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

M.A. GURURANI*

Department of Biology, College of Science, United Arab Emirates University, Al Ain, UAE

*Corresponding author: E-mail: gururani@uaeu.ac.ae

**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...
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 Tsimpli-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, Aklan 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 (Aklan 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 (Aklan 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 phytagar. 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 cm³ boiling water and the filtrate was collected. The electrical conductivity (EC) of the filtrate was measured and labelled as ECc. The filtrate was then heated to 55 °C for 30 min and the EC was measured again and labelled as ECa. Finally, the filtrate was again kept in boiling water for 10 min and EC was measured which was labelled as ECb. Electrolyte leakage [%] was calculated as (ECb - ECa/ECc) × 100.

For estimation of MDA content, 0.5 g of leaf tissue was homogenized in 5 cm³ of 50 mM buffer (0.07 % m/v, NaH₂PO₄· 2 H₂O and 1.6 % Na₂HPO₄· 12 H₂O), and centrifuged at 20 000 g and 4 °C for 30 min. Then, 4 cm³ of 20 % trichloroacetic acid containing 0.5 % thiobarbituric acid was added to 1 cm³ 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 cm³ of 3 % (m/v) sulfosalicylic acid. The homogenate was filtered, and 2 cm³ of the homogenate was mixed with 2 cm³ of acid-ninhydrin, and 2 cm³ 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 cm³ of toluene and the absorbance of the extracts was measured at 520 nm in a spectrophotometer (Shimadzu UV-1100). 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’s TruScript™ 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 (Fₐ) and the minimal fluorescence (F₀) of sampled leaves were used to calculate the Fₐ/F₀ ratio (primary photochemistry of PS II) as shown in Table 1 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 Biolizer 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 ≤ 0.05) in the chlorophyll content of WT and D200 plants was noted after 2 weeks of salinity stress (Fig. 1A). 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. 1B). Electrolyte leakage in WT plants was 51 and 64 % higher than that of D200-81 and D200-82 salt-stressed plants, respectively (Fig. 1C). 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. 1D). 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. 1E).

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.

| Plant height [cm] | Root length [cm] | Leaves per plant | Fresh mass [g] |
|------------------|------------------|------------------|----------------|
| WT | 12.95±1.10     | 6.75±0.77       | 13.13±1.40     | 12.59±1.09     |
| WT-NaCl | 9.82±1.01     | 2.74±0.80       | 14.26±1.79     | 8.84±0.96     |
| D200-81 | 13.50±1.38     | 7.23±0.82       | 13.20±1.69     | 14.34±0.99     |
| D200-81-NaCl | 11.60±1.08     | 4.16±0.70       | 12.66±2.02     | 11.07±1.12     |
| D200-82 | 13.07±1.38     | 5.85±1.83       | 12.93±1.90     | 12.73±1.35     |
| D200-82-NaCl | 11.92±1.18     | 4.44±0.91       | 12.00±1.81     | 11.23±1.22     |
higher in D200-82 plants exposed to salinity stress (Fig. 2A–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. 3A). 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. 3B). 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. 3C). As expected, both $F_v/F_m$ and the PI values of WT and D200 plants under normal conditions, showed no significant difference (Fig. 3B, C).

The Chl a fluorescence transients were further analyzed by the so-called “JIP-test” (Strasser and Stürbert 1998) to deduce six functional parameters that specified the photosynthetic performance of the WT and D200 plants under normal and stressed conditions (Fig. 3D). The biophysical parameters thus evaluated included the quantum yields and efficiencies ($\phi_{Po}$, $\phi_{Eo}$, $\phi_{Ro}$, $\phi_{Do}$, $\delta_{Ro}$, and $\psi_{Eo}$). 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 $\phi_{Po}$, $\phi_{Eo}$, $\phi_{Ro}$, $\phi_{Do}$ 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
The parameter $\delta_{Ro}$ represents the efficiency of an electron to reduce the end electron acceptor at the acceptor side of PS I. Similarly, $\psi_{Eo}$ denotes the excitation transfer efficiency of the electron transport chain. Significantly higher values of $\delta_{Ro}$ and $\psi_{Eo}$ 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. 1A). 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. 1B), 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. 1C) 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).
GURURANI et al.

noted that D200 plants accumulate a significantly lower amount of MDA than the WT plants under salinity stress (Fig. 1D), 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. 1E). 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. 2A-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 (Sirhind et al. 2016).

