

Chaetocin Affect Photosynthesis of *Nicotiana tabacum* under Abiotic Stress

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ABSTRACT

Epigenetic regulation of gene expression plays an important role in plant growth, development, and response to various environmental stresses. Due to increasing pollution and global warming, the environmental condition is getting worse for living organisms and plants. The growth and productivity of crop plants worldwide are severely affected by abiotic stress condition. In present study, we studied the effect of histone methyltransferase inhibitor chaetocin in tobacco plant under different abiotic stresses like cold, heat, drought and salinity. In our present study, we have found that photosynthesis is lowered in control plants by chaetocin and it is further lowered under abiotic stresses in the presence of chaetocin. Our study will help understand the epigenetic regulation of genes involved in photosynthesis and identify stress-responsive genes to understand the mechanism that reduces crop yield.

Keywords: Abiotic stress, Chaetocin, Histone methyltransferase.

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INTRODUCTION

Stress in plants refers to any external condition that is not ideal for its growth. Due to sessile nature, plants have to deal with these external environmental conditions that are changing continuously. Stress in plants can lead to a reduction in their growth, damage them or result in death. These stress conditions pose a severe threat for agriculture crops as the crop yield decreases significantly (Wang *et al.*, 2003; Wania *et al.*, 2016). Abiotic stress consists of different environmental conditions like high temperature, cold, light, salinity, deficit or excessive water, radiation, etc. Due to increasing pollution and global warming, the environmental condition is getting worse for living organisms and plants. Growth and productivity of crop plants worldwide are severely affected by Abiotic stress conditions. Plants response to abiotic stress is a complex process (Skirycz *et al.*, 2010; Cramer *et al.*, 2010), resulting in temporary or permanent changes in plant physiology. Abiotic stress response in plants is interconnected and occurs in expression changes in number of genes. Our present study uses histone modification inhibitors to study plant physiology concerning gas exchange parameters i.e photosynthesis, transpiration, and water use efficiency.

Epigenetic regulation of gene expression consists of DNA methylation and post-translational Histone modifications. Histone modifications consist of acetylation, methylation, ubiquitylation sumoylation of lysine and arginine residues (Strahl and Allis, 2000). In this study, we focused on histone methylation which was discovered in 1960 at the ϵ -amino group of lysine residue in histone protein (Allfrey and Mirsky, 1964; Murray, 1964). Histone methylation on lysine residue can be mono-, di-, tri-methylated, whereas methylation on arginine residue can be mono-, di-methylation symmetrical or dimethylation asymmetrical (Greer and Shi, 2012). Histone methylation on lysine is tightly regulated by methyltransferases (KMTs) and Demethylases (KDMs). First histone KMT (KMT1A) was discovered in the year 2000 by Thomas jenuwein and coworkers (Rea *et al.*, 2000). KMTs contain an enzymatic SET domain made up of 130 amino acids and are the largest among

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the two KMTs family (Dillon *et al.*, 2005). The second class of KMTs does not contain SET domain (van Leeuwen *et al.*, 2002). Both the KMTs class of enzymes uses S-adenosyl-L-methionine as the methyl group donor (Dillon *et al.*, 2005). KMTs are very specific in lysine methylation.

On the other hand the first histone demethylase LSD1/KDM1A was discovered by Yang shi and coworkers (Shi *et al.*, 2003) and contain Flavin adenine dinucleotide (FAD) dependant amine oxidase domain for demethylation (Shi *et al.*, 2004). Other classes of demethylases were also observed that contain JmjC (jumonji C) and JmjN domain (Shi and Whetstone, 2007; Chen *et al.*, 2006).

Chaetocin is a fungal metabolite from *Chaetomium minutum* with antimicrobial and cytostatic activity (Weber, 1972). It is a molecular dimer of two five-membered rings cis fused. It is a competitive inhibitor of S-adenosylmethionine and acts as an inhibitor for lysine-specific methyltransferase making this compound useful in epigenetic gene regulation studies (Greiner *et al.*, 2006). Chaetocin is specific for Su(var)3-9, a methylase of histone H3 at lysine 9 and is best suited for heterochromatin-mediated gene silencing (Greiner *et al.*, 2006). In the present study we have used Chaetocin to study the effect of histone methylation on the physiology of plants as well as under various abiotic stresses. This study will help us provide information about how epigenetic regulation of genes affects plant physiology and their response in abiotic stress.

