm^3 of glacial acetic acid. The reaction mixtures were then incubated for 1 h and the reaction was stopped by cooling the tubes on ice. The chromophore-containing phase was extracted with 4 cm^3 of toluene and the absorbance of the extracts was measured at 520 nm in a spectrophotometer (*Shimadzu UV-3600*). A standard curve was prepared using known concentrations of proline. Final proline content on a fresh mass basis [µg g^{-1}(f.m.)] was determined using the standard curve.

Expression analysis of genes encoding ROS-scavenging enzymes: After two weeks of NaCl-induced salinity, leaf samples were collected for expression analysis of genes encoding APX, CAT and SOD enzymes. Total RNA from *Arabidopsis* rosette was isolated using an RNA isolation kit (*Norgen Biotek*, Ontario, Canada). RNA samples were quantified using *Nanodrop* and 1 µg RNA was used for cDNA synthesis using *Norgen'sTruScript*™ kit (*Norgen Biotek*). The cDNA was diluted 10-folds before using as a template for RT-qPCR analysis which was performed using the *Mx3000P* qPCR system (*Agilent Technologies*, Santa Clara, CA, USA) and *LF TaqPCR SYBR Mix* (*Applied Biosystems*, Bedford, MA, USA). Gene-specific primers (Table 1 Suppl.) were designed using the *PRIMER3* program. Relative transcription of each gene was calculated with respect to *GAPDH* as described earlier (Ghosh *et al.* 2017). Mean values were obtained from three biological replicates.

Chlorophyll *a* fluorescence measurements: Chl *a* fluorescence was recorded on fully expanded leaves of WT and D200 plants grown under NS and NaCl conditions. The intact plants were kept in a dark room for at least 1 h prior to the measurements using a pocket *PEA* fluorimeter (*Hansatech Instruments*, Norfolk, UK). Chl *a* fluorescence values from WT-NS plants served as controls. Actinic radiation (3 000 µmol photons m^{-2} s^{-1}) is used by the device and the fluorescence is measured at 685 nm. Five randomly selected leaves from each of the triplicate pots for each treatment were used for making the measurements. The maximal fluorescence (F_m) and the minimal fluorescence (F_0) of sampled leaves were used to calculate the F_v/F_m ratio (primary photochemistry of PS II) as shown in Table 2 Suppl. Additionally, the fluorescence readings were analyzed using the JIP test equations (Strasser 1981, Strasser and Stirbet 1998, Kalaji *et al.* 2011, Zivcak *et al.* 2014) on a *Biolyzer* software program (http://www.fluoromatics.com/biolyzer_software-1.php). The formulas and definitions of the parameters used in the analysis are described in Table 2 Suppl.

Statistical analysis: All experiments in this study were done at least three times and the data were analyzed using *Origin 8.1* software (www.originlab.com). Statistical differences were determined using one-way analysis of variance (*ANOVA*) followed by Tukey's multiple comparison tests. Standard error was calculated using the *n* values for each experiment. Error bars with different letters in the figures indicate significant differences at $P < 0.05$.

Results

Wild type and transgenic D200 *Arabidopsis* plants subjected to NaCl-induced salt stress exhibited a significant difference in terms of their morphology, physiology, and biochemical profile (Table 1, Fig. 1). The difference between WT and D200 plants grown under stressed conditions was prominent in terms of plant height, root length and fresh mass. No significant difference was found in the number of leaves per plant. D200-81 and D200-82 plants showed an 18 and 21 % increase in plant height, 51 and 62 % increase in root length and 25 and 27 % increase in fresh mass, respectively, compared to WT plants after two weeks of salinity stress (Table 1). Similarly, a significant difference ($P \leq 0.05$) in the chlorophyll content of WT and D200 plants was noted after 2 weeks of salinity stress (Fig. 1*A*). Transgenic lines D220-81 and D200-82 accumulated about 75 and 60 % higher total chlorophyll content, respectively, compared to the WT plants subjected to NaCl treatment. Average stomatal conductance was found 70 and 67 % higher in D200-81 and D200-82 plants, respectively, compared to WT plants subjected to NaCl-induced salt stress (Fig. 1*B*). Electrolyte leakage in WT plants was 51 and 64 % higher than that of D200-81 and D200-82 salt-stressed plants, respectively (Fig. 1*C*). Also, the accumulation of MDA under salt stress was in WT plants 79 and 51 % higher than in D200-81 and D200-82 plants, respectively (Fig. 1*D*). On the other hand, D200-81 and D200-82 plants accumulated 39 and 49.8 % higher proline content respectively, compared to WT plants after two weeks of salinity stress (Fig. 1*E*).

