EFFECT OF SALT STRESS ON GROWTH AND PHOTOSYNTHESIS PHYSIOLOGY OF TWO TOBACCO CULTIVARS

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**Abstract:** Saltstress is one of the most serious abiotic stress and the cultivation area affected by salt stress is growing, which seriously affects the plants growth and development. In the current study, chlorophyll content, gas exchanges, chlorophyll fluorescence, and genes transcriptional changes were compared in two local cultivars [(HonghuaDaJinYuan (H), Zhongyan100 (Z)] of tobacco (*Nicotiana tabacum*), in response to salt treatments (0, 100 mM and 150 mM NaCl) for 10 days. Salt-induced stress significantly affected the fresh weight (FW) of the cultivars, and the lowest FW was measured at 150 mM. Likewise, the photosystem II (PSII) quantum yield (QY\_Lss) and photochemical quenching coefficient (qP\_Lss) were comparatively highly decreased in Z than H, by the salt treatments. In addition, the photosynthetic rate and stomatal conductance were highly decreased in Z than H cultivar. However, chlorophyll content was significantly enhanced in H at both levels of salinity, while significantly decreased in Z at 150 mM. Taken all together, better gas exchanges, higher expression of photosynthesis-related and salt-responsive genes, suggested H as tolerant cultivar to salinity than Z cultivar.

**Keywords:** Tobacco (*Nicotiana tabacum),* Varieties; Salinity; Physiological and Molecular responses

1. Introduction

Salinity is one of the most destructive abiotic stresses affecting crop production all over the world. Salinity causes land degradation which results in a reduction of cultivated land area and ultimately a decline in productivity and quality of crops (Machado & Serralheiro, 2017). It has been evaluated that overall about 20% of cultivated and 33% of irrigated land is affected by salinity (Shrivastava & Kumar, 2015). Moreover, the salinity-affected areas are increasing 10% annually for several reasons, especially low precipitation which is aggravated by irrigation with saline water and poor cultural practices (Jamil *et al*., 2011). It has been assessed that over half of the arable land would be salinized by the year 2050 under current cultivation practices (Najar *et al*., 2019).

Plant adaptation to environmental conditions is a complex set of processes that occurs at molecular, cellular, physiological, and biochemical levels. Stress conditions lead to metabolic imbalance resulted in excessive formation of reactive oxygen species (ROS), causing oxidative stress (Sharma *et al*., 2019). In response to salt stress, the adaptive strategies by plants include biosynthesis of compatible solutes, activation of the antioxidant defense system, and salt stress responsive-related genes (Kumar *et al*., 2017; Gupta & Huang, 2014). The role of some stress-responsive genes has been reported, conferring tolerance and adaptation to abiotic stresses including salinity stress like *NHX* (Yokoi *et al*., 2002), *SOS* (Yang *et al*., 2019). In addition, chloroplast-encoded *psbA* and *RBCL* genes play a critical role in defending photosystem-II against oxidative damage during external stimuli (Rintämaki *et al*., 1996). Overexpressed *psbA* transgenic tobacco confers abiotic stresses tolerance by regulating antioxidant defense systems, photosynthetic capability, and stress defense responsive genes (Huo *et al*., 2016).

The combination of multicolor fluorescence imaging (MFI) and kinetic chlorophyll fluorescence (KCF) are effective techniques to early detect plant responses to abiotic stress (Yao *et al*., 2018). Based on pulse amplitude modulation (PAM), KCF quench kinetics, probing the performance of photosynthetic apparatus (Yao *et al*., 2018). Using the KCF, a previous study evaluated the photosynthetic efficiency of two varieties of perennial grass under salt stress (Dąbrowski *et al*., 2016). Yao *et al*. (2018) assessed the phenotypic responses of two genotypes to drought stress in Arabidopsis. The Plant defense response not only reflects on the photosynthesis but also the phenolic compounds produced under stress (Yao *et al*., 2018). Previous studies applied multicolor fluorescence for evaluating drought tolerance in tobacco varieties (Khan *et al*., 2019) water and nutrient deficiencies (Hsiao *et al*., 2010; Tremblay *et al*., 2012), fruit qualities, and pathogen attack (Ortiz-Bustos *et al*., 2016).