MATERIALS AND METHODS

Plant Materials

Our study used *Nicotiana tabacum* as it is considered a model plant to study various developmental processes. *Nicotiana tabacum* cv. Petite Havana SR1 (NTPH) were grown under 16 hours of light and 8 hours of dark period in a glasshouse at 25°C.

Histone Methyltransferase Inhibitor

Histone methyltransferase inhibitor Chaetocin was used. Chaetocin was given to four-week-old plantlets and different stress conditions to observe the change in gas exchange parameters. Chaetocin was given at a concentration of 0.1M with media.

Different Stress Treatment

One-month-old plantlets were exposed to different stress conditions for the study. For drought conditions, water was withdrawn from Tobacco plants for 10 days. One-month-old plants were supplemented with 300mM NaCl in growth media to create salinity stress condition for 10 days. For cold conditions plants were exposed to 4°C temperature for 8 hours. Plants were kept at 37°C for 8 hours to create heat conditions.

For the experiment with inhibitor, 0.1 M chaetocin was added in growth media and was given to plants. All the four stress condition of Heat, Cold, Drought and Salinity were carried out on plants grown in the presence of inhibitor. The condition for all the stress remained same as of the plants without inhibitor.

Gas Exchange Measurement

Photosynthetic rate (A), Transpiration rate (E) and Stomatal conductance (gs) was measured with an LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE) with red and blue LED light sources. Measurement were taken at 400µmol CO₂ mol⁻¹ air. Water use efficiency (WUE) was calculated by taking ratio of (A) to (E).

RESULTS AND DISCUSSION

Change in Photosynthesis, Transpiration and Stomatal Conductance in Presence of Inhibitor

Nicotiana tabacum plantlets of one month were treated with Inhibitor chaetocin for ten days. Physiological parameters Photosynthesis, transpiration, stomatal conductance were measured. Fig. 1 shows Photosynthesis, Transpiration, and Stomatal conductance in the presence of inhibitor. Photosynthesis was decreased significantly in presence of

