Journal of Animal and Veterinary Advances 11 (21): 3898-3907, 2012
ISSN: 1680-5593

Photosynthetic Properties of Different Drought Resistant Chicory Strains and their Responses to Drought Stress

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Abstract: The photosynthetic properties and photosynthetic responses to drought stress in the pot experiment of three chicory (*Cichorium intybus* L.) strains with different drought resistance, space-mutagenesis-bred strains PA-82 and PA-43 and their initial strain PA-57 were studied using Li-6400 portable photosynthesis system. The results showed that diurnal time courses of net Photosynthetic rate (Pn), Transpiration rate (Tr), stomatal Conductance (Cond) presented as double-peak curves. There was an obvious midday Pn depression, which was caused by nonstomatal factors. Highest Pn, Tr and Cond values were observed in PA-82 and lowest corresponding values in PA-43 ($p<0.05$). Intercellular CO_2 Concentration (Ci) of three strains showed an opposite course to that of Pn which reached its valley at 10:00-12:00. PA-82 had the highest Water Use Efficiency (WUE), followed by PA-57, then PA-43. Light Saturation Points (LSP) of three strains were between 1207-1264 μmol/m^2/sec and Light Compensation Points (LCP) were between 14.55-14.85 μmol/m^2/sec, both indicating good adaptation to changes of light intensity. Pn, Tr, Cond, maximal photochemical efficiency of PS II in dark adaptation (Fv/Fm), potential photochemical efficiency (Fv/F0) and coefficient of Photochemical quenching (qP) decreased with the aggravation of water deficit. Among the three strains, the parameters of PA-43 dropped to a greater extent at a highest speed, followed by PA-57 then PA-82. With the aggravation of water deficit, Ci and co-efficient of nonphotochemical quenching (qN) increased to a greater extent at a highest speed in PA-43. The results suggest that PA-82 and PA-57 have higher resistance against severe water deficit in comparison with PA-43.

Key words: *Cichorium intybus* L., drought stress, photosynthesis, chlorophyll fluorescence, resistance, China

INTRODUCTION

Photosynthesis is the most essential physiological process in the growth and development for plants which is closely related to their first productivity. The daily changes of photosynthesis can be measured by analyzing specific parameters like net Photosynthesis rate (Pn), Transpiration rate (Tr), stomatal Conductance (Cond), intercellular CO_2 Concentration (Ci) and stomatal limitation value (Ls). A plant's photosynthetic capacity varies according to its genetic traits. As a result, the difference in plant material would cause disparity in daily photosynthetic capacity. Therefore, a study on the changing characteristics of photosynthesis can provide theoretical and practical evidence to the analysis of plant productivity. Stomata are apparatus with complicated regulatory functions in plants which optimally regulate photosynthesis and transpiration simultaneously and affect Water Use Efficiency (WUE) greatly. They not only prevent excessive water loss caused by transpiration but also ensure acquisition of sufficient CO_2 for plant photosynthesis, so as they serve as a key link to regulate the material and energy exchanges between water loss and carbon acquisition in the soil-plant-atmosphere continuum. The study on the responses of stomatal conductance to the environmental factors will serve as a basis to understand the mechanisms of chicory's adaptation to different environmental conditions. WUE is a parameter that reflects photosynthesis and transpiration comprehensively and is defined as the ratio of Pn to Tr within a given time, namely, the amount of accumulative dry matter per unit of water loss by transpiration. The physiological and the ecological parameter used to describe the relationship between the plant production and the assimilated CO_2. It reflects the amount of assimilated CO_2 per unit weight of water used by a plant. In a similar environmental condition, a high WUE in the plant suggests the high efficiency of water utilization, good water conservation capacity and high productivity in drought. Therefore, WUE acts as an important parameter in crop farming, strain selection and evaluation of productivity in drought conditon. Light response

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curve, on the other hand, reflects the variation of photosynthetic rate of a plant with the changes of light intensity. Light Saturation Point (LSP) and Light Compensation Point (LCP) reflects on the requirement of a plant to lighting conditions indicating the utilization capacity of intensive light and weak light, respectively. Generally, a sciad has low LSP and LCP and a heliophyte is the opposite. A plant with low LCP and high LSP is highly adaptive to the light conditions whereas a plant with high LCP and low LSP is poorly adaptive to the light conditions.

Photosynthesis System II (PS II) is the first protein complex in the serial photochemical reactions which catalyzes light-driven oxidation of water and reduction of plastoquinone in the photosynthesis (Nelson and Ben-Shem, 2004). It is believed that PS II plays a significant role in plants' responses to environmental stress (Baker, 1991). In the recently years, determination of chlorophyll fluorescence has been used in a variety of areas in plant physiology and physiological ecology. Development of fluorescence measuring instruments, helps the researchers not only can study the effects of different environmental stresses on photosynthesis (Andrews *et al*., 1995; Tsonev *et al*., 1999) but also to investigate the absorbance, transportation, dissipation and distribution of light energy in photosynthesis systems using the methods of chlorophyll fluorescence dynamics. Chlorophyll fluorescence dynamics technology is therefore regarded as a quick and non-intrusive probe to study the photosynthesis pattern. Under the condition of water deficit, the effect of water stress on PS II can be evaluated by measuring various parameters like chlorophyll initial Fluorescence (F0), maximal Fluorescence (Fm), coefficient of Photochemical quenching (qP) and coefficient of Nonphotochemical quenching (qN) and by calculation of variable Fluorescence in dark adaptation (Fv), maximal photochemical efficiency of PS II in dark adaptation (Fv/Fm) and potential photochemical efficiency (Fv/F0) (Flexas *et al*., 1998; Inamullah, 2005; Zlatev, 2009).

Li-6400 portable photosynthesis system is equipped with two independent absolute open-path non-diffusible infra-red analyzers for the determination of absolute concentrations of CO_2 and H_2O, respectively. In the system, the environmental factors around the leaf such as CO_2 concentration, H_2O concentration, atmospheric temperature, relative humidity, light intensity and temperature in leaf chamber could be regulated automatically or manually. If equipped with 6400-40 fluorescence leaf chamber, the system can be used to measure simultaneously the gas exchange, respiratory and fluorescence parameters and it can automatically generate the light response curve and CO_2 response curve from every data collection process. This system has been widely used in field measurements of photosynthesis in various environmental conditions (Flexas *et al*., 2007; Yang *et al*., 2008; Wen *et al*., 2011).

Chicory (*Cichorium intybus* L.) is a perennial herbaceous plant which belongs to Compositae family. It is high-quality forage with high production, high nutrition value, good palatability and good post-mowing regeneration capacity (Lambert *et al*., 2004; Kidane *et al*., 2010). The production performance of chicory and its utility as forage has been intensively studied in China. The relationship between nutritious dynamics and production of Puna chicory during different growth and development periods has been investigated. The use of chicory as a forage for pigs, chickens and sheep has also been reported. However, there have been limited studies on its photosynthetic and transpirative properties and their responses to different environmental changes. Devacht and colleagues investigated the effect of cold stress on early vigour, photosynthesis, chlorophyll A fluorescence and pigment content of industrial chicory (*C. intybus*) and also the effect of cold stress and anthocyanin on industrial chicory using chlorophyll fluorescence imaging (Devacht *et al*., 2007, 2008, 2009). Monti *et al*. (2005) studied the relationship between Pn, Cond and water conditions (Monti *et al*., 2005). Currently researchers have succeeded in developing space mutagenesis breeding of chicory. But the photosynthetic properties and photosynthetic responses to drought stress of the initial strain (PA-57) and the mutant strains (PA-82 and PA-43) have yet need to be comprehensively studied, especially in terms of chlorophyll fluorescence changes. In the earlier studies, researchers have compared leaf xeromorphic structure and percentages of withered leaves of the three strains and found out that PA-82 was the most drought-resistant strain, followed by PA-57 and PA-43. In the recent years, due to climate change the crop production worldwide is heavily affected by the water shortage which has become a major limiting factor for the high-efficient and the sustainable development of farming in many regions. To ensure an appropriate cultivation and propagating techniques for chicory and to provide high-quality forage to stockbreeding in arid regions, it is necessary to systematically investigate photosynthetic properties of these chicory strains under the condition of drought stress. In the present study, the diurnal changes of photosynthesis parameters of three chicory strains with different drought resistance and their photosynthetic responses and the changes in chlorophyll fluorescence parameters in water deficit were studied with an attempt to elucidate the relationship between water status and photosynthetic physiology in chicory, to understand its anti-drought mechanisms and to provide photosynthetic evidences for its breeding, cultivation, propagation and utilization.

MATERIALS AND METHODS

The three strains, one is Puna chicory (PA-57) and its two mutant strains bred with space mutagenesis breeding (PA-82 and PA-43) were selected for the study. Plants were planted in plastic pots with a diameter of 40 cm and a height of 30 cm. Each pot was filled with 8 kg of topsoil from experimental field that was pulverized, blended but not sterilized. Each pot was sowed with 20 seeds that were full, uniform in size and free of pests and diseases. Each strain was grown in 12 pots. Upon the emergence of all seedlings, thinning was carried out to a final plant population of 10 seedlings per pot.

Treatments: For all the strains, 4 different treatments of water conditions were set according to a randomized complete-block design in the experiment with four replicates for each. The field moisture capacity of treatment A (no drought stress) was controlled at 75-80%, treatment B (mild drought stress) at 65-70%, treatment C (moderate drought stress) at 55-60% and treatment D (severe drought stress) at 40-45%. Before treatment, the soil in each pot was saturated with water and were allowed it to dry naturally until the field moisture capacity reached the indicated treatment levels and then maintained. Water gradients formed on 10th June and drought stress started. The amount of water in each pot was control by using the weighing method. Every day the water was supplemented through pierced holes in the soil at 17:00 in the evening pots were moved to plastic greenhouse during rainy weather conditions. The parameters were measured after the field moisture capacity of the treatments has reached the indicated levels for 15 days.

Measured parameters and methods

The measurements of daily photosynthetic progression: Net Photosynthesis rate (Pn, $\mu mol/m^2/sec$), Transpiration rate (Tr, mmol $H_2O/m^2/sec$), stomatal Conductance (Cond, mol $H_2O/m^2/sec$), intercellular CO_2 concentration (Ci, μmol CO_2/mol), Photosynthetically Active Radiation (PAR, $\mu mol/m^2/sec$), atmospheric Temperature (Ta,°C), Relative Humidity (RH, %) and atmospheric CO_2 concentration of each treatment were measured in sunny weather using Li-6400 Portable Photosynthesis System. The measuring duration was between 8:00 and 20:00 with an intermittence of 1 h. Data were collected from the third mature leaf counted from the top of the healthy plants of similar performance. Five plants were selected and their mean was calculated. The formulae used for calculation of WUE was as follows: WU E = Pn/Tr. Stomatal limitation value (Ls) was calculated by using the equation:

$$Ls = \frac{(Ca - Ci)}{Ca - J}$$

Where:

Ca = Atmospheric CO_2 concentration
Ci = Intercellular CO_2 concentration

Both the datas were exported from Li-6400 System. J was CO_2 compensation point which was negligible here.

Measurement of light response curve: Parameters of chicory such as Pn, Cond and Ci were measured during 9:00-11:00 at a CO_2 concentration of (400±10) $\mu mol\ mol^{-1}$ when the photon irradiation gradient in leaf chamber was set as 2000, 1800, 1600, 1400, 1200, 900, 700, 500, 200, 150, 100, 50 and 0 $\mu mol/m^2/sec$. The response progressions were fitted in the equations $y = a+bx$ and $y = ax^2+bx+c$, respectively to calculate LCP and LSP.

Photosynthetic responses of chicory in drought stress: Pn ($\mu mol/m^2/sec$), Tr (mmol $H_2O/m^2/sec$), Cond (mol $H_2O/m^2/sec$) and Ci (μmol CO_2/mol) of chicory were measured during 9:00-11:00 with Li-6400 Portable Photosynthesis System after the field moisture capacity of the treatments had reached the indicated levels for 15 days. During the measurement of photosynthetic responses, the light intensity was set at 1200 $\mu mol/m^2/sec$ and CO_2 concentration at 400 μmol CO_2/mol.

Measurements of chlorophyll fluorescence parameters: The chlorophyll fluorescence parameters of functioning leaves in the four treatments were measured by using Li-6400 Portable Photosynthesis System after 24 h dark adaptation of the leaves. The parameters measured were initial Fluorescence (Fo), maximal Fluorescence (Fm), coefficient of Photochemical quenching (qP) and coefficient of Nonphotochemical quenching (qN). Variable fluorescence in dark adaptation (Fv = Fm-Fo), maximal photochemical efficiency of PS II in dark adaptation (Fv/Fm) and potential photochemical efficiency (Fv/Fo) were also calculated.

Data analysis: All data and statistical analysis was conducted using Excel and SPSS Software. Graphs were processed in MS Excel.

RESULTS AND DISCUSSION

Daily changes of major meteorological factors: Photosynthetically Active Radiation (PAR) increased gradually after sunrise. It peaked at around 10:00 in a day and then decreased. The range of changing was 763.61-1418.96 μmol/m^2/sec. Atmospheric temperature started rising in the early morning. It reached a summit at 13:00 and started to decline with a range of changing from 26.10-34.46°C. There was a relatively high concentration of atmospheric CO$_2$ in the early morning hour and reached a valley at approximately 10:00 before starting to rise again. It peaked at 13:00 and rose slightly with a fluctuation range of 383.34-388.43 μmol mol^{-1}. The data were collected during hot and sunny weather season. Changes of meteorological factors were summarized in Table 1. Diurnal time courses of photosynthetic properties in chicory.

Diurnal time courses of Pn, Tr and Cond in leaves of different chicory strains: Figure 1a shows the diurnal time courses of Pn in chicory leaves that were presented as double-peak curves in all three strains. The first peak appeared at 10:00 and the valley at 12:00, followed by the second peak at 14:00. Of the three strains, PA-83 displayed the highest peak value of Pn (31.2 μmol CO$_2$/m^2/sec) whereas PA-43 had the lowest peak value of Pn (27.3 μmol CO$_2$/m^2/sec). Figure 1b shows the diurnal time courses of Tr in the chicory leaves which has double-peak curves with a similar pattern to that of Pn. The first peak appeared at 11:00 and the second peak at 16:00 both were 1-2 h behind the peaks of Pn curve, respectively. PA-83 showed the highest peak value of Tr (5.6 μmol/m^2) and PA-43 had the lowest peak value of Tr (4.5 μmol/m^2/sec). Figure 1c shows the diurnal time courses of Cond in chicory leaves which has double-peak curves with a similar pattern to that of Pn. The first peak appeared at 10:00 and the second at 14:00. PA-83 has shown the highest peak value of Tr (0.44 mol/m^2/sec) and PA-43 showed the lowest peak value of Tr (0.31 mol/m^2/sec).

The diurnal time courses of Ci and Ls in leaves of different chicory strains: Figure 1d shows the diurnal time courses of Ci in chicory leaves which reached their valleys at 10:00 and their peak at 12:00. The time courses were of a pattern opposite to that of Pn and Cond. Farquhar and Sharkey suggested that the trends of Ci and Ls were two reliable criteria when judging the decrease of Pn which could be caused by stomatal factors or nonstomatal factors like: decrease in Ci and increase in Ls indicates that reduced stomatal conductance was responsible for Pn depression whereas increase in Ci and decrease in Ls indicates that nonstomatal factors plays a major role (Farquhar and Sharkey, 1982). The trends of Ls and Ci were analyzed. It is shown in Fig. 2 that Ls values of Pa-57, PA-82 and PA-43 declined and Ci values rose at 12:00. It indicates that reduction in Pn was caused by nonstomatal factors. Moreover, Pn and Cond of chicory leaves dropped to the lowest point at 12:00 in three strains suggesting that nonstomatal factors were responsible for the midday depression of Pn.

Diurnal time courses of WUE in different chicory strains: As shown in Fig. 3, the WUE time courses of the three strains had a similar pattern to that of Pn time courses which were presented as double-peak curves. WUE of the studied strains started rising at 8:00 in the morning, peaked at 10:00 and then declined to the first valley at 12:00. WUE rose again until it reached the

Table 1: Changes of meteorological factors

Photosynthetically active radiation (μmol/m^2/sec)	Atmospheric CO$_2$ concentration (μmol/mol)	Relative humidity (%)	Atmospheric temperature (°C)
763.61-1418.96	383.34-388.43	42.93-59.27	26.10-34.46

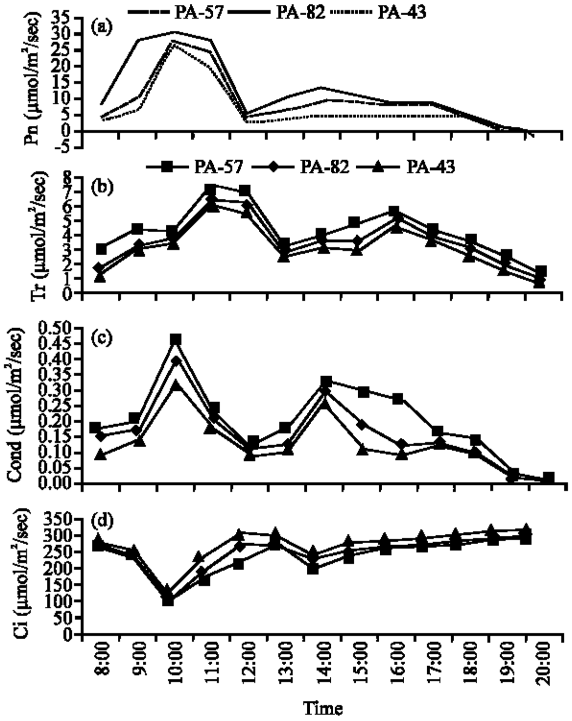

Fig. 1: a) Diurnal time courses of net photosynthetic rate in leaves of three chicory strains. b) Diurnal time courses of net transpiration rate in leaves of three chicory strains. c) Diurnal time courses of stromatal conductance in leaves of three chicory strains. d) Diurnal time courses of intercellular CO$_2$ concentration in leaves of three chicory strains