Tobacco is widely used as a model plant in scientific studies and gaining significances as a crop in bioresource technology. Thus tobacco is a nonfood economic crop that has both agriculture and scientific significance. The biocatalytic conversion of nicotine into useful hydroxylated-pyridine compounds provides an alternative green strategy for using tobacco as a biomass resource (Yu *et al*., 2017). Salinity decreases whole plant biomass of tobacco by suppressing the photosynthetic activity of the plant (Taylor, 2006).

In recent years, various studies have focused on disease resistance, drought tolerance, and salt tolerance in transgenic tobacco (Sharma *et al*., 2019; Agarwal *et al*., 2018; Wang *et al*., 2018; Garg *et al*., 2017). However, a comprehensive study has not yet been conducted to evaluate the tobacco cultivars to salt stress. Here, this study aims to comparatively evaluate and examine their performances at physiological and molecular levels to salt stress. As selection criteria, the data could be used for the possible breeding programs under stressful conditions (Akinci *et al*., 2004).

2. Materials and Methods

2.1. Plant materials, growth conditions, and stress treatments

This study was conducted on two local cultivars of tobacco; H and Z, seeds provided by the Tobacco Research Institute of Chinese Academy of Agricultural Sciences. The seeds were sown in expanded vermiculite and were cultivated in a growth room under 25/18 oC and 16/8 h (day/night) conditions. The seeds were watered with tap water up to emergence and then with one-quarter strength Hoagland solution (HS). Fifteen days old seedlings of uniform size and vigor were transferred to 2 L black trays covered by floating board with small holes. The Hoagland solution (1/4 HS, pH 5.7) containing 1.5 mM KNO3, 1 mM Ca(NO3)2.4H2O, 0.25 mM (NH4)2HPO4, 0.25 mMMgSO4.7H2O for macronutrients and 12.5 µM KCl, 6.25 µM H3BO3, 0.5 µM MnSO4.H2O, 0.5 µM ZnSO4.7H2O, 0.125 µM CuSO4.5H2O, 0.125 µM H2MoO4 (85% MoO3), 5 µM Fe-EDTA for micronutrients. The solution was replaced after two days and the seedlings were aerated for 15 min to maintain the nutrients concentration balance and optimal oxygen content throughout the experiment. Twenty-five days old seedlings were exposed to NaCl stress (100 mM and 150 mM) and to prevent any osmotic shock, salt was gradually added. The seedlings were sampled for analysis after ten days of starting salt treatment. The schematic diagram of the conducted experiment is given in Figure 1.



**Figure 1.** Schematic diagram of salt stress treatment of two tobacco cultivars. HS and DAE represent Hoagland solution and days after emergence respectively.

2.2. Determination of Fresh and dry biomass per plant

Plant fresh weight (FW) was measured by taking the whole plant randomly and immediately weighed. After measuring FW, the plants were dried in a drying oven at 80 oC for two days and weighed to determine the dry weight (DW) (Shi *et al*., 2020). Six plants were taken per replicate.

2.3. Photosynthetic parameters

2.3.1. Determination of Chlorophyll content

The method described in a previous study (Khan *et al*., 2019), was followed to measure the chlorophyll content. The second leaf from the top was taken as a sample from each replicate (n = 6 plants).Leaf discs from the middle of the leaf with an area of 1.08 cm2 were taken and were cut into narrow strips and put in 10 ml glass tubes. Then 5 ml 80% acetone was added in the tubes and the tubes were put in the dark until the color of the strips changed from green to white, to completely extract the chlorophyll. For pigments quantification, UV-spectrophotometer (UV-2600, Shimadzu Corporation, Kyoto, Japan), was used for the solution absorption at 645, and 663 nm. Acetone (80%) was used as blank. The pigments concentrations (in µg/ml/cm2) were determined according to the following formulae:

|  |  |
| --- | --- |
| Chla = (12.7 x A633) – (2.69 x A645) | (1) |
| Chlb = (22.9 x A645) – (4.86 x A663) | (2) |
| Chlt = 0.5 (Chla + Chlb)/leaf disc area | (3) |
| Leaf disc area = 1.08 cm2 | (4) |

Where A633 and A645 are the absorbance of the solution at 633 and 645nm respectively.