Real-time quantitative PCR (qPCR) was performed for estimating the mRNA transcription of *APX*, *CAT*, and *SOD* in WT and D200 *Arabidopsis* lines. The expressions of all three genes in D200-81 and D200-82 were found significantly higher compared to that of WT after 2 weeks of salinity stress. The expressions of *APX* and *SOD* were higher in D200-81 plants while the expression of *CAT* was

Table 1. Differences in growth parameters between wild type (WT) and transgenic *Arabidopsis* plants expressing potato *D200* gene exposed to NaCl-induced salt stress. Means ± SEs from three independent assays with five replicates for each treatment. Different letters in each column indicate significant differences ($P \leq 0.05$) between WT and D200 plants according to Tukey's test ($n = 5$).

	Plant height [cm]	Root length [cm]	Leaves per plant	Fresh mass [g]
WT	12.95±1.10^a	6.75±0.77^a	13.13±1.40^a	12.59±1.09^a
WT-NaCl	9.82±1.01^b	2.74±0.80^b	12.26±1.79^a	8.84±0.96^b
D200-81	13.50±1.38^a	7.23±0.82^c	13.20±1.69^a	14.34±0.99^c
D200-81-NaCl	11.60±1.08^c	4.16±0.70^d	12.66±2.02^a	11.07±1.12^d
D200-82	13.07±1.38^a	5.85±1.83^e	12.93±1.90^a	12.73±1.35^a
D200-82-NaCl	11.92±1.18^c	4.44±0.91^d	12.00±1.81^a	11.23±1.22^d