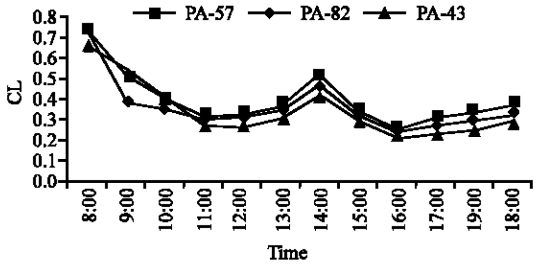

Fig. 2: Diurnal time courses of stromatal limitation value in leaves of three chicory strains

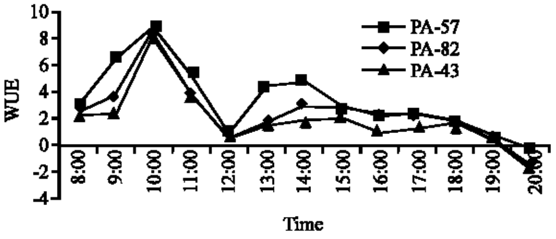

Fig. 3: Diurnal time courses of water use efficiency in leaves of three chicory strains

second peak at 14:00 before it fell. PA-82 showed a high WUE value ($p<0.05$), representing the highest water saving capacity, drought productivity and drought resistance among the three strains. PA-57 took the second place with moderate water saving capacity and drought productivity. The lowest WUE value was found in PA-43, implying poor water saving capacity, low productivity in drought and drought resistance.

Responses of different chicory strains to changes of light intense: Pn was tested at a series of PAR, using the red and blue light sources installed in Li-6400 System. The light response curves of the three chicory strains (Fig. 4a and b) shows that in a given range of PAR, Pn in leaves of different chicory strains increased with the ascending of PAR until light intensity reached a certain point (light saturation point, LSP) where Pn stopped increasing and was saturated; photosynthesis of chicory was inhibited by excessive high light intensity and Pn dropped to different extents in the tested strains, presenting as binary curves. LSP of three chicory strains can be generated from light response curves and Light Compensation Point (LCP) was calculated with the regression equations (Table 2 and 3). When the light intensity was <200 μmol/m^2/sec, Pn had a linear relationship with PAR and while calculating, it was fitted into a linear equation.

As shown in Table 4, the range of LSP in three chicory strains was 1207-1264 μmol/m^2/sec and the range of LCP was 14.55-14.85 μmol/m^2/sec. A low LCP and a high LSP indicates a high apparent quantum yield, thus a good adaptation to light intense. LCP is one key parameter to evaluate the plant's utilization capacity of weak light. The lower the LCP, the higher the utilization capacity of weak light is. When LCP exceeds LSP, Pn is no longer limited by photochemical reactions but by enzymatic process and CO_2 supply. Maximum quantum efficiency is the maximal light use efficiency before the appearance of photoinhibition and reflects a plant's maximum utilization capacity of light quantum. The Apparent Quantum Yield (AQY) of PA-57, PA-82 and PA-43 were 0.049, 0.055 and 0.043, respectively. These similar AQY values ($p>0.05$) indicate a high use efficiency of intensive light in the studied chicory strains.

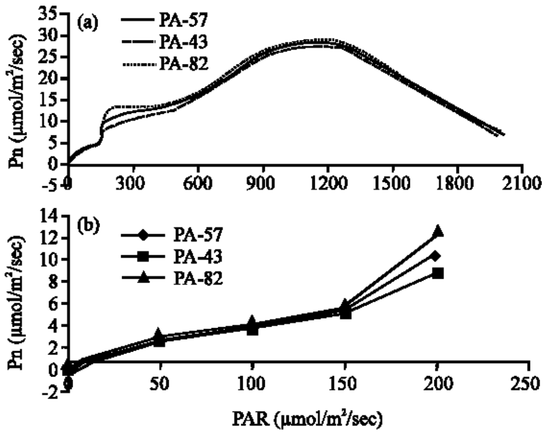

Fig. 4: a) Light response curves of three chicory strains. b) Light response curves of three chicory strains at a low light intensity of 200 μmol/m^2/sec

Table 2: Binary regression equations of Pn-PAR in three chicory strains

Strains	Binary regression equation	Value	Multiple correlation coefficient
PA-57	$y = -2E{-}05x^2+0.0497x-0.9475$	1242	R = 0.947
PA-82	$y = -2E{-}05x^2+0.0506x-0.5507$	1264	R = 0.936
PA-43	$y = -2E{-}05x^2+0.0483x-1.3061$	1207	R = 0.912

Table 3: Unary linear regression equations of Pn-PAR in three chicory strains

Strains	Unary regression equation	Multiple correlation coefficient
PA-57	$y = 0.0488x-0.72$	R = 0.929
PA-82	$y = 0.0552x-0.82$	R = 0.872
PA-43	$y = 0.0426x-0.62$	R = 0.964

Responses of Pn, Tr, Ci and Cond in leaves of different chicory strains to drought stress. Figure 5a shows the Pn values of different chicory strains at different water status. Under the condition without drought stress, PA-82 had the highest Pn value (28.7±0.12 μmol/m^2/sec), followed by PA-57 (Pn = 4.8±0.09 μmol/m^2/sec). PA-43 had the lowest Pn value (19.8±0.10 μmol/m^2/sec). Pn of the three chicory strains