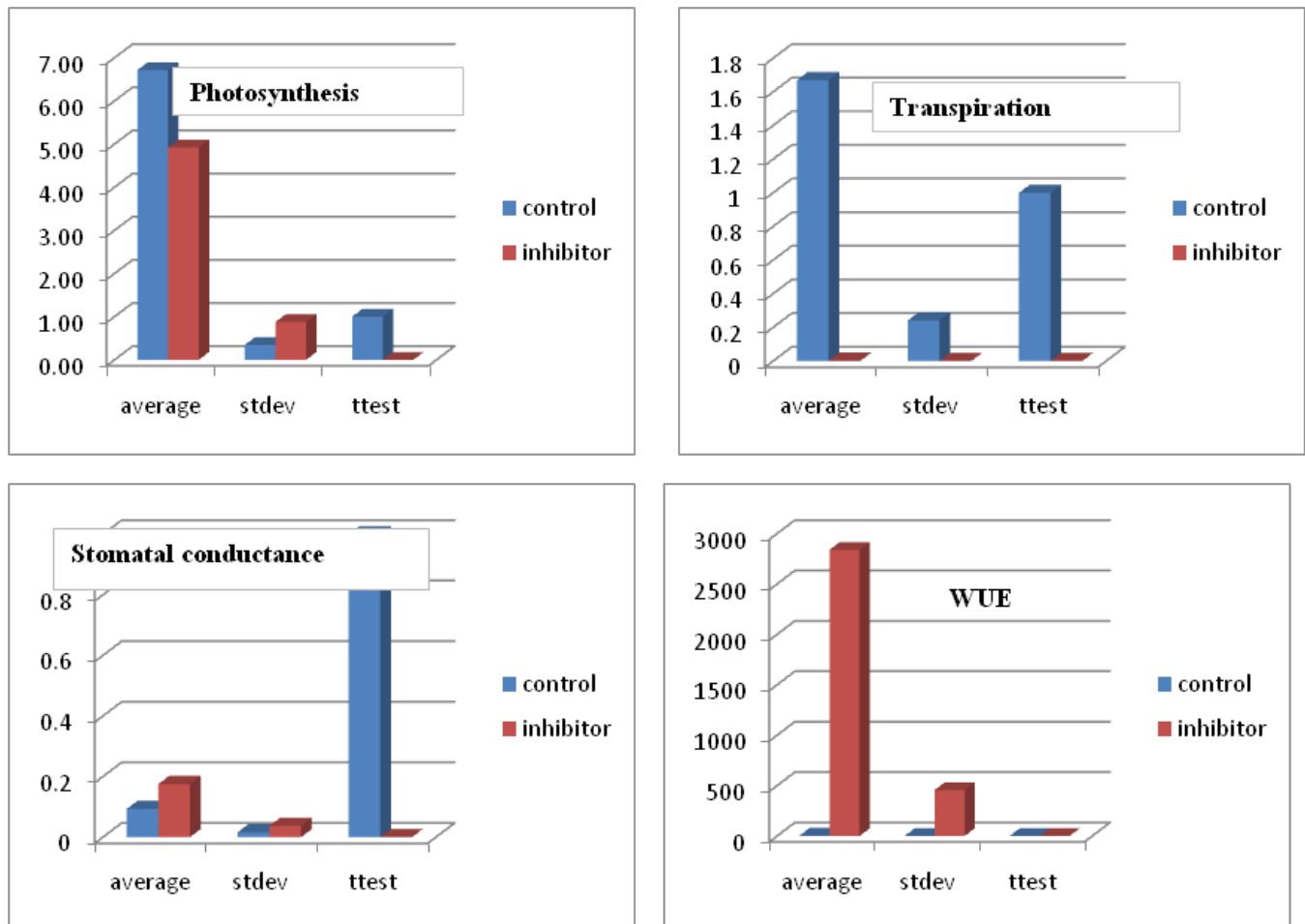


Fig. 1: Photosynthesis, Transpiration, Stomatal conductance and Water use efficiency (WUE) in presence of inhibitor.

inhibitor concerning control. Transpiration rate was also decreased significantly whereas stomatal conductance was increased. Fig. 1 shows the water use efficiency of both control and inhibitor. Water use efficiency was increased significantly in plants with inhibitor in comparison with Control plants.

Comparative Physiological Response under Drought Stress in Presence of Inhibitor

Nicotiana tabacum plants were grown in the presence of inhibitor Chaetocin, and then they were subjected to different abiotic stresses along with plants without inhibitors. Different Gas exchange values i.e. photosynthesis, transpiration and stomatal conductance. Fig. 2 shows the different parameters under drought condition.

In the comparative study of Gas exchange parameters photosynthesis and transpiration increased under drought stress in the presence of inhibitor concerning drought stress without inhibitor. Whereas Stomatal conductance was increased under drought stress in the presence of inhibitor in comparison with drought condition. Water use efficiency was calculated and is shown in Fig. 2. Presence of inhibitor increased the water use efficiency under drought condition concerning drought condition without inhibitor.

Comparative Physiological Response under Salinity Stress in Presence of Inhibitor

Nicotiana tabacum plants were subjected to Salinity stress for ten days using 300mM NaCl. For Inhibitor treatment plant were grown with 0.1M concentration of inhibitor for ten days and were subjected to salinity condition. Fig. 3 shows photosynthesis, transpiration and stomatal conductance. It is clearly indicated that photosynthesis and transpiration in salinity with inhibitor were increased compared to salinity without inhibitor. whereas stomatal conductance was increased under salinity condition with inhibitor. In case of water use efficiency shown in Fig. 3, salinity without inhibitor has higher water use efficiency concerning salinity in the presence of inhibitor.

Comparative Physiological Response under Heat Stress in Presence of Inhibitor

For Heat stress, inhibitor plants were grown with a 0.1M inhibitor condition for ten days and then subjected to 37° for 16 hours in the growth chamber. Fig. 4 shows the photosynthesis, transpiration and stomatal conductance. The results show that inhibitor presence has increased photosynthesis under heat condition, but transpiration was decreased significantly in the presence of inhibitor under heat stress. As results obtained in