The impact of abiotic stresses on the photosynthetic machinery can be assessed using Chl a fluorescence analysis, which 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 (Čiček et al. 2018). In this study, the OJIP transient curves altered in response to the NaCl-induced salinity stress, as shown in Fig 3A. 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, Čiček et al. 2018). A strong decrease in the PI but a non-significant decrease in sensitive-salt and tolerant chickpea plants in response to salinity stress was reported by Čiček et al. (2018). We observed a similar trend in terms of PI values. However, in contrast to the findings of Čiček et al. (2018), we recorded a sharp decline in the Fv/Fl values as well. The D200 lines recorded significantly higher values of PI and Fv/Fl compared to WT under salinity stress conditions (Fig. 3B,C). Overexpression of maize PsbA gene in tobacco was reported to induce drought tolerance with increased antioxidant enzyme activity and higher Fv/Fl 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 Fv/Fl 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 Fv/Fl and PI values, the D200 plants showed improved quantum yields and efficiencies as shown in Fig. 3D. The average values of φo, ϕp, ϕo, φp, δp, and ψp 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 φp) compared to effects on the electron transport chain close to PS I (indicated by φo) 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.

References

Ábrahám, É., Hourtou-Cabassa, C., Erdei, L., Szabados, L.: Methods for determination of proline in plants. In: Sunkar R. (ed.) Plant Stress Tolerance. Methods in Molecular Biology (Methods and Protocols): Plant Stress Tolerance, Vol. 639. Pp. 317-331. Humana Press, 2010.

Akilan, S., Halima, T.H., Sasi, S., Kappachery, S., Baniekal-Hiremath, G., Venkatesh, J., Gururani, M.A.: Evaluation of osmotic stress tolerance in transgenic Arabidopsis plants expressing Solanum tuberosum D200 gene. - J. Plant Interact. 14: 79-86, 2019.

Alyammahi, O., Gururani, M.A.: Chlorophyll a fluorescence analysis reveals differential response of photosynthetic machinery in melatonin-treated oat plants exposed to osmotic stress. - Agronomy 10: 1520, 2020.

Baker, N.R.: Chlorophyll fluorescence: a probe of photosynthesis in vivo. - Annu. Rev. Plant Biol. 59: 89-113, 2008.

Butt, H.I., Yang, Z., Geng, Q., Chen, E., Wang, X., Zhao, G., Ge, X., Zhang, X., Li, F.: GaMYB85, an R2R3 MYB gene, in transgenic Arabidopsis plays an important role in drought tolerance. - BMC Plant Biol. 17: 1-17, 2017.

Cao, K., Yu, J., Xu, D., Ai, K., Bao, E., Zou, Z.: Exposure to lower red to far-red light ratios improve tomato tolerance to salt stress. - BMC Plant Biol. 18: 10-15, 2018.

Chen, Y., Jiang, J., Chang, Q., Gu, C., Song, A., Chen, S., Dong, B., Chen, F.: Cold acclimation induces freezing tolerance via antioxidative enzymes, proline metabolism and gene expression changes in two chrysanthemum species. - Mol. Biol. Rep. 41: 815-822, 2014.

Choudhury, F.K., Rivero, R.M., Blumwald, E., Mittler, R.: Reactive oxygen species, abiotic stress and stress combination. - Plant J. 90: 856-867, 2017.

Chovancek, E., Zivcak, M., Botyanszka, L., Hauptvogel, P., Yang, X., Misheva, S., Hussain, S., Brestic, M.: Transient heat waves may affect the photosynthetic capacity of susceptible wheat genotypes due to insufficient photosystem I photoprotection. - Plants 8: 282, 2019.

Čiček, N., Oukarroum, A., Strasser, R.J., Schansker, G.: Salt stress effects on the photosynthetic electron transport chain in two chickpea lines differing in their salt stress tolerance. -
Photosynth. Res. 136: 291-301, 2018.

Davletova, S.: Cytosolic ascorbate peroxidase 1 is a central component of the reactive oxygen gene network of Arabidopsis. - Plant Cell 17: 268-281, 2005.