Table 4: Photosynthetic characteristic parameters of chicory

Strains	Maximum net photosynthetic rate (P-max) (μmol/m^2/sec)	Apparent Quantum Yield (AQY) (CO$_2$/photon)	Light Saturation Point (LSP) (μmol/m^2/sec)	Light compensation point (LCP) (μmol/m^2/sec)
PA-57	29.92	0.049	1229	14.75
PA-82	31.45	0.055	1247	14.85
PA-43	27.85	0.043	1202	14.55

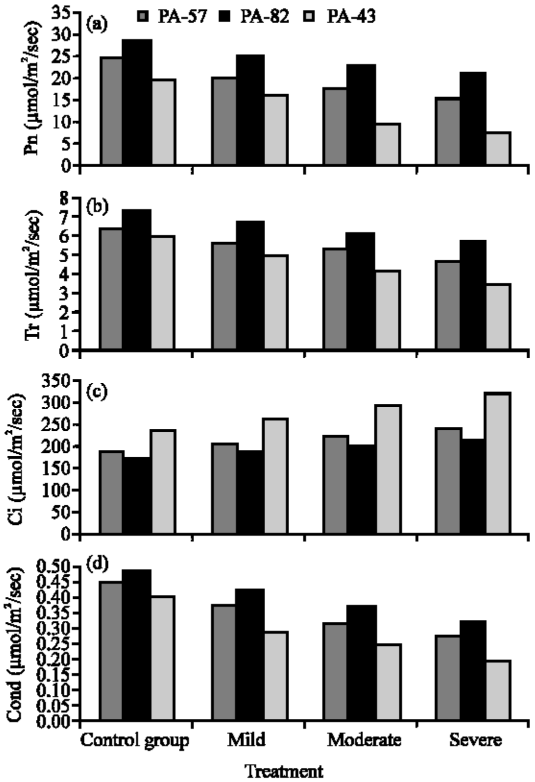

Fig. 5: a) Net photosynthesis rate of three chicory strains under different drought stress. b) Transpiration rate of three chicory strains under different drought stress. c) Intercellular CO$_2$ concentration in leaves of three chicory strains under different drought stress. d) Stromatal conductance in leaves of three chicory strains under different drought stress

dropped dramatically when the drought stress became severe. Pn of PA-43 dropped at a faster rate than those of the other two strains. Especially in conditions of moderate and severe drought stress, Pn of PA-43 decreased by 51 and 62%, respectively to the values <10 μmol/m^2/sec. Other two strains underwent less reduction in Pn. In PA-57, Pn is decreased by 18, 27 and 37% under mild, moderate and severe drought stress, respectively when compared with control (treatment A). While Pn of PA-82 dropped by 11, 19 and 25% under mild, moderate and severe drought stress respectively when compared with control. The changes in Tr of three chicory stains at different water status was illustrated in Fig. 5b. Under the condition without drought stress, PA-82 had the highest Tr (7.3±0.08 μmol/m^2/sec), followed by PA-57 (Tr = 6.3±0.05 μmol/m^2/sec) then PA-43 (Tr = 5.9±0.07 μmol/m^2/sec). When the severity of drought stress was increased, Tr level in the three strains fell substantially. Tr of PA-43 dropped at the highest rate by 16.9, 31 and 41% under mild, moderate and severe drought stress, respectively in comparison with control whereas other two strains underwent less reduction in Tr. Tr of PA-57 declined by 11, 16 and 25% under mild, moderate and severe drought stress respectively in comparison with control. While in PA-8, Tr dropped by 8, 15 and 21% under mild, moderate and severe drought stress, respectively in comparison with control. Results of variance analysis showed that in each strain, there was significant differences among Tr values of chicory at different water status ($p<0.05$) indicating a marked effect of drought stress in soil on chicory plants. Figure 5c shows changes in Ci values of different chicory strains at different water status. When there was no drought stress, PA-43 had the highest Ci (237±1.02 μmol/mol) followed by PA-57 (Ci = 189±1.05 μmol/mol) then PA-82 (Ci = 175±1.09 μmol/mol). With the aggravation of drought stress, Ci in three strains showed a trend of ascending. Among the three strains, Ci of PA-43 rose at a fastest speed and increased by 13, 25 and 35% under mild, moderate and severe drought stress respectively when compared with control. Other two strains underwent less increasing rate in Ci. Ci of PA-57 rose by 11, 22 and 27% under mild, moderate and severe drought stress respectively in comparison with control. And Ci of PA-82 was increased by 9, 16 and 24% under mild, moderate and severe drought stress respectively when compared with control. Results of variance analysis showed that in each strain, there were significant differences among Ci values of chicory at different water status ($p<0.05$). The changes in Cond of three chicory stains at different water status are shown in Fig. 5d. In the absence of drought stress, PA-82 had the highest Cond among three strains (4.4±0.03 mol/m^2/sec), followed by PA-57 (Cond = 4.0±0.05 mol/m^2/sec) and then PA-43 (Cond = 3.6±0.06 mol/m^2/sec). When the severity of drought stress increased, Cond in three chicory strains dropped. Among them, Cond in PA-43 dropped at the highest speed and decreased by 27, 38 and 53% under mild, moderate and severe drought stress, respectively when compared to control. Other two strains underwent relatively less reduction in Cond. Cond of PA-57 descended by 15, 27 and 37.5% and Cond of PA-82 by 14, 25 and 34% under mild, moderate and severe drought stress respectively in comparison with control. Results of variance analysis showed that in each strain, there were significant differences among Cond values of chicory at different water status ($p<0.05$). Resonpses of chlorophyll fluorescence in leaves of different chicory strains to drought stress.

Changes of Fv/Fm and Fv/Fo leaves of different chicory strains: Fv/Fm is generally used to evaluate the primary conversion efficiency of light energy of PS II and indicates the utilization capacity of PS II and the extent of photoinhibition. Fv/Fo reflects, on the other hand, the potential activity of PS II. Both are the important parameters that reflect the photochemical conditions. Regardless of species and growth conditions, Fv/Fm seldom changes when there is no environmental stress. But the parameter changes dramatically in leaves undergo photoinhibition and is therefore a good parameter and probe. In the study, Fv/Fm and Fv/Fo of three strains decreased substantially to different extents under drought stress (Fig. 6a and b). Fv/Fm and Fv/Fo of PA-82 had a smallest decline and those of PA-43 underwent the largest reduction. In other words, the strain that has low resistance against drought stress had a larger extent of decrease in Fv/Fm and Fv/Fo and its chlorophyll fluorescence is affected by drought stress to a larger extent. In comparison with control, the rates by which Fv/Fm in three strains decreased under mild, moderate and severe drought stress are as follows: PA-57 (8.2, 11.3 and 16.7%); PA-82 (5.6, 9.8 and 10.5%); PA-43 (10.6, 17.2 and 26.3%). In comparison with control, the rates by which Fv/Fo in three strains decreased under mild, moderate and severe drought stress are as follows: PA-57 (8.7, 15.2 and 28.3%); PA-82 (6.7, 11.1 and 17.6%); PA-43 (17.2, 30.3 and 36.8%). The above data shows that in low drought-resistant chicory strains, drought stress can reduce the primary conversion efficiency of light energy of PS II and the damage potential activity of PS II to a greater extent as compared to high drought-resistant chicory strains.

Effect of drought stress on qP and qN in leaves of chicory strains: Coefficient of Photochemical quenching (qP) reflects the redox conditions of primary electronic receptor QA in PS II and the number of open centers in PS II. A high qP value represents a good electronic transporting activity (Kramer *et al.*, 2004). Reduction in qP under drought stress indicates the inhibition of the electronic transferring from the oxidization side to reaction centers in PSII. qN reflects the portion of the energy absorbed by antenna pigments that is not used for photochemical electronic transferring but dissipated as heat. The escalated qN helps dissipate excessive exciting energy and emolliates the environmental effects on photosynthesis. Nonphotochemical quenching is therefore a self-protection mechanism which protects photosynthetic apparatus.

As shown in Fig. 7a, qP values of three chicory strains under drought stress exhibited a trend of

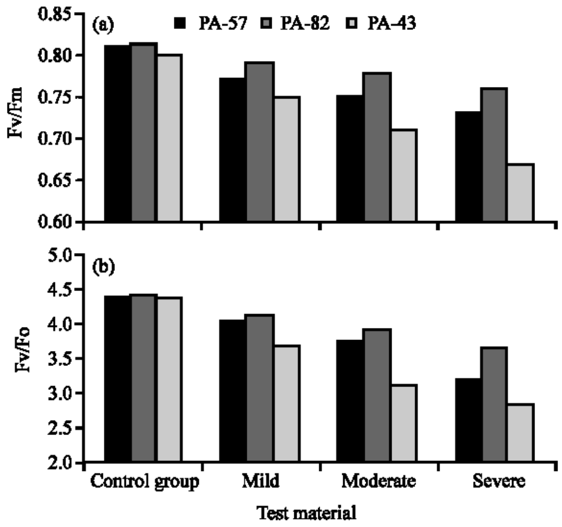

Fig. 6: a) Effect of water stress on Fv/Fm. b) Effect of water stress on Fv/Fo

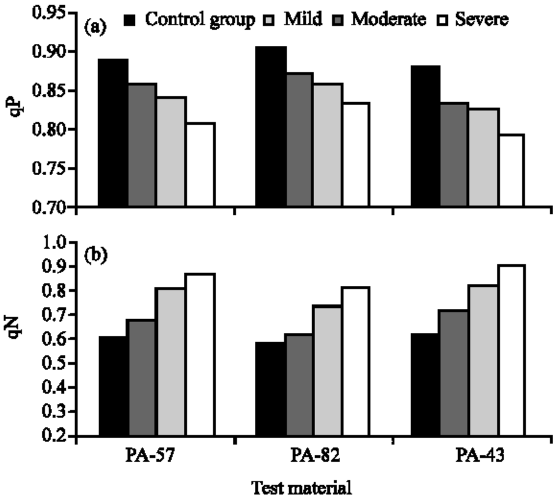

Fig. 7: a) Effect of water stress on coefficient of photochemical quenching. b) Effect of water stress on coefficient of nonphotochemical quenching

reduction. Under mild drought stress, qP values of PA-82 and PA-57 underwent a small reduction and decreased by 5.8 and 6.7%, respectively as compared to control. The qP value of PA-43 dropped by 11.3% compared to control. The qP values of three chicory strains declined substantially under moderate drought stress with the highest reduction in PA-43 (by 25.4%) and the lowest reduction in PA-82 (8.9%) as compared with control. Under severe drought stress, qP values of PA-82, PA-57 and PA-43 fell dramatically by 11.5, 14.6 and 21.2% in comparison with control. Results of variance analysis showed that in PA-57 and PA-43, there was significant differences among qP values of different treatments ($p<0.05$). For PA-82, the differences of qP values between treatments of moderate and severe drought stress were not significant ($p>0.05$) but there was a significant difference among qP values of other treatments ($p<0.05$). These results show that drought stress influenced the electronic transferring activity in PS II of chicory leaves to a larger extents. Among the tested strains, PA-82 underwent the smallest reduction in qP indicating a lesser influence by drought stress whereas PA-57 was affected by drought stress to a greater extent and PA-43 to the greatest extent.

The qN reflects the portion of the energy absorbed by antenna pigments that is not used for photochemical electronic transferring but dissipated as heat (Henriques, 2009). The qN increases when a plant is under environmental stress. The results showed that qN values in all three chicory strains increased when compared to control. PA-57 and PA-82 increased largely in qN than PA-43 did (Fig. 7b). These data indicate that there is high degree of openness of reaction centers in PS II of the high drought-resistant chicory strain which ensures high electronic transferring and heat dissipating capacities, thus protects photosynthetic apparatus from excessive light energy.