2.3.2. Leaf gas exchange

Gas exchange was measured following the method of Su *et al*. (2017) using a LI-6400 portable photosynthesis analyzer (LI-COR, Lincoln, NE, USA). The net photosynthetic rate (Pn) expressed in µmol m−2 s−1 and stomatal conductance (Gs) in mmol m−2 s−1 were determined on second fully developed leaves with 800 µmol m−2 s−1 photosynthetic photon flux density, 500µmol s−1 flow rate, leaf temperature 30±2◦C, and relative humidity 45±3%.

2.4. Determination of chlorophyll fluorescence and multicolor imaging

The chlorophyll fluorescence parameters and images of plants were obtained with a PAM fluorescence imaging system (FluorCam FC 800, Photon Systems Instruments, Brno, Czechia) after 10 days of starting salt stress. With an optimized quenching protocol as followed by Yao *et al*. (2018), the chlorophyll fluorescence images of the seedlings were acquired after dark adaptation for 20 min until the PSII reaction centers opened. Here we used a destructive method to quantity the chlorophyll fluorescence of the second leaf from the top of the plant.

Likewise, for multicolor fluorescence analysis and imaging also second fully expanded leaf from the top was selected from control and salt-stressed seedlings of the studied genotypes and were excited by the using UV (300-400 nm) LED panel. The multicolor fluorescence images were detected in blue (BF-440 nm) and green (GF-520 nm) wavelength regions (Khan *et al*., 2019).

2.5. RNA extraction and qRT-PCR

Total RNA was extracted by the phenol-based method described in a previous study (Shi *et al*., 2020). To examine the expression of salt responsive genes, PSII-related genes, chlorophyll biosynthesis, and vegetative growth-related genes in the seedlings under normal and salinity treatments, a qRT-PCR analysis was performed. cDNA was synthesized from 1 µg total RNA using HiScript® III-RT SuperMix for qPCR (+gDNA wiper) (Vazyme). The PCRs were performed on the Light cycler 96 (Roche Diagnostics, Germany), using ChamQTM SYBR® qPCR Master Mix (Vazyme). The amplification reactions were performed at a total volume of 20 µL, containing 10 µL 2×ChamQ SYBR qPCR Master Mix, 7.2 µL ddH2O, 0.8 µL reverse and forward primers (10 µM), and 2 µL cDNA (diluted 10 times after synthesis). PCR was performed as follows: 95°C for 1 min, then 40 cycles of 95°C for 10 s and 60°C for 30 min. The expression of the tobacco actin *NtL25* gene was used as an internal positive control (Schmidt & Delaney, 2010). The list of primers used for qRT-PCR is presented in Table 1. Three biological repeats were taken for quantification.

**Table 1. Primers list used for qRT-PCR**

| Primer name | Sequence (5’-3’) | Sequence (5’-3’) |
| --- | --- | --- |
| qRT-PCR primers | Forward | Reverse |
| *NHX1*  *SOS1*  *CHLH1*  *TTG2*  *psbA*  *RBCL*  *NtL25* | GGCGTCTGAGCTGGCTTCTA  GGCAGGGGTCTCCAGAGAAG  CACGGCTGATGCCACATTCC  AGATGGACAAGGCCGAGCAG  GCGGCTCCCTATTCAGTGCTA  GTTGTGGAGCCTGGGACTGT  CAAAAGTTACATTCCACCG | GCGCAAAGGAGTGCCACAAA  TGCATTGCCCGGGTAGACAT  TGCGTCAAGCCTCACAGTCT  TCCCGCAGCTGCAATGGAAT  ACTACAGGACAAGCAGCTAGGAAT  ACTCGATGCCAAGCAATGCG  TTTCTTCGTCCCATCAGGC |

2.6. Statistical analysis

The data were analyzed by two-way ANOVA using Statistix 8.1 software (Analytical Software, Tallahassee, FL, USA). Differences among the means of the treatments of the two cultivars were presented as standard deviation (±SD) using the least significant difference (LSD) test at p = 0.05 level of significance. The graphs were drawn using OriginPro 9.1 (OriginLab Corporation, Northampton, MA, USA).

3. Results

3.1. Effect of salinity on tobacco plant biomass and morphology

Salinity treatments greatly affected the morphology of the cultivars (Figure 2A). Under no-treatment control (CK), the growth of the two genotypes was similar, however, under salinity treatments, the growth of Z cultivar was highly affected comparatively with the H cultivar. After being treated with T1 for 10 days, the first pair of leaves in Z cultivar were observed yellow and the top second pair of leaves were slightly yellow. While, in T2 treatment, together with yellowing, slightly wilting was observed in Z cultivar, however, H cultivar remained normal.