Du, H., Wang, N., Cui, F., Li, X., Xiao, J., Xiong, L.: Characterization of the beta-carotene hydroxylase gene DM2 conferring drought and oxidative stress resistance by increasing xanthophylls and ascorbic acid synthesis in rice. - Plant Physiol. 154: 1304-1318, 2010.

El-Esawi, M.A., Elansary, H.O., El-Shanhorey, N.A., Abdel-Hamid, A.M., Ali, H.M., Elshikh, M.S.: Salicylic acid-regulated antioxidant mechanisms and gene expression enhance rosemary performance under saline conditions. - Front. Physiol. 8: 1-14, 2017.

Fu, J., Huang, B.: Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. - Environ. exp. Bot. 45: 105-114, 2001.

Ghosh, R., Gururani, M.A., Popandopoulou, L.N., Mishra, R.C., Park, S.C., Jeong, M.J., Bae, H.: Expression analysis of sound vibration-regulated genes by touch treatment in Arabidopsis. - Front. Plant Sci. 8: 2017.

Gill, S.S., Tuteja, N.: Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. - Plant Physiol. Biochem. 48: 909-30, 2010.

Gollery, M., Harper, J., Cushman, J., Mittler, R., Girke, T., Zhiu, J. K., Bailey-Serres, J., Mittler, R.: What makes species unique? The contribution of proteins with obscure features. - Genome Biol. 7: 57, 2006.

Gururani, M.A., Mohanta, T.K., Bae, H.: Current understanding of the reprogramming of phytohormones and photosynthesis under environmental stress. - Int. J. mol. Sci. 16: 19055-19085, 2015a.

Gururani, M.A., Venkatesh, J., Ganesan, M., Strasser, R.J., Han, Y., Kim, J.I., Lee, H.Y., Song, P.S.: In vivo assessment of cold tolerance through chlorophyll a fluorescence in transgenic zoysiagrass expressing mutant phytochrome A. - PLoS ONE 10: e0127200, 2015b.

Gururani, M.A., Venkatesh, J., Ghosh, R., Strasser, R.J., Popandopoulou, L.N., Bae, H.: Chlorophyll a fluorescence evaluation of PEG-induced osmotic stress on PSI activity in Arabidopsis plants expressing SIP1. - Plant Biosyst. 152: 945-952, 2018.

Gururani, M.A., Venkatesh, J., Tran, L.S.P.: Regulation of photosynthesis during abiotic stress-induced photoinhibition. - Mol. Plant 8: 1304-1320, 2015c.

Huo, Y., Wang, M., Wei, Y., Xia, Z.: Overexpression of the maize psl4 gene enhances drought tolerance through regulating antioxidant system, photosynthetic capability, and stress defense gene expression in tobacco. - Front. Plant Sci. 6: 1-10, 2016.

Hussain, S., Zhand, J.H., Zhong, C., Zhu, L.F., Cao, X.C., Yu, S.M., James, A.B., Hu, J.J., Jin, Q.Y.: Effects of salt stress on rice growth, development characteristics, and the regulating ways: a review. - J. integr. Agr. 16: 2357-2374, 2017.

Ibrahimova, U., Zivcak, M., Ya, D., Rastogi, A., Allakhverdiev, S.I., Yang, X., Brestic, M.: Electron and proton transport in wheat exposed to salt stress: is the increase of the thylakoid membrane proton conductivity responsible for decreasing the photosynthetic activity in sensitive genotypes? - Photosynth. Res. 150: 1-17, 2021b.

Kalaji, H.M., Govindjee, Bosa, K., Koscielniak, J., Zúk-Golaszewska, K.: Effects of salt stress on photosystem II efficiency and CO2 assimilation of two Syrian barley landraces. - Environ. exp. Bot. 73: 64-72, 2011.

Kapil, H.M., Oukarroum, A., Alexandrov, V., Kouzamanova, M., Brestic, M., Zivcak, M., Samborska, J.A., Cermez, M.D., Allakhverdiev, S.I., Goltsev, V.: Identification of nutrient deficiency in maize and tomato plants by in vivo chlorophyll a fluorescence measurements. - Plant Physiol. Biochem. 81: 16-25, 2014.

Kappachery, S., Y., J.W., Banekal-Hiremath, G., Park, S.W.: Rapid identification of potential drought tolerance genes from Solanum tuberosum by using a yeast functional screening method. - Comp. rend. Biol. 336: 530-545, 2013.