The decrease in Pn can be ascribed to stomatal and nonstomatal factors. Farquhar and Sharkey earlier proposed that the trends of Ci and Ls were the two reliable criteria when judging whether the decrease of Pn was caused by stomatal factors or nonstomatal factors: decrease in Ci and increase in Ls indicates that reduced stomatal conductance was responsible for Pn depression whereas increase in Ci and decrease in Ls indicates that nonstaomatal factors plays a major role. Nonstaomatal factors also include increase in intercellular space between mesophyll cells and CO_2 diffusion resistance, reduction in PS II and photophosphorylation activities, decrease in RuBP carboxylase and FBPaes activities and the blockage of RuBP regeneration (Farquhar and Sharkey, 1982). Researchers observed in the study an increased Ci and decreased Ls when the Pn dropped at 12:00 of the day suggesting that nonstomatal factors could be responsible for the suppressed photosynthesis. In other words, the midday Pn suppression in chicory was caused by nonstomatal factors. Furthermore, Tr time course reached its peak at 12:00 in the studied strains. Chicory plants were probably influenced by high atmospheric temperature at noon which leads to strong transpiration and declined water potential. The photophosphorylation was subsequently suppressed, resulting in the reduction in Pn.

Under the same growing conditions, a high WUE means good water use efficiency, water saving capacity and productivity in drought. WUE is therefore an important parameter in crop farming, strain selection and evaluation of plant productivity under drought stress (Kumar *et al*., 2001). PA-82 had the highest WUE among all the three strains, suggesting that it has a high water saving capacity and productivity under drought stress. A-57 had a moderate WUE value which indicates that it has modest water saving capacity and productivity in drought stress. A lowest WUE value was found in PA-43 which indicates its low water saving capacity and productivity under drought stress.

The ratio of the variable chlorophyll fluorescence to maximal fluorescence (Fv/Fm) is an important parameter in studying the plant under stress which reflects the excitation energy catching efficiency of open PS II and can be reduced by any environmental stress that affects the PS II efficiency. The value of Fv/Fm ratio was 0.80-0.83 in higher plants when there is no environmental stress (Bjorkman and Demmig, 1987). In the present research, the range of the ratio Fv/Fm was 0.808-0.812 in chicory plants that were not subjected to drought stress. With the progression of drought stress, the ratio Fv/Fm in three chicory strains decreased indicating that drought stress have caused the damage to PS II and its potential activity center and suppressed the light energy primary conversion efficiency of PS II, there by inhibiting photosynthetic primary reaction and which in turn affected the transferring of photosynthetic electrons from PS II reactive center to QA, QB and PQ pools. The order of reduction in the ratio Fv/Fm was PA-43>PA-57 >PA-82, suggesting that drought stress influenced the photosynthetic efficiency of PS II in PA-82 to a smallest extent and influenced that of PA-43 to a largest extent.

Serious photoinhibition can result in damaging reactions such as degradation of reaction centers. In many cases, non-radiant energy dissipation is a protecting process to prevent reaction centers from the damage. All higher plants are protected by sophisticated nonphotochemical quenching mechanisms which can consume light energy, catch protein complexes (LHCII and LHC I) and absorb excessive light energy through non-radiant energy dissipation (Badger *et al*., 2000). The extent of pf heat dissipation can be evaluated with qN (Tezara *et al*., 2005; Henriques, 2009). The results showed that there was substantial decrease in light energy primary conversion efficiency of PS II in the studied chicory plants indicating the presence of significant photoinhibition. However, the increased qN as a self-protection mechanism provided protection to the photosynthetic apparatus. Of the three strains, the largest increase of qN was found in PA-43 as compared to control and the largest increase of qN was found in PA-82. These results suggest that the electronic transferring and heat dissipation capacities of PS II in the highly drought-resistant strain (PA-82) were higher on comparison with the low drought-resistant strain (PA-43). The damage caused to photosynthetic apparatus was therefore reduced and the drought resistance enhanced.

The photosynthetic properties and their responses to drought stress varied in chicory strains of different drought resistance capacity. PA-82, the strain bred using space mutagenesis breeding technology had the highest Pn, Tr, Cond and WUE values. Smallest extent of decrease in Fv/Fm, Fv/F0, qP and the highest extent of increase in qN were observed in PA-82. From the findings researchers could say, PA-82 is more drought-resistant than its initial strain PA-57 and space-mutagenesis-bred strain PA-43. This is consistent with the conclusion about the drought resistance of the strains drawn from the earlier study on their leaf xeromorphic structure. In conclusion, the high drought resistance of the space-mutagenesis-bred strain PA-82 is associated with photosynthetic properties, chlorophyll fluorescence characteristics of photosynthetic centers and the xeromorphic structure of its leaves.

CONCLUSION

The diurnal time courses of Pn, Cond and Tr in three chicory strains were presented as double-peak curves. The first and second peaks of Tr occurred at 1-2 h behind those of Pn. PA-82 had the highest Pn, Tr and Cond values and the corresponding values in PA-43 were lowest among the three strains. PA-43 had the highest Ci value and the lowest Ci value was found in PA-82. Ci value was higher in the morning and at evening in all studied strains with a daily changing pattern that is opposite to that of Pn. PA-82 had the highest WUE value, followed by PA-57 and PA-43.

Three chicory strains had low LSP and high LCP values and the differences among the strains were not significant indicating a higher adaptation capacity to the light intensity.

Pn, Tr and Cond in three strains decreased to different extents under drought stress. In terms of Pn, Tr and Cond in the three strains, PA-82 underwent smallest and slowest decreases whereas PA-43 underwent the largest and fastest decreases. It was found that Pn in three strains was suppressed at 12:00. By analyzing the changes in Ls and Ci, researchers conclude that the midday suppression of Pn was caused by nonstomatal factors.