According to our ANOVA results, the biomass of the tobacco cultivars; in terms of fresh/dry weight (g plant−1) was significantly affected by the salt treatments irrespective of the genotypes. In H cultivar, a significant declined of 42% and 51% fresh weight (FW) in T1 and T2 treated seedlings was observed compared with untreated, respectively. However, the percent drop of 64% was noted in Z cultivar with T2 salinity stress, and 47% in T1 compared with untreated seedlings (Figure 2B). While there is no significant difference among the dry weights of the treated and non-treated seedlings in the genotypes studied (Figure 2C).

Significant changes were found between cultivars, salinity treatments, and their interaction in the chlorophyll content (Figure 2D). In H cultivar, the chlorophyll content significantly enhanced in T1 and T2 compared with zero salt stress, and the increase was estimated as 25% and 24% in T1 and T2 respectively. In Z cultivar, the chlorophyll content significantly decreased (16%) in T2 seedlings compared with control, however, there is no statistical difference in chlorophyll content of T1 and control seedlings.



**Figure 2.** (**A**) Growth phenotypes of the two cultivars H and Z under control, T1 (100 mM), and T2 (150 mM) salinity treatments. Values shown in **B**, **C**, and **D** are mean values of fresh and dry weight per plant and chlorophyll content, respectively. Error bars represent the standard deviation of three replicates, with different letters, indicate significant differences. \*p<0.05, \*\*p<0.01, and ns represent non-significant.

3.2. Effect of salinity on chlorophyll and multicolor fluorescence

To compare the photosynthetic fluorescence performance of the two *Nicotiana tabacum* cultivars; H, Z various chlorophyll fluorescence parameters were measured after 10 days of salt stress. Figure 3A portrayed the steady-state quantum yield (QY\_Lss) response of two cultivars under salt stress. Salt stress significantly decreases the QY\_Lss in the two cultivars. In H, 17% and 15% decline was observed in 100 mM (T1) and 150 mM NaCl (T2) treatment in comparison with control, respectively. Similarly, a higher percentage reduction in QY\_Lss was observed in Z with 18% in T1 and 29% in T2 treated seedlings, as compared with control seedlings. Photochemical quenching coefficient (qP\_Lss) data shown in Figure 3B were progressively reduced with 12% and 13% in T1 and T2 treated H seedlings, in comparison with control, respectively. Similarly, the reduction of qP\_Lss in Z is significant at both T1 and T2 with a 17% and 26% decline, respectively. Salinity treatments resulted in 27 and 50% significant increase in NPQ\_Lss by T1 and T2 of H cultivar, respectively. In Z cultivar, a significant increase was observed in NPQ\_Lss with 24% and 50% in T1 and T2, respectively. However, no statistical differences were found between the cultivars (Figure 3C).

Likewise, there was no statistical difference between non-photochemical quenching coefficients (qN\_Lss) of the two genotypes. However, salinity stress considerably enhanced the qN\_Lss in H cultivar by 18% (T1) and 27% (T2) compared with control. In Z cultivar, it was enhanced by 65% in T1 and 28% in T2 compared with control, correspondingly (Figure 3D).

The representative chlorophyll fluorescence images of QY\_Lss and NPQ\_Lss are shown in Figure 4. The signal intensity of QY\_Lss was severely decreased and more obvious in Z than H cultivar at both T1 and T2. Moreover, the signal intensity was decreased from the middle of the leaf to its margin. In contrary, the signal intensity of NPQ\_Lss was increased and spread from the middle to the whole leaf canopy, which exhibited spatial heterogeneity.



**Figure 3.** Mean values of actual quantum yield (QY\_Lss) (A), photochemical quenching coefficient (qP\_Lss) (B), non-photochemical quenching (NPQ\_Lss) (C), and non-photochemical quenching coefficient (qN\_Lss) (D). H and Z represent HonghuaDaJinYuan and Zhongyan100 respectively. T1 and T2 stand for 100 mM and 150 mM NaCl stress accordingly. Error bars represent the standard deviation of three replicates, with different letters indicate significant difference. \*p<0.05, \*\*p<0.01, and ns represent non-significant.



**Figure 4.** Representative chlorophyll fluorescence images of actual quantum yield QY\_Lss and NPQ\_Lss for control (CK) and salt treatment after 10 days of salt treatments. T1 represents 100 mM NaCl stress. T2 represents 150 mM NaCl treatment. The color code depicted at the right of the images ranges from black (minimum value) to red (maximum value). H and Z represent HonghuaDaJinYuan and Zhongyan100 cultivars respectively.



**Figure 5:** Multicolor fluorescence values of blue fluorescence (BF) (**A**) and green fluorescence (GF) (**B**) for control and salt treatment at day 10 of salt treatments. T1 represents 100 mM NaCl stress. T2 represents 150 mM NaCl treatment. H and Z represent HonghuaDaJinYuan and Zhongyan100 cultivars respectively. Error bars represent the standard deviation of three replicates, with different letters indicate significant difference. \*p<0.05, \*\*p<0.01, and ns represent non-significant.

In this study, BF and GF parameters were selected from the full data (Figure 5), which represents the phenolic compounds produced in stress (Yao *et al*., 2018). The salinity treatments significantly influenced the BF and the values were enhanced in both genotypes (H and Z) compared with control. In H, the percent increase in BF value was 23% and 10%, while in Z, 6%, and 9% by T1 and T2, respectively. Similarly, the GF values were significantly enhanced by 28% and 21% in H, while 15% and 16% by T1 and T2, compared with control. However, statistically, there were no differences between the genotypes. The representative multicolor images of BF and GF are shown in Figure 6, exhibited that the signal intensity of BF and GF enhances in leaf upon 10 days of salt treatments, starting from leaf tip and margins to the middle of the leaf, which shows spatial heterogeneity.



**Figure 6.** Representative multicolor fluorescence images of blue fluorescence (BF) and green fluorescence GF for control (CK) and salt treatments (T1 =100 mM, T2 = 150 mM) after 10 days of salt treatments. H and Z represent HonghuaDaJinYuan and Zhongyan100 cultivars respectively. The color code depicted at the right of the images ranges from black (minimum value) to red (maximum value).

3.3. Changes in gas-exchange parameters of tobacco cultivars under salt stress

The dynamic changes in the net photosynthetic rate (Pn) and stomatal conductance (Gs) after 10 days of salt treatments were quantified in both cultivars as shown in Figure 7. Leaf net photosynthetic rate was significantly declined in both cultivars by salinity treatments. There are no significant differences between the genotypes. In H cultivar, compared with no-treatment control (CK), the percent decline in Pn was 72% by T1 and 74% by T2 respectively. While in Z cultivar, Pn was largely decreased with 78 and 94% by T1 and T2 respectively (Figure 7A). Significant differences were found between cultivars, salinity treatments, and their interaction in the stomatal conductance. In H cultivar, the stomatal conductance (Gs) was decreased (52%) by T2 only, while in Z cultivar, reduced by both T1 and T2 (74% and 88% respectively) compared with CK.



**Figure 7.** Values shown in **A** and **B**, are mean values of net photosynthetic rate and stomatal conductance respectively. H and Z represent HonghuaDaJinYuan and Zhongyan100 respectively. T1 and T2 stand for 100 mM and 150 mM NaCl stress accordingly. Error bars represent the standard deviation of three replicates, with different letters indicate significant difference. \*p<0.05, \*\*p<0.01, and ns represent non-significant.

3.4. Transcriptional changes of several categories genes under salinity

Transcript levels of salt stress-related (*NHX1*, *SOS1*), photosynthesis-related (*psbA*, *RBCL*), chlorophyll biosynthesis-related gene (*CHLH1*), and vegetative growth-related gene (*TTG2*) were detected by qRT-PCR in H and Z cultivar after 10 days of salt stress (Figure 7). The transcript levels of all the studied genes in the two cultivars were significantly affected by salt stress. The expression of salt stress-responsive genes *NHX1* enhanced by 4 and 7 times in H by T1 and T2 salinity treatments, respectively, while in Z, its expression enhanced with 3 times in both T1 and T2. Likewise, *SOS1* significantly enhanced by 3 and 4 times in H, while 4 times in Z cultivar, by T1 and T2 respectively. Similarly, the PSII-related gene *psbA* was significantly enhanced (6 and 20 times in H, 4, and 3 times in Z) by T1 and T2 correspondingly. However, there are no significant differences between the expressions of *RBCL* of the two genotypes. The transcript of *CHLH1* significantly enhanced in T1 (1.2 times), however, 0.6 times decreased in T2 compared with CK in H. While in Z cultivar, the expression of *CHLH1* reduced significantly by both T1 (0.6 times) and T2 (0.4 times). The relative expression of *TTG2* was also significantly enhanced by the T1 (4 and 9 times) and T2 (5 and 6 times) compared with CK in H and Z, respectively.



**Figure 8.** Values shown in **A-F** are mean values of relative expression of *NHX1*, *SOS1*, *psbA*, *RBCL*, *CHLH1,* and *TTG1* gene respectively. H and Z represent HonghuaDaJinYuan and Zhongyan100 cultivars of tobacco respectively. T1 and T2 stand for 100 mM and 150 mM NaCl stress accordingly. Error bars represent the standard deviation of three replicates, with different letters indicate significant difference. \*p<0.05, \*\*p<0.01, and ns represent non-significant.

4. Discussion

Salinity treatments considerably reduced the fresh weight of the two cultivars studied. Similar results in a salt-induced decrease in fresh biomass have been recorded in many crop plants e.g., *Solanum lycopersicum* L. (Zribi et al., 2009), *Carthamus tinctorius* L. (Erdal & Cakirlar, 2014), *Vigna radiate* L. (Ullah *et al*., 2016), *Vicia faba* L. (Qados, 2011), *Oryza sativa* L. (Jamil *et al*., 2012), *Brassica napus* L. (Mohamed *et al*., 2020) and *Cucumis sativus* L. (Ahmad *et al*., 2020). In general, salt stress causes both reduced cell division and cell elongation (Pitann *et al*., 2009) which is primarily due to salt-induced perturbance in the nutrients uptake, high accumulation of reactive oxygen species (Ashraf, 2009), cytoplasmic enzyme inhibition, turgor loss (Pitann *et al*., 2009) and hormonal imbalance (Ashraf *et al*., 2010; Iqbal & Ashraf, 2011) which consequently affects plant growth in terms reduce yield or biomass production (Ahmad *et al*., 2012).

The salinity stress often damages the photosynthetic apparatus as a result of the loss of chlorophyll, damages the light-harvesting complex, and suppress the PSII activity (Chaves *et al*., 2009). Previous experiments documented that salinity decreases the chlorophyll content (Ashraf & Harris, 2013), however, it depends on the tolerance of the plant species (Stefanov *et al*., 2016). Severe salinity may cause a set of physiological interactions that enable the plant to withstand the stress (Pandolfi *et al*., 2012). In our study, salinity stress enhanced chlorophyll content in tolerant cultivar, which is supported by a previous study (Shah *et al*., 2017) and this could be used as a biochemical indicator of salt-tolerant in plants. To maintain the proper functioning of the photosynthesis system under moderate salinity stress, the plant enhances the biosynthesis of chlorophyll pigment (Shah *et al*., 2017).