Kaur, G., Ashtr, B.: Proline: a key player in plant abiotic stress tolerance. - Biol. Plant. 59: 609-614, 2015.

Kurepin, I.V., Ivanov, A.G., Zaman, M., Pharis, R.P., Allakhverdiev, S.I., Hurry, V., Hùner, N.P.: Stress-related hormones and glycinebetaine interplay in protection of photosynthesis under abiotic stress conditions. - Photosynth. Res. 126: 221-235, 2015.

Lee, J., Bae, H., Jeong, J., Lee, J.Y., Yang, Y.Y., Hwang, I., Martinova, E., Lee, Y.: Functional expression of a bacterial heavy metal transporter in Arabidopsis enhances resistance to and decreases uptake of heavy metals. - Plant Physiol. 133: 589-596, 2003.

Luhua, S., Ciftci-Yilmaz, S., Harper, J., Cushman, J., Mittler, R.: Enhanced tolerance to oxidative stress in transgenic Arabidopsis plants expressing proteins of unknown function. - Plant Physiol. 148: 280-292, 2008.

Mathur, S., Jajoo, A., Mehta, P., Bharti, S., Designs of elevated temperature-induced inhibition of photosystem II using chlorophyll a fluorescence induction kinetics in wheat leaves (Triticum aestivum). - Plant Biol. 13: 1-6, 2011.

Nath, K., Jajoo, A., Poudyal, R.S., Timilsina, R., Park, S.K., Aro, E.M., Nam, H.G., Lee, H.: Towards a critical understanding of the photosystem II repair mechanism and its regulation during stress conditions. - FEBS Lett. 587: 3372-81, 2013.

Ning, W., Zhai, H., Yu, J., Liang, S., Yang, X., Xing, X., Hau, J., Pang, T., Yang, Y., Bai, X.: Overexpression of Glycine soja WRKY20 enhances drought tolerance and improves plant yields under drought stress in transgenic soybean. - Mol. Breed. 37: 19, 2017.

Oh, M.-H., Sun, J., Oh, D.H., Zielinski, R.E., Clouse, S.D., Huber, S.C.: Enhancing Arabidopsis leaf growth by engineering the BRASSINOSTEROID INSENSITIVE1 receptor kinase. - Plant Physiol. 157: 120-131, 2011.

Oukarroum, A., Madidi, S., El Schansker, G., Strasser, R.J.: Probing the responses of barley cultivars (Hordeum vulgare L.) by chlorophyll a fluorescence measurements. - Plant Physiol. Biochem. 81: 609-614, 2015.

Paillotin, G.: Movement of excitations in the photosynthetic domains of photosystem II. - J. theor. Biol. 58: 237-252, 1976.

Rastogi, A., Zivek, M., Tripathi, D.K., Yadav, S., Kalaji, H.M., Brestic, M.: Phytotoxic effect of silver nanoparticles in Triticum aestivum: improper regulation of photosystem I activity as the reason for oxidative damage in the chloroplast. - Photosynthetica 57: 209-216, 2019.

Rastogi, A., Kovar, M., He, X., Zivcak, M., Kataria, S., Kalaji, H.M., Skalicky, M., Ibrahimova, U.F., Hussain, S., Mbarki, S., Brestic, M.: JIP-test as a tool to identify salinity tolerance in sweet sorghum genotypes. - Photosynthetica 58: 3333-343, 2020.

Redillas, M.C.F.R., Strasser, R.J., Jeong, J.S., Kim, Y.S., Kim, J.K.: The use of JIP test to evaluate drought-tolerance of transgenic rice overexpressing OsNAC10. - Plant Biotechnol. 336: 530-545, 2013.
SALINITY STRESS TOLERANCE IN TRANSGENIC \textit{ARABIDOPSIS}

\textbf{131}

Saibi, W., Feki, K., Ben Mahmoud, R., Brini, F.: Durum wheat dehydrin (DHN-5) confers salinity tolerance to transgenic \textit{Arabidopsis} plants through the regulation of proline metabolism and ROS scavenging system. - \textit{Planta} \textbf{242}: 1187-1194, 2015.

Sasi, S., Venkatesh, J., Daneshi, R., Gururani, M.A.: Photosystem II extrinsic proteins and their putative role in abiotic stress tolerance in higher plants. - \textit{Plants} \textbf{7}: 100, 2018.