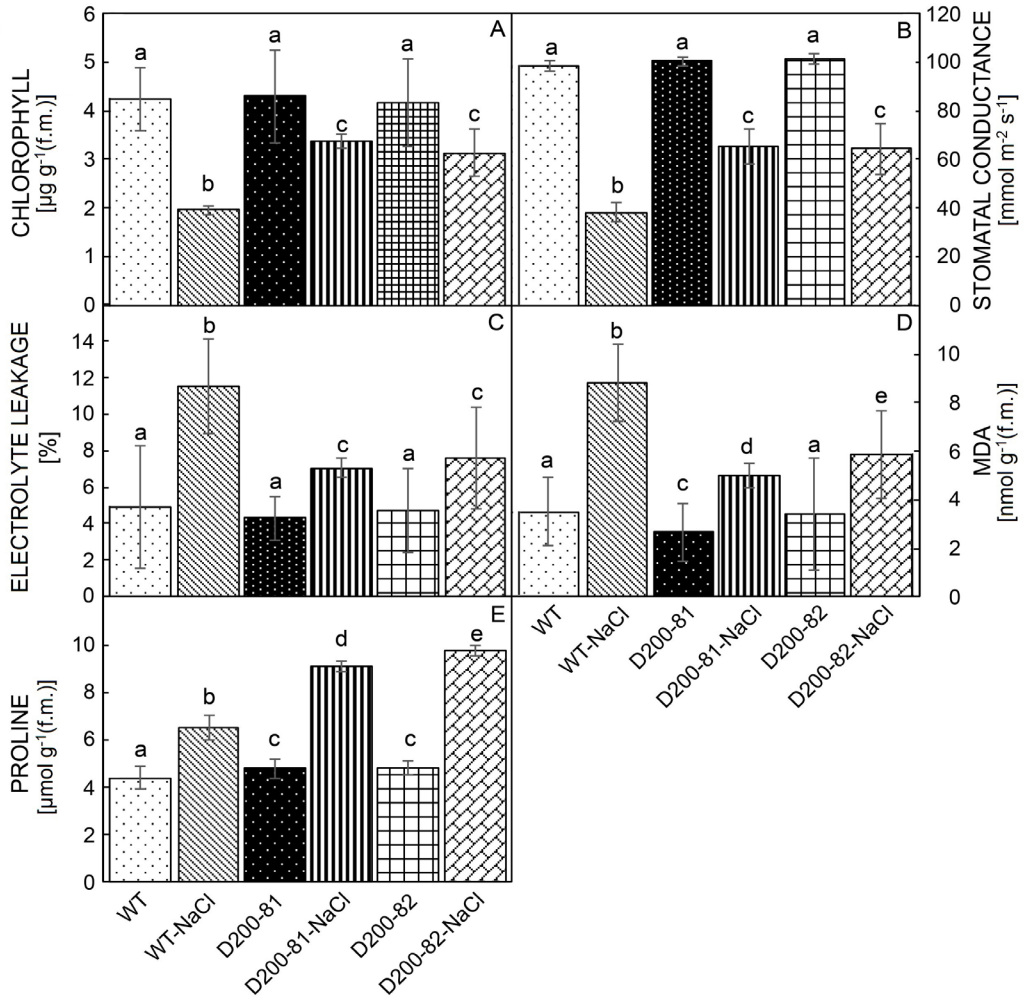

Fig. 1. Estimation of total chlorophyll content (*A*), stomatal conductance (*B*), electrolyte leakage (*C*), malondialdehyde (MDA; *D*), and proline content (*E*) in wild type (WT) and transgenic *Arabidopsis* plants expressing potato *SlD200* gene (D200-81 and D200-82) under normal and salinity stress conditions induced by 200 mM NaCl. Means ± SEs, $n = 3$; different letters indicate significant differences ($P \leq 0.05$) between the treatments after performing Tukey's test.

higher in D200-82 plants exposed to salinity stress (Fig. 2*A*-*C*).

Chlorophyll *a* fluorescence analysis was done to estimate the photosynthetic performance of WT and D200 plants under normal and stressed conditions. The Chl *a* fluorescence transient of the dark-adapted leaves are illustrated on a logarithmic scale from 20 µs to 1 s (Fig. 3*A*). A typical transient curve was observed in all the samples with similar maximum variable fluorescence ($F_v = F_m - F_0$), indicating that all the plants were photosynthetically active. The F_v/F_m values denote the maximum quantum yield of PS II (Strasser and Govindjee 1991). These values ranged between 0.83 (WT) and 0.84 (D200-81) under non-stressed conditions and from 0.7 (WT) and 0.78 (D200-81) under salinity stress conditions. A sharper decline in F_v/F_m in WT exposed to NaCl-induced salinity stress compared to the D200 plants was recorded, suggesting that D200 plants resisted the salinity stress markedly better than the WT plants (Fig. 3*B*). These findings were further substantiated with the estimation of the performance index (PI). The PI values indicate the overall vigour of plant samples (Mathur *et al.* 2011). The average PI value of D200-81 and D200-82 plants was 85 and 53.8 % higher, respectively, compared to the PI of WT plants after two weeks of salinity stress (Fig. 3*C*). As expected, both F_v/F_m and the PI values of WT and D200 plants under normal conditions, showed no significant difference (Fig. 3*B*,*C*).

The Chl *a* fluorescence transients were further analyzed by the so-called "JIP-test" (Strasser and Stirbet 1998) to deduce six functional parameters that specified the photosynthetic performance of the WT and D200 plants under normal and stressed conditions (Fig. 3*D*). The biophysical parameters thus evaluated included the quantum yields and efficiencies (ϕ_{Po}, ϕ_{Eo}, ϕ_{Ro}, ϕ_{Do}, δ_{Ro}, and ψ_{Eo}). The values of the parameters were normalized to those of the WT plants. The definitions and formulas of these parameters are shown in Table 2 Suppl. The parameters ϕ_{Po}, ϕ_{Eo}, ϕ_{Ro}, ϕ_{Do} represent the quantum yields of primary photochemistry, electron transport, thermal dissipation, and reduction of end electron acceptor at the PS I acceptor side, respectively. These yields were significantly higher in

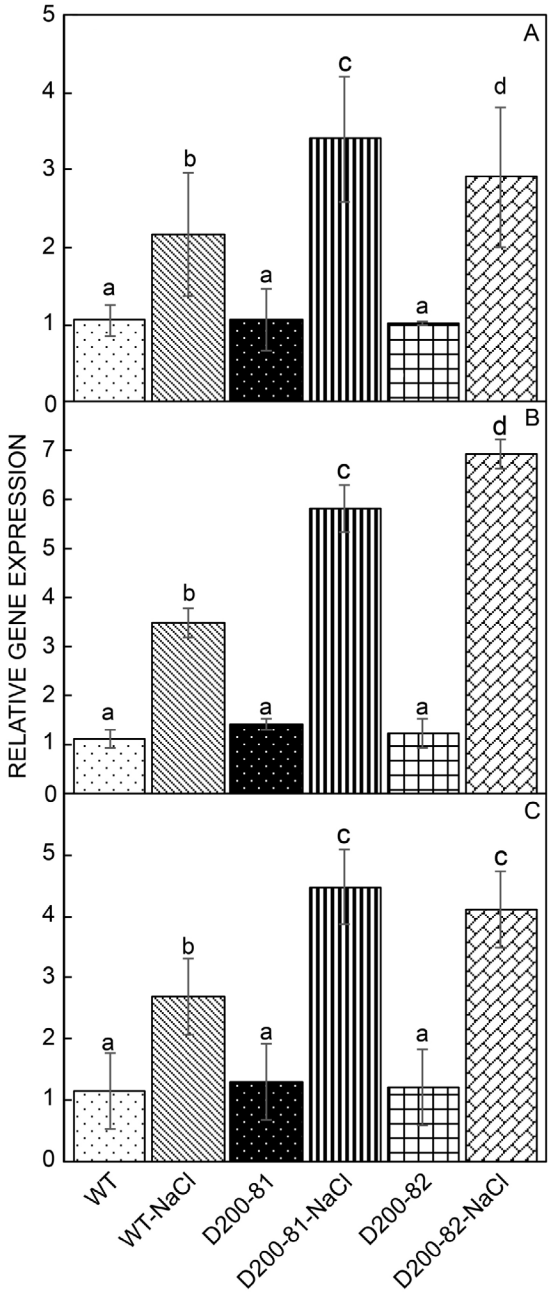

Fig. 2. The expression of the genes encoding ascorbate peroxidase (*APX*, *A*), catalase (*CAT*, *B*), and superoxide dismutase (*SOD*, *C*) in wild type (WT) and transgenic *Arabidopsis* plants expressing potato *D200* gene (D200-81 and D200-82) under normal and salt stress conditions induced by 200 mM NaCl. Fold expression values are normalized to those of the *GAPDH* control. Means ± SEs, $n = 5$; different letters indicate significant differences ($P \leq 0.05$) between the treatments after performing Tukey's test.

D200-81 and D200-82 plants compared to the WT plants under salt stress (Fig. 3*D*). The parameter δ_{Ro} represents the efficiency of an electron to reduce the end electron acceptor at the acceptor side of PS I. Similarly, ψ_{Eo} denotes the excitation transfer efficiency of the electron transport chain. Significantly higher values of δ_{Ro} and ψ_{Eo} in D200 plants under salinity stress indicated that the electron transport efficiency was higher in these plants compared to WT plants.