Fv/Fm and Fv/Fo ratios dropped substantially in three chicory strains under drought stress. PA-82 underwent the smallest reduction and PA-43 had a largest reduction in both Fv/Fm and Fv/Fo. Reduced qP and increased qN were found in three chicory strains under drought stress.

ACKNOWLEDGEMENTS

This research is supported by the innovation ability construction project grant (No. (2009) 4013) and the agriculture research project grant (NY[2009]3083), GNSF (20112065). Researchers appreciate the valuable comments from other members of the laboratories.

REFERENCES

Andrews, J.R., M.J. Fryer and N.R. Baker, 1995. Characterization of chilling effects on photosynthetic performance of maize crops during early season growth using chlorophyll fluorescence. J. Exp. Bot., 46: 1195-1203.

Badger, M.R., S. von Caemmerer, S. Ruuska and H. Nakano, 2000. Electron flow to oxygen in higher plants and algae: rates and control of direct photoreduction (Mehler reaction) and rubisco oxygenase. Philos Trans. R. Soc. Lond. B., 355: 1433-1446.

Baker, N.R., 1991. A possible role for photosystem II in environmental perturbations of photosynthesis. Physiol. Plantarum, 81: 563-570.

Bjorkman, O. and B. Demmig, 1987. Photon yield of O_2 evolution and chlorophyll fluorescence at 77k among vascular plants of diverse origins. Planta, 170: 489-504.

Devacht, S., P. Lootens, I. Roldan-Ruiz, L. Carlier and J. Baert *et al*., 2008. The use of chlorophyll fluorescence imaging to evaluate the effect of cold stress for industrial chicory. Commun. Agric. Applied Biol. Sci., 73: 137-140.

Devacht, S., P. Lootens, J. Baert, J. Van Waes, E. Van Bockstaele and I. Roldan-Ruiz, 2009. Influence of anthocyanin on the photosynthetic performance of industrial chicory under cold stress conditions. Commun Agric. Applied Biol. Sci., 74: 121-126.

Devacht, S., P. Lootens, L. Carlier, J. Baert, J. Van Waes and E. Van Bockstaele, 2007. Effect of cold stress on early vigour, photosynthesis, chlorophyll A fluorescence and pigment content of industrial chicory. Commun. Agric. Applied Biol. Sci., 72: 165-169.

Farquhar, G.D. and T.D. Sharkey, 1982. Stomatal conductance and photosynthesis. Annu. Rev. Plant Physiol., 33: 317-345.

Flexas, J., A. Diaz-Espejo, J.A. Berry, J. Cifre, J. Galmes, R. Kaldenhoff, H. Medrano and M. Ribas-Carbo, 2007. Analysis of leakage in IRGA's leaf chambers of open gas exchange systems: quantification and its effects in photosynthesis parameterization. J. Exp. Bot., 58: 1533-1543.

Flexas, J., J. Escalona and H. Medrano, 1998. Down-regulation of photosynthesis by drought under field conditions in grapevine leaves. Func. Plant Biol., 25: 893-900.

Henriques, F.S., 2009. Leaf chlorophyll fluorescence: Background and fundamentals for plant biologists. Bot. Review, 75: 249-270.

Inamullah, I.A., 2005. Adaptive responses of soybean and cotton to water stress. II. Changes in CO2 assimilation rate, chlorophyll fluorescence and photochemical reflectance index in relation to leaf temperature. Plant Prod. Sci., 8: 131-138.

Kidane, A., J.G. Houdijk, S. Athanasiadou, B.J. Tolkamp and I. Kyriazakis, 2010. Effects of maternal protein nutrition and subsequent grazing on chicory (*Cichorium intybus*) on parasitism and performance of lambs. J. Anim. Sci., 88: 1513-1521.

Kramer, D.M., G. Johnson, O. Kiirats and G.E. Edwards, 2004. New fluorescence parameters for the determination of q(a) redox state and excitation energy fluxes. Photosynth Res., 79: 209-218.

Kumar, B., D. Pandey, C. Goswami and S. Jain, 2001. Effect of growth regulators on photosynthesis, transpiration and related parameters in water stressed cotton. Biol. Plantarum, 44: 475-478.

Lambert, M.G., D.A. Clark and A.J. Litherland, 2004. Advances in pasture management for animal productivity and health. New Zeland. Vet. J., 52: 311-319.

Monti, A., M.T. Amaducci, G. Pritoni and G. Venturi, 2005. Growth, fructan yield, and quality of chicory (*Cichorium intybus* L.) as related to photosynthetic capacity, harvest time, and water regime. J. Exp. Bot., 56: 1389-1395.

Nelson, N. and A. Ben-Shem, 2004. The complex architecture of oxygenic photosynthesis. Nat. Rev. Mol. Cell Biol., 5: 971-982.

Tezara, W., O. Marin, E. Rengifo, D. Martinez and A. Herrera, 2005. Photosynthesis and photoinhibition in two xerophytic shrubs during drought. Photosynthetica, 43: 37-45.

Tsonev, T., V. Velikova, M. Lambreva, D. Stefanov, 1999. Recovery of the photosynthetic apparatus in bean plants after high-and low-temperature induced photoinhibition. Bulgarian J. Plant Physiol., 25: 45-53.

Wen, G., L. Zhang, R. Zhang, Z. Cao, G. Zhou, H. Huang and M. Wong, 2011. Temporal and spatial dynamics of carbon fixation by moso bamboo (*Phyllostachys pubescens*) in subtropical China. Bot Rev., 77: 271-277.

Yang, H.B., S.Q. An, O.J. Sun, Z.M. Shi, X.S. She, Q.Y. Sun and S.R. Liu, 2008. Seasonal variation and correlation with environmental factors of photosynthesis and water use efficiency of Juglans regia and Ziziphus jujuba. J. Integr. Plant Biol., 50: 210-220.

Zlatev, Z., 2009. Drought-induced changes in chlorophyll fluorescence of young wheat plants. Biotechnology, 23: 437-441.