Shah, K., Nahakpam, S.: Heat exposure alters the expression of SOD, POD, APX and CAT isozymes and mitigates low cadmium toxicity in seedlings of sensitive and tolerant rice cultivars. - \textit{Plant Physiol. Biochem.} \textbf{57}: 106-113, 2012.

Shan, D.P., Huang, J.G., Yang, Y.T., Guo, Y.H., Wu, C.A., Yang, G.D., Gao, Z., Zheng, C.C.: Cotton GhDREB1 increases plant tolerance to low temperature and is negatively regulated by gibberellic acid. - \textit{New Phytol.} \textbf{176}: 70-81, 2007.

Sirhindi, G., Mir, M.A., Ab-Allah, E.F., Ahmad, P., Gucel, S.: Jasmonic acid modulates the physio-biochemical attributes, antioxidant enzyme activity, and gene expression in \textit{Glycine max} under nickel toxicity. - \textit{Front. Plant Sci.} \textbf{7}: 1-12, 2016.

Strasser, R.J.: The grouping model of plant photosynthesis: heterogeneity of photosynthetic units in thylakoids. - In: Akoyunoglou, G. (ed.): Photosynthesis: Structure and Molecular Organisation of the Photosynthetic Apparatus, Vol. 3, pp. 727-737, Balaban International Science Services, Philadelphia, 1981.

Strasser, R.J., Govindjee: The F_{o} and the O-J-I-P fluorescence rise in higher plants and algae. - In: Argyroudi-Akoyunoglou J.H. (ed.) Regulation of Chloroplast Biogenesis. pp. 423-426, Springer, Boston 1991.

Strasser, R.J., Stirbet, A.D.: Heterogeneity of photosystem II probed by the numerically simulated chlorophyll \textit{a} fluorescence rise (O-J-I-P). - \textit{Math. Comput. Simul.} \textbf{48}: 3-9, 1998.

Strasser, R.J., Tsimilli-Michael, M.: Stress in plants, from daily rhythm to global changes, detected and quantified by the JIP-test. - \textit{Chim. Nouv.} \textbf{75}: 3321-3326, 2001.

Sullivan, C., Ross, W.: Selecting for drought and heat resistance in grain sorghum. - In: Mussell, H., Staple, R. (ed.): Stress Physiology in Crop Plants. pp. 263-281. John Wiley and Sons, New York 1979.

Thach, L.B., Shapcott, A., Schmidt, S., Critchley, C.: The OJIP fast fluorescence rise characterizes \textit{Graptofylhum} species and their stress responses. - \textit{Photosynth. Res.} \textbf{94}: 423-436, 2007.

Varghese, N., Alyammahi, O., Nasereddine, S., Alhassani, A., Gururani, M.A.: Melatonin positively influences the photosynthetic machinery and antioxidant system of \textit{Avena sativa} during salinity stress. - \textit{Plants} \textbf{8}: 610, 2019.

Vuletic, V., Spanic, V.: Characterization of photosynthetic performance during natural leaf senescence in winter wheat: multivariate analysis as a tool for phenotypic characterization of photosynthetic performance during natural leaf senescence in winter wheat. - \textit{Photosynthetica} \textbf{57}: 116-128, 2019.

Yusuf, M.A., Kumar, D., Rajwanshi, R., Strasser, R.J., Tsimilli-Michael, M., Sarin, N.B.: Overexpression of gamma-tocopherol methyl transferase gene in transgenic \textit{Brassica juncea} plants alleviates abiotic stress: physiological and chlorophyll \textit{a} fluorescence measurements. - \textit{Biochim. biophys. Acta} \textbf{1797}: 1428-38, 2010.

Zhang, S., Xu, Z.S., Li, P., Yang, L., Wei, Y., Chen, M., Li, L., Zhang, G., Ma, Y.: Overexpression of \textit{TaHSF3} in transgenic \textit{Arabidopsis} enhances tolerance to extreme temperatures. - \textit{Plant mol. Biol. Rep.} \textbf{31}: 688-697, 2013.

Zivcak, M., Brestic, M., Kalaji, H.M.: Photosynthetic responses of sun- and shade-grown barley leaves to high light: is the lower PSII connectivity in shade leaves associated with protection against excess of light? - \textit{Photosynth. Res.} \textbf{119}: 339-354, 2014.