Discussion

Despite numerous efforts, abiotic stresses continue to remain major constraints for plant production. It is important to characterize novel proteins with unknown functions, so they can be used to engineer stress-resilient crops in future. Previous studies have indicated that up to 40 % of genes among all the known eukaryotes encode for proteins with unknown function (Gollery *et al.* 2006). However, many studies suggested that several such genes could be of great importance in plant growth, development, and abiotic stress tolerance in higher plants. Transgenic *Arabidopsis* plants expressing proteins of unknown function that constitutively expressed in response to oxidative stress were generated. Some of the transgenic lines were reported to have significantly increased oxidative stress tolerance (Luhua *et al.* 2008). Identification of abiotic stress-responsive genes is critical for the rapid development of stress-tolerant cultivars. In an earlier study, *StD200* encoding a hypothetical protein was identified as a potential candidate gene responsive to multiple abiotic stresses (Kappachery *et al.* 2013). Transgenic *Arabidopsis* plants expressing *StD200* exhibited improved osmotic stress tolerance as reported in our previous study (Akilan *et al.* 2019). In this work, the D200 plants showed improved resistance against salinity stress as reflected by the physiological and biochemical analyses. Higher Chl accumulation in D200 plants compared to the WT plants under stressed conditions was noted (Fig. 1*A*). A similar accumulation of Chl has been reported in numerous studies in *Arabidopsis* under salinity (Saibi *et al.* 2015), drought (Butt *et al.* 2017), heavy metals (Lee *et al.* 2003), and high temperature (Zhang *et al.* 2013). Abiotic stresses such as salinity and drought are known to inhibit photosynthesis by restricting stomatal conductance. Therefore, stomatal conductance is a critical parameter in evaluating the stress tolerance in plant samples. In our study, D200 plants exhibited a significantly higher stomatal conductance than the WT plants under salinity stress (Fig. 1*B*), indicating that the overexpression of potato *D200* in *Arabidopsis* plants conferred salinity stress tolerance. Transgenic soybean plants expressing *WRKY20* were reported to confer drought resistance and improve plant yield. Increased tolerance in transgenic plants corroborated with significantly higher stomatal conductance (Ning *et al.* 2017). Akin to stomatal conductance, electrolyte leakage is another crucial factor that is frequently used to evaluate the abiotic stress resistance in higher plants as it indicates the membrane permeability and allows researchers to estimate the membrane damage. A significantly reduced electrolyte leakage was estimated in D200 plants compared to WT plants (Fig. 1*C*) that pointed toward D200-induced salinity stress tolerance in the transgenic lines. Abiotic stress factors cause an increase in the production of ROS, that in turn increases the accumulation of MDA, a significant marker of membrane lipid peroxidation (Gill and Tuteja 2010). We

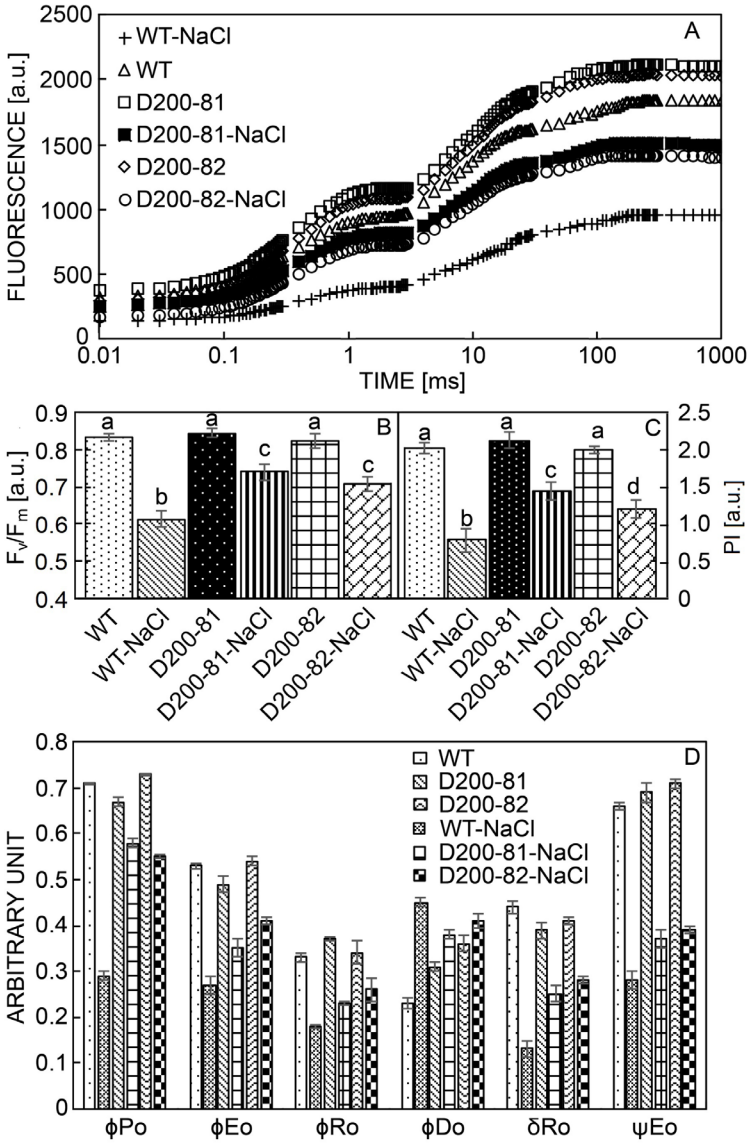

Fig. 3. *A* - Fast chlorophyll *a* fluorescence kinetics OJIP of the dark-adapted leaves. Transient polyphasic curves of each line represent the means of 15 measurements (5 measurements per plant taken from 3 different plants). *B* - Quantum yield of PS II determined as F_v/F_m. *C,D* - Performance index (PI, *C*), and quantum yield and efficiencies (*D*) in wild type (WT) and transgenic *Arabidopsis* plants expressing potato *D200* gene (D200-81 and D200-82) under normal and salt stress conditions induced by 200 mM NaCl. The details of each parameter are given in Table 2 Suppl. Means ± SEs of three independent assays with four replicates in each treatment. Different letters in *B* and *C* indicate significant differences ($P \leq 0.05$) between treatments; Tukey's test ($n = 9$).

noted that D200 plants accumulate a significantly lower amount of MDA than the WT plants under salinity stress (Fig. 1*D*), thus indicating higher intactness of the membrane in these plants. Similar findings of reduced accumulation of MDA in stress-resistant plants have been documented previously (Du *et al.* 2010, Cao *et al.* 2018). Proline is an extensively studied amino acid that is involved in several processes during stressed conditions in higher plants. It is involved in the stabilization of membranes and proteins as well as in the detoxification of ROS (Kaur and Asthir 2015). Therefore, an increase in proline accumulation is considered an indicator of improved stress tolerance in plants. The D200 plants accumulated significantly higher amounts of proline compared to WT plants under NaCl-induced salt stress (Fig. 1*E*). A significant increase in proline accumulation was recently reported in sorghum plants exposed to salinity stress induced by 100 mM NaCl indicating its positive effects on stress tolerance (Rastogi *et al.* 2020). Our findings are in agreement with previous studies where increased proline content in plants under stress is correlated with improved stress tolerance (Shan *et al.* 2007, Oh *et al.* 2011, Alyammahi and Gururani 2020).

The detoxification of cellular ROS is performed by non-enzymatic factors such as ascorbates, tocopherols, proline, *etc*. Additionally, several enzymes are also involved in the ROS-scavenging in plant cells. These enzymes include APX, CAT, and SOD which are well-known to participate

in the ROS-detoxification in chloroplast, mitochondria, and peroxisomes (Gill and Tuteja 2010). Our expression analysis of genes encoding these three enzymes revealed that the mRNA accumulation of all three enzymes was markedly higher in both D200 lines compared to that of WT plants under salinity stress (Fig. 2*A-C*). Previous studies have demonstrated a similar change in *APX*, *SOD*, and *CAT* gene expression in various plants in response to high temperature (Shah and Nahakpam 2012), cold (Chen *et al.* 2014), salinity (El-Esawi *et al.* 2017), and heavy metal toxicity (Sirhindi *et al.* 2016).

The impact of abiotic stresses on the photosynthetic machinery can be assessed using Chl *a* fluorescence analysis, that provides great details on the sequential energy fluxes of photosynthetic events. These details are crucial for the plants' overall vitality especially under challenging stress conditions (Çiçek *et al.* 2018). In this study, the OJIP transient curves altered in response to the NaCl-induced salinity stress, as shown in Fig 3*A*. Although the decline in the curves was observed in WT and D200 lines, the sharpest decline was exhibited in the WT plants after 2-weeks of salinity stress. Nevertheless, the typical OJIP curve indicated that all the plants were photosynthetically active as was described in previous reports (Kalaji *et al.* 2011, Redillas *et al.* 2011, Gururani *et al.* 2015b, Çiçek *et al.* 2018). A strong decrease in the PI but a non-significant decrease in salt-sensitive and salt-tolerant chickpea plants in response to salinity stress was reported by Çiçek *et al.* (2018). We observed a similar trend in terms of PI values. However, in contrast to the findings of Çiçek *et al.* (2018), we recorded a sharp decline in the F_v/F_m values as well. The D200 lines recorded significantly higher values of PI and F_v/F_m compared to WT under salinity stress conditions (Fig. 3*B,C*). Overexpression of maize *PsbA* gene in tobacco was reported to induce drought tolerance with increased antioxidant enzyme activity and higher F_v/F_m values indicating improved maximal photochemical efficiency of PS II (Huo *et al.* 2016). Similarly, PI values are considered an important indicator of plants' overall physiology and vigour (Oukarroum *et al.* 2007). Higher PI values in D200 plants subjected to salinity stress compared to that of WT plants further indicated that these plants not only resisted PEG-induced drought stress (Akilan *et al.* 2019) but also alleviated the damage to photosynthetic components during salinity. Our findings are in line with several previous studies which described F_v/F_m and PI as important markers to assess abiotic stress tolerance in higher plants (Thach *et al.* 2007, Mathur *et al.* 2011, Yusuf *et al.* 2010, Varghese *et al.* 2019). Similar to the F_v/F_m and PI values, the D200 plants showed improved quantum yields and efficiencies as shown in Fig. 3*D*. The average values of ϕ_{Po}, ϕ_{Eo}, ϕ_{Ro}, δ_{Ro}, and ψ_{Eo} in the D200 plants compared to those of WT plants after two-week exposure to salt stress clearly indicated that the D200 plants showed improved primary photochemistry of PS II, efficient electron transport, and excitation transfer efficiency to the electron transport chain. Similar findings have been documented in mustard (Yusuf *et al.* 2010), wheat (Vuletic and Spanic 2019), oat (Alyammahi and Gururani 2020), maize, and tomato plants (Kalaji *et al.* 2014) exposed to different stresses. Based on these findings, it appears that the protection of the PS II electron acceptors in D200 plants is not indisputable (indicated by ϕ_{Eo}) compared to effects on the electron transport chain close to PS I (indicated by ϕ_{Ro}) in which the salt stress effect was the most severe and the overexpression of *D200* led to almost two times higher efficiency further confirming the protective role of *D200* gene against oxidative damage, primarily affecting PS I and the ameliorative effects may be associated with higher membrane stability as described earlier (Chovancek *et al.* 2019, Rastogi *et al.* 2019, Ibrahimova *et al.* 2021b).

Taken together, our findings described here suggest that the overexpression of the *StD200* gene in *Arabidopsis* not only led to improved drought stress tolerance (Akilan *et al.* 2019) but also alleviated salinity stress by limiting the damage to photosynthetic components accompanied by improved ROS-scavenging activity. Further molecular characterization of the *D200* gene is underway, which could provide more details on the putative functions of the protein.

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