Fluorescence of chlorophyll could be regarded as excellent and effective parameters for characterizing plant resistance to stresses (Najar *et al*., 2019; Pineda *et al*., 2008). In this study, chlorophyll fluorescence measurement was also performed. PSII electron transport quantum yield, which determines the actual quantum yield of the photosystem, designated as QY\_Lss. The QY\_Lss significantly declined with salt stress in the cultivars, which may be attributed to accelerated senescence or to the effects of the salts on the reaction centers of the PSII directly (Najar *et al*., 2019). However, the percent decline in H cultivar is comparatively less than the Z cultivar, which shows tolerance of the cultivar to salinity. Results of the photochemical quenching coefficient (qP\_Lss) showed that salt stress progressively closed the reaction centers of PSII in the cultivars. However, in H cultivar, the percent decrease is less than the Z cultivar compared with control. Similar results were observed for salt stress tolerance comparison study (Najar *et al*., 2019). Non-photochemical quenching (NPQ\_Lss) is that part of light energy which is absorbed by antenna pigment and dissipated as thermal energy instead to be used in electron transport, was reported higher in both cultivars under salt stress. Previous studies also reported higher NPQ value under salinity stress in all genotypes of Rape plants (Pak *et al*., 2009). Najar *et al*. (2019) reported that salinity stress resulted in higher NPQ value by affecting electron transport from PSII to PSI and the light energy was dissipated as heat. Zhao *et al*. (2017) showed similar results under moderate and high light conditions in rice and reported that NPQ improves photosynthesis by alleviating photo-damage via ROS production. Beis & Patakas, (2012) reported higher NPQ value in a drought-tolerant variety of grapevine, which dissipates the excess energy efficiently via the xanthophyll cycle. The non-photochemical quenching coefficient (qN\_Lss) describe the processes quench singlet-excited chlorophylls that dissipate excessive excitation energy as heat, thus helping to regulate and protect photosynthesis against excess light energy (Müller *et al*., 2001). In our study qN\_Lss was significantly increased by the salinity treatments in H and Z cultivars, suggests that PSII reaction centers and light-harvesting complexes suffered seriously that the photoinhibition was activated (Laisk *et al*., 2014) and the plants efficiently dissipated thermal energy to protect leaves from serious damage due to excessive energy excitation in the reaction centers (Yuan *et al*., 2014; Jiang *et al*., 2017). The corresponding chlorophyll fluorescence images, which showed spatial variations from the middle to the margin of the leaf might imply greater restriction of photosynthesis on these sites (Khan *et al*., 2019).

Multicolor fluorescence imaging is a useful way to consider for detecting plant responses to abiotic stresses (Pineda *et al*., 2008). Salinity treatments significantly enhanced BF and GF in both cultivars and also exhibited spatial heterogeneity in their fluorescence images (Figure 6). This might be attributed to the accumulation of blue-green fluorescence emitting substances (phenolic compounds) and the production of intermediatory compounds during chlorophyll breakdown (Yao *et al*., 2018; Pineda *et al*., 2008; Perez-Bueno *et al*., 2016) which is an adaptation and tolerance mechanism to salinity.

Photosynthetic parameters under salt stress are good indicators of salinity tolerance in plants. As shown in Figure 7, the net photosynthetic rate (Pn) and stomatal conductance (Gs) significantly decreased upon 10 days of salt stress. However, the percent reduction is less in H cultivar than in Z cultivar, compared with CK. The stomatal conductance, exhibits gas exchange, significantly decreased in H at T2 only, which indicates that the stomata are maintained open at T1, showing tolerance of the cultivar to moderate salt stress. While in sensitive Z cultivar, the stomatal conductance significantly inhibited at both T1 and T2. Similar results were found for evaluating salt tolerance between two *Medicago truncutula* genotypes (Najar *et al*., 2019). While in sensitive Z cultivar, it was significantly inhibited at both levels of salinity. This changing trend was supported by comparisons of the chlorophyll content between H and Z cultivars, which shows a significant increase in chlorophyll content in H cultivar, while a reduction in Z cultivar (Figure 5C). Besides, a higher percent increase in chlorophyll biosynthesis (*CHLH1*) and chloroplast-encoded PSII-related genes (*psbA*, *RBCL*) expression indicated that the H has better photosynthetic performance than Z cultivar. Our results are in agreement with Fracasso *et al*. (2016) and Su *et al*. (2017), reported similar differences in chlorophyll content, photosynthetic parameters, and PSII-related genes expression comparing stress tolerance between *Sorghum bicolor* and *Nicotiana tabacum* genotypes, respectively. Meanwhile, higher percent of salt stress-responsive genes (*NHX1* and *SOS1*) expression, suggesting that H unveiled a better genetic basis against salt stress than Z cultivar. In addition, higher expression of the genes might be an adaptive mechanism in response to salt stress (Mojtaba & Rabiei, 2019; Yue *et al*., 2012).

5. Conclusions and forward look

Salt stress influences plants in many ways. Tobacco cultivars exhibited different features in terms of plant growth, biomass, photosynthetic-related physiology, and differences in genes transcriptional levels in response to salt stress. In the current study, H cultivar had better photosynthetic efficiency, which led to less biomass reductions with higher chlorophyll content after 10 days of salt stress. Meanwhile, comparatively higher expression of photosynthesis-related and salt responsive genes in H unveiled a better genetic basis against salt stress than Z cultivar. Based on our results it can be concluded that H is a potential candidate for enhancing salt tolerance in tobacco through breeding and introgression programs.

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