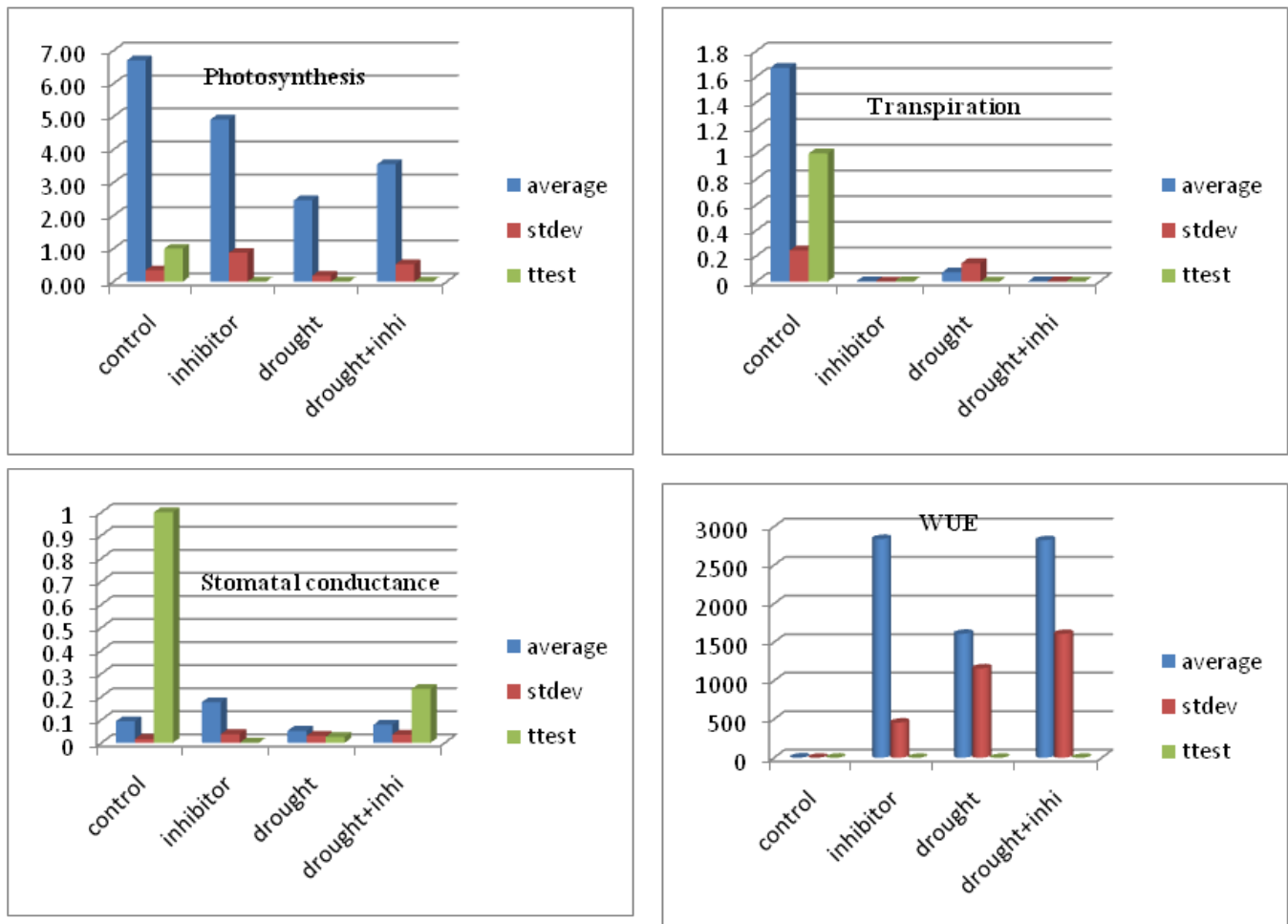


Figure 2: Photosynthesis, transpiration, stomatal conductance and water use efficiency (WUE) under 10 days of drought stress.

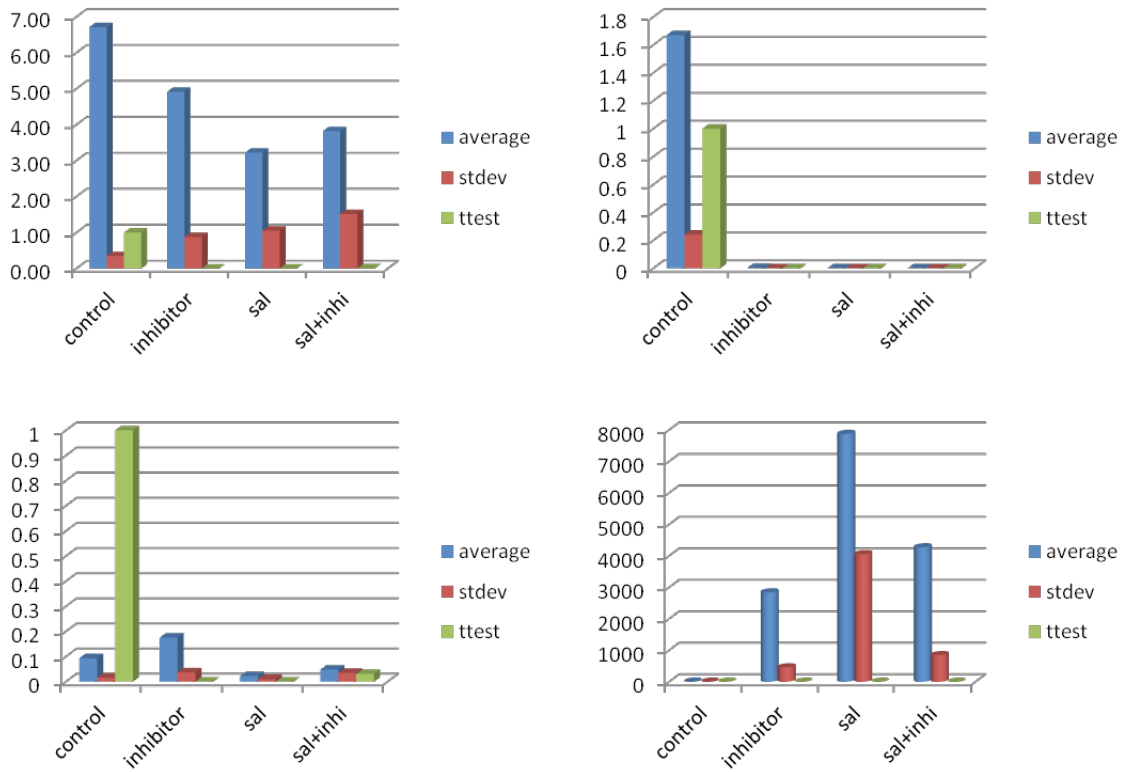


Fig. 3: Photosynthesis, transpiration, stomatal conductance and water use efficiency (WUE) under 10 days of salinity stress.

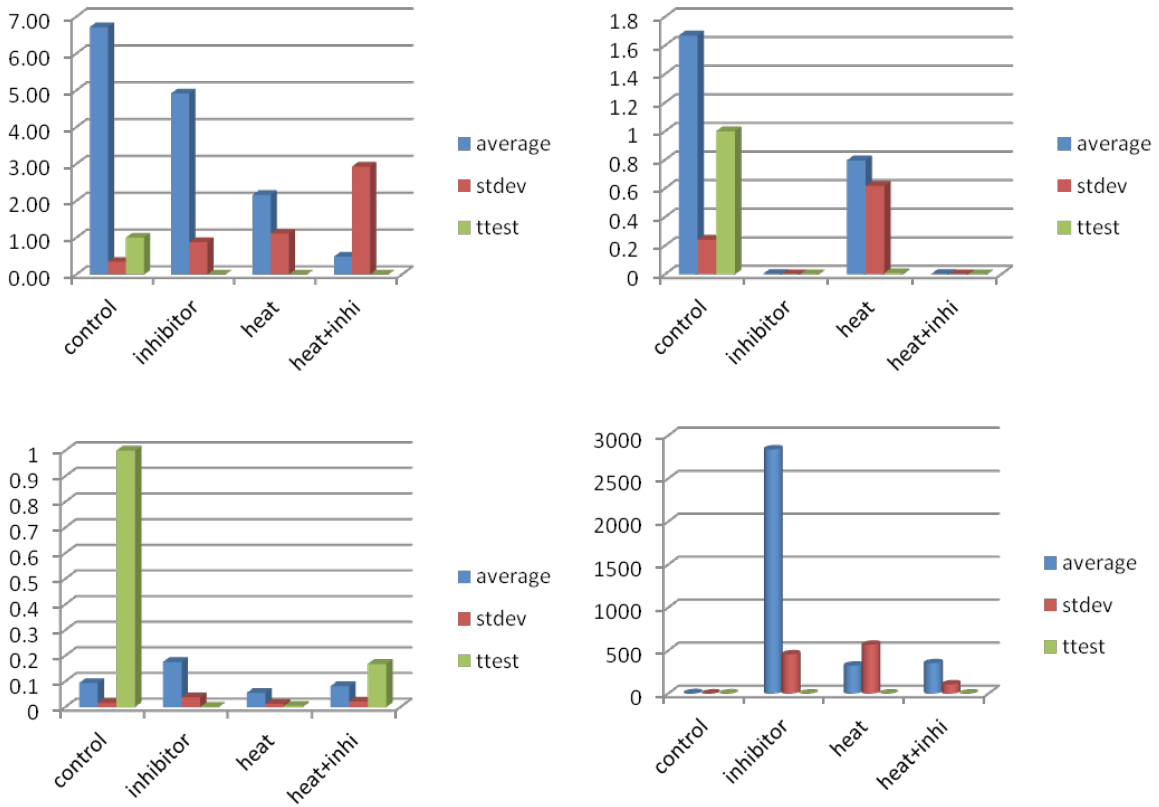


Fig. 4: Photosynthesis, transpiration, stomatal conductance and water use efficiency (WUE) under 8 hours of heat at 37°C

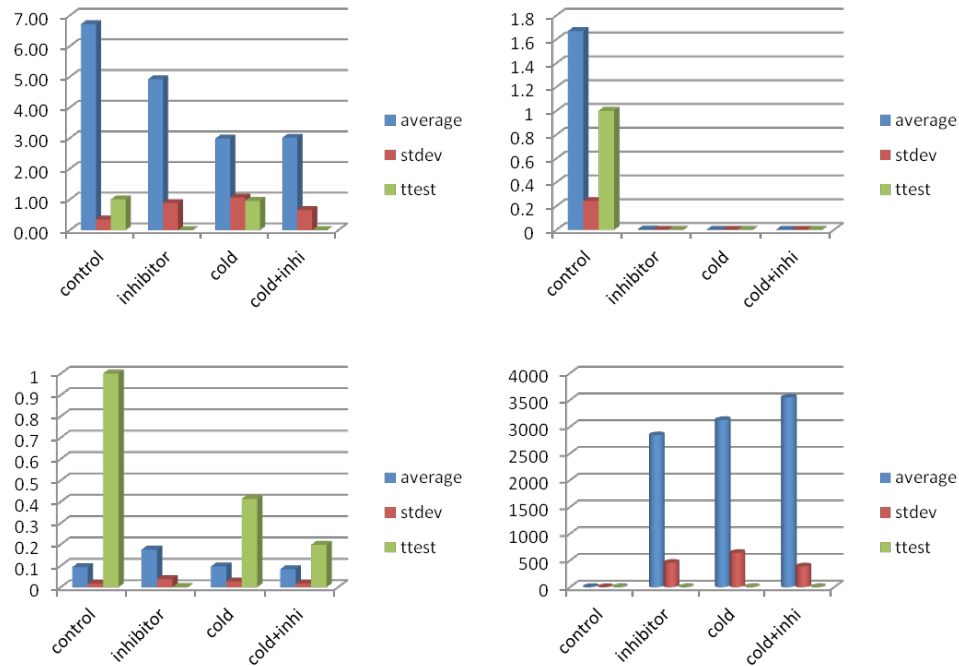


Fig. 5: Photosynthesis, transpiration, stomatal conductance and water use efficiency (WUE) under 8 hours of cold at 4°C

salinity and drought stress, stomatal conductance has increased in inhibitor under heat condition. Water use efficiency has been calculated and Fig. 4 shows decreased WUE in inhibitor under heat condition.

Comparative Physiological Response under Cold Stress in the Presence of Inhibitor

Tobacco plants were subjected to chilling temperature of 4 °C for 8 hours. For cold stress in the presence of inhibitor plants were supplemented with inhibitor for 10 days and then subjected to cold stress. Result of gas exchange parameters shows changes under cold stress in presence inhibitor. Water use efficiency was higher in cold stress in the presence of inhibitor as shown in Fig. 5.

CONCLUSIONS

Our present study concluded that Histone methyltransferase inhibitor chaetocin affects the photosynthesis rate in *Nicotiana tabacum* plants. This can further result in identification of genes that are epigenetically regulated by histone methylation. Under different abiotic stress photosynthesis rate is further decreased in presence of inhibitor. A group of genes governs plants response to various abiotic stresses but their regulation is complicated or they act in different ways in different stress. It can be concluded that histone methylation is affecting genes involved in different abiotic stresses. Genes involved in photosynthesis or stress responsive pathway can be characterized in future studies.

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