

THE EFFECT OF ECTOPIC EXPRESSION OF PINE GLUTAMINE
SYNTHETASE (GS1A) ON WATER USE EFFICIENCY IN TRANSGENIC
POPLAR

by

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A Dissertation submitted to the

Graduate School-Newark

Rutgers, The State University of New Jersey

in partial fulfillment of the requirements

for the degree of

Master of Science

Graduate Program in Biology

written under the direction of

Dr. Edward G. Kirby and Dr. Karina V.R. Schäfer

and approved by

Newark, New Jersey

October 2014

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ABSTRACT OF THE THESIS

The effect of ectopic expression of pine glutamine synthetase (*GS1a*) on water use efficiency in transgenic poplar

By ARMAN YALCIN

Glutamine synthetase (GS) is a key factor in the assimilation of nitrogen in plants. Hybrid poplar (*Populus tremula X P. alba*, INRA 717-1-B4) ectopically expressing pine GS display increased growth rates, increased nitrogen use efficiency, and resistance to drought. In order to assess mechanisms associated with observed drought tolerance of GS poplars, we studied the ability of the GS poplars to perform photosynthesis under control and water-limiting conditions, as well as their nitrogen and carbon isotope content. Included in this study were carboxylation efficiency, maximum assimilation rate, quantum yield of photosynthesis, maximum electron transport rate, dark respiration, triose phosphate utilization, light compensation point, intrinsic water use efficiency, stomatal conductance, and nitrogen-use efficiency. Rooted cuttings (12-18 months) were grown in a growth chamber (24°C; 16 hour photoperiod; approx. $330 \mu\text{mol m}^{-2} \text{s}^{-1}$). Well-watered conditions were defined as a soil moisture value of over $0.40 \text{ m}^3 \cdot \text{m}^{-3}$, and drought conditions were defined as having a value of less than $0.20 \text{ m}^3 \cdot \text{m}^{-3}$. The results showed significant differences between the GS

transgenic and wild type. Under drought and control conditions, GS poplars showed carboxylation efficiencies similar to wild type grown under well-watered and drought conditions. Photosynthetic capacities were not different regardless of condition or genotype. However, the GS poplars showed less water use efficiency when compared to wild types, but higher nitrogen use efficiency. These data suggests that, compared to the wild type, GS poplars display a tradeoff between water and nitrogen use efficiency, and are characterized by slightly enhanced photosynthesis, even under drought-stress.

Acknowledgements

I would like to thank everyone who helped with this project:

- Dr. Kirby and Dr. Schäfer for editing my work, funding, help with statistical analyses, and patience throughout the study.
- Nick Carlo, for help with the statistical software and the LI-COR.
- Walter Preiss, for keeping the growth chamber pest-free.
- Rajan Tripathee, for help with the LI-COR.
- The Rutgers University Research Council and ArborGen Inc. for funding.

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Introduction

Often times, a limiting factor in the growth of plants is the availability of nitrogen in the soil. For this reason, trees thrive where inorganic nitrogen, such as ammonium and nitrate ions, are present in the soil in significant amounts, as they are the main sources of usable nitrogen (Wolt and Wolt, 1994). Woody and herbaceous plants acquire inorganic nitrogen in the same way from the soil. This uptake of nitrogen is governed by the active uptake of nitrogen through plant roots and passive loss of nitrogen back to the soil. The rate at which nitrogen is actively taken up into the root cells is determined by the inorganic nitrogen transport systems, which are encoded by several genes that have been identified in model organisms such as *Arabidopsis* (Glass et al., 2002). The rate of nitrate uptake varies between species due to varying regulation. This regulation is affected by the concentration of nitrate in the soil, the status of the plant with regards to carbon and nitrogen, the accumulation of amino acids in the roots, and the cytokinin levels that up-regulate nitrate uptake at a transcriptional level (Gessler et al., 2004).

Nitrite and nitrate reductase are responsible for the conversion of nitrate ions to ammonium ions; which mainly takes place in photosynthetically active tissues. The ammonium ions are then incorporated into the organic pool of molecules, which reactions are initially catalyzed by glutamine synthetase (GS).

This is significant because GS is responsible for the incorporation of ammonium ions into glutamine, which is an amino acid precursor to glutamate and all other nitrogen-containing compounds.

GS is the central enzyme responsible for nitrogen metabolism in plants (Figure 1), and plays a fundamental role in plant growth and development. GS produces glutamine by catalyzing the ATP-dependent addition of ammonium to the γ -carboxyl group of glutamate (Kirby et al., 2011). There are various sources of ammonium that GS can assimilate into glutamine, including uptake from the soil, reduction of nitrate or nitrite, photorespiration, recycling of ammonium released from senescing tissues, and de-amination of phenylalanine by phenylalanine ammonia-lyase (Kirby et al., 2011).

In angiosperms there are two forms of GS, cytosolic GS (GS1) and chloroplastic GS (GS2). GS2 is responsible for producing glutamine in young leaves, while GS1 mainly produces glutamine for intercellular transport. Thus, the two isoforms of GS have different roles in the plant, and are differentially expressed in the leaves, stems, vascular tissues, and roots. Molecular analysis has shown that GS1 is encoded by a small family of genes, while GS2 is encoded by a single gene (Castro-Rodríguez et al., 2011). The role of GS has been well studied and it has been introduced into many model organisms and has conferred increased growth rate and drought resistance to them (Gallardo et al., 1999; Kirby et al., 2006). The *Populus* species has been used as a model system for the genetic engineering of forest trees because of its ease of

propagation, small genome size, ease of transformation, and availability of the whole genome sequence in the third generation (<http://www.phytozome.net/poplar.php>). Thus, the study of GS inserted in *Populus* is an effective avenue by which to study its effect. Since GS plays an important role in the nitrogen content of plants, and photosynthetic capacity is known to be proportional to leaf nitrogen content (Seeman et al., 1987), it is possible that plants expressing GS have altered photosynthetic capacity. The amount of carbon assimilated per unit of nitrogen present in the plant is defined as nitrogen use efficiency (NUE). Plant photosynthetic capacity is also correlated to the amount of water available to the plant. The amount of carbon assimilation per unit of water lost is described as water use efficiency (WUE) (Eamus 1991). This value can be determined instantaneously by the plants photosynthetic parameters such as assimilation and transpiration, or over a span of time via carbon isotopic ratios present in plant tissues (Farquhar et al., 1989). Due to the nature of gas exchange at the stomata, increased stomatal conductance may increase the NUE, but also decrease the WUE by causing excess water to be lost to the atmosphere (Field et al., 1983). A plant's photosynthetic capacity depends on the balance of carbon assimilation and water loss (Field et al., 1983), as well as the availability of water (Katul et al., 2010). Despite the fact that GS is a limiting factor in growth and development in poplar (Castro-Rodriguez et al., 2011), there may exist a tradeoff between WUE and NUE (Field et al., 1983).

The mechanism by which these two efficiencies are related is the availability of water. Plants that are grown under water stress are expected to have higher carbon isotopic ratios than those grown in optimal water conditions **(Farquhar and Sharkey 1982)**. There have been several studies showing a negative correlation between rainfall and carbon isotopic ratios across functional groups **(Stewart et al., 1995; Hartman and Danin, 2010; Swap et al. 2004)**. However, the relationship between water and nitrogen isotopic ratio is less straightforward because plants do not fix it directly from the atmosphere – it is taken up from the soil or through a symbiotic relationship with nitrogen fixing microorganisms and can vary due to plant physiology **(Hartman and Danin, 2010)**. There are also studies showing a negative correlation between water availability (or rainfall) and nitrogen isotopic ratios among plant functional groups **(Swap et al., 2004; Heaton 1987; Handley et al., 1999; Amundson et al., 2003)**. Therefore, transgenic poplars ectopically expressing the GS1 gene, that have increased nitrogen metabolism, may have altered nitrogen isotopic ratios, and thus altered NUEs. Previous studies have shown that plants grown in areas with little rainfall increase the nitrogen content in their leaves in order to increase their light use efficiency, compared to those plants that grow in regions with greater rainfall (Field et al., 1983). Given this, the water availability to a C₃ plant during its growth can be measured using the C/N ratio present in the leaves.

This mechanism by which increasing the NUE or WUE of a plant is of significant interest, as it may improve the performance and growth rate of crop

and forest species under drought conditions or select for those plants that are better adapted to this adverse condition. When soil moisture levels are decreased, a common response is a decrease in photosynthesis, transpiration, and leaf conductance (Farquhar et al., 1989).

In this study, the photosynthetic ability and water use efficiencies of transgenic poplars ectopically expressing pine GS was compared to non-transgenic poplars, under control and water-limiting conditions. Specifically, data on the maximum carboxylation rate (V_{cmax}), maximum assimilation rate (A_{max}), CO_2 compensation point, quantum yield of photosynthesis, light compensation point, dark respiration rate, maximum electron transport rate (J_{max}), and triose phosphate utilization (TPU) were collected over two trials in order to have an adequate number of repetitions and to confirm reproducible results. These photosynthetic parameters were estimated from light response (net CO_2 assimilation rate, A , versus light intensity, PPFD) and A/C_i curves (net CO_2 assimilation rate, A , versus calculated substomatal CO_2 concentration, C_i) for poplars under each condition, and the intrinsic WUEs were calculated via stable isotope analysis. We hypothesized that, compared to non-transgenic poplars, the transgenic line of poplars will demonstrate higher photosynthetic abilities under water-limiting conditions, as well as higher intrinsic WUE due to their enhanced ability to resist drought conditions via ectopic expression of GS1.

Materials and Methods

Plant material

A hybrid poplar clone was studied (INRA 7171-B4, *Populus tremula* L. × *P. alba* L.) and a fast growth transgenic line derived from this clone that overexpress the pine cytosolic glutamine synthetase (*GS1a*) gene (Gallardo et al. 1999). Rooted cuttings were planted in 6-inch pots containing a peat-based commercial growth medium (Metro-Mix 200, Scotts, Marysville, OH) with supplemented iron and Scotts Peters water-soluble fertilizer, and raised in a growth chamber supplying a 16-h photoperiod (24–25 °C) (Figure 2). Five replicate samples, as well as three duplicate samples of each plant type were sampled for each of two trials, to determine photosynthetic capacities during the control and water-limiting treatments.

Water status

Soil water potential was measured with a Theta Meter HH1 and Theta Probe ML2 (Delta-T devices, Cambridge, England). Control water conditions were defined as a soil moisture value of greater than $0.40 \text{ m}^3 \cdot \text{m}^{-3}$, and drought conditions were defined as a soil moisture level less than $0.20 \text{ m}^3 \cdot \text{m}^{-3}$ (González 2003). We used soil water as a proxy measure of plant water status (Acevedo, et al., 1971; El-Khatib et al., 2004), because the study plants were small and continued harvesting of leaves to measure leaf water potential would likely have significantly affected plant source–sink relationships and, hence, photosynthetic activity (Thomas and Strain 1991).

Water-limiting treatment

Water stress was applied to the plants by withholding irrigation until the soil moisture level was less than $0.20 \text{ m}^3 \cdot \text{m}^{-3}$. Plants that were under the control water level treatment were irrigated regularly so that the soil moisture level was maintained above $0.40 \text{ m}^3 \cdot \text{m}^{-3}$.

Gas exchange measurements

Photosynthetic gas exchange measurements were made with an open flow infrared gas analyzer system LI-6400XT portable photosynthesis system (Li-Cor Inc., Lincoln, NE). Leaf tissue (6 cm^2) was enclosed in the cuvette and exposed to $2000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ photosynthetic photon flux density (PPFD) from a red/blue LED light source attached to the cuvette. Leaf temperature and cuvette CO_2 concentration were maintained at $20 \text{ }^\circ\text{C}$ and 400 ppm, respectively. Leaves were allowed to equilibrate to cuvette conditions for a minimum of 3 minutes before the measurements were made. Following these measurements, standard light response and A/C_i curves were measured using the leaves present on three different nodes on each plant. These curves were plotted in Sigmaplot (SPSS Inc., version 11, Chicago, IL USA). For the A/C_i curves, light levels were maintained at $2000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and CO_2 concentrations began at 400 ppm, were reduced gradually to 50 ppm, and then increased gradually until 1600 ppm when maximum photosynthetic rates were achieved. From the A/C_i data, based on theory and equations from Farquhar et al. (1980), non-linear regressions were used to estimate data for the Rubisco-limited portion of the curve (V_{cmax}), as well as for the electron transport limited portion (J_{max}), and triose phosphate utilization (TPU). These values were corrected for temperature based on theory from

Bernacchi et al. (2003). Light response curves were produced by maintaining the CO₂ concentration in the cuvette at 400 ppm and incrementally reducing the light level from 2000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ to 0 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Exponential curves were fitted to determine maximum assimilation at saturating light levels (A_{max}), light compensation points (x-intercept) and dark respiration rates (y-intercept). Linear regressions were also used to estimate quantum yield between 0 and 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The instantaneous WUE (iWUE) was also calculated using assimilation and conductance data, as well as transpiration data that was collected. Three-way analysis of variance tests were performed in Sigmaplot to determine significant differences in the variation of these parameters between the wild type and transgenic plants under control and drought conditions between two trials.

Isotope analysis

Leaf samples were collected in 2012 and 2013 from each group in the study, under control conditions and after withholding of irrigation to induce water-limiting conditions (where applicable). The leaf samples taken were mature leaves that were not used during the collection of data via the LiCor. The samples were dried in a 60°C oven over several days, finely ground using a ball mill, and sealed in aluminum capsules for determination of carbon isotopic ratios and carbon and nitrogen concentrations via the University of California Davis Stable Isotope facility. Leaf mass per area (LMA in g m^{-2}) and percent nitrogen per area (N_{area} in g m^{-2}) were calculated using these leaf samples and their nitrogen contents. Carbon isotope discrimination (Δ ; ‰) of each sample was calculated using the following equation (Farquhar et al. 1989):

$$\Delta = \left(\frac{\delta^{13}a - \delta^{13}c}{1000 + \delta^{13}c} \right) * 1000$$

where $\delta^{13}a$ is the isotopic concentration of the source air ($\delta^{13}a = -12.9$). Intrinsic water use efficiency ($WUE_{isotope}$) was also estimated using the following equation (Farquhar et al. 1980):

$$WUE_{isotope} = \frac{c_a}{1.6} * \left(\frac{27 - \Delta}{27 - 4.4} \right)$$

Where c_a is the ambient CO₂ concentration (390 ppm), the value 1.6 is the ratio of water vapor to carbon dioxide diffusivity, the value 27 is the discrimination of Rubisco to C¹³, and the value 4.4 is the diffusive discrimination of C¹³ in air through the stomata. NUE was also calculated from the nitrogen content and assimilation of the leaves. Sigmaplot was used to perform ANOVA tests to determine if there were significant differences in the variation due to plant type and water availability between two trials, between transgenic and non-transgenic, and control and drought leaves.

Results

Photosynthetic Data

In order to determine differences in photosynthetic activity between drought-stressed and well-watered transgenic and wild type poplars, data extracted from the LICOR measurements were subjected to three-way ANOVAs.

From the A/C_i curves (Figure 3), the maximum carboxylation rates, maximum electron transport rates, and triose phosphate utilization rates were determined for both genotypes under both conditions. These values were subjected to a three-way ANOVA to determine differences between the transgenic and wild type poplars under both conditions between two trials (Table 1). The two trials are shown separately as most of the parameters between them are significantly different. The data suggest that maximum carboxylation rate (V_{cmax}) is not significantly different among the test conditions and genotypes. Both genotypes show higher values for maximum electron transport rate (J_{max}) under well-watered conditions as compared to the drought plants. Triose phosphate utilization (TPU) is higher in transgenic plants under control conditions compared to wild types, but lower under water-limiting conditions.

From the light response curves (Figure 4), light compensation points, maximum assimilation (A_{max}), dark respiration rates, quantum yield values, and instantaneous water use efficiencies ($iWUE$) were also determined via assimilation and conductance data, as well as transpiration data for GS transgenic and wild type poplars under both conditions. These values were also subject to a three-way ANOVA to determine any differences between the poplar genotypes under both conditions (Table 1). The data suggest that the light compensation point is not significantly different among the plants due to condition or genotype. Maximum assimilation was significantly higher for plants under control conditions when compared to their drought counterparts. However, no significant differences in A_{max} were observed between each genotype under the

same conditions. Quantum yield values were also significantly higher for plants under control conditions, but not significantly different between the two genotypes. The dark respiration rate is not significantly different between condition or genotype. Instantaneous WUE (iWUE) was also calculated using the photosynthetic data. The iWUE values were calculated from assimilation and conductance, and assimilation and transpiration taken from the light response curves. They were subjected to three-way ANOVAs to determine differences between GS and wild type poplars under both conditions (Table 2). The iWUEs calculated from transpiration, as well as conductance, are significantly different among condition and type in both trials; with the wild type drought plants being higher than transgenic drought and the control transgenic being higher than the control wild type.

Leaf Isotope Analysis

The WUE of the poplars were determined via the data obtained from stable isotope analysis. These values were calculated using the methodology of Farquhar et al., 1989 and Farquhar et al., 1980, and the C^{13} content of the leaves sampled from each plant. These data show that $WUE_{isotope}$ is significantly lower in transgenic plants compared to wild types, regardless of condition. The Δ is significantly higher among transgenic plants compared to wild types, except for the drought transgenic plants of trial 1, which display lower Δ . The nitrogen and carbon concentration are not significantly different between trials, condition, or

genotype. Leaf mass per area (LMA) and nitrogen mass per area (N_{area}) are higher among wild types in trial 1, but are not significantly different in trial 2. In trial 1 the transgenic plants display lower N_{area} than wild types, with the plants in the drought condition being significantly higher in this regard. The LMA for the plants are slightly smaller than expected, and is most likely due to the young age of the leaves that were used in the study (3 to 6 months). In both trials, NUE was higher among the control conditions compared to their drought counterparts. However, the NUE was only slightly significantly different between the two genotypes ($p = 0.09$), with the transgenic plants displaying higher NUE.

Discussion

In comparison of the photosynthetic parameters for transgenic and wild type poplars, it was found that light compensation point and maximum carboxylation rate (V_{cmax}) values were not statistically significant between the groups at both conditions. This suggests that neither genetics nor water availability affects the maximum carboxylation rates and activity of Rubisco in these poplars. Compared to other studies, these poplar have less V_{cmax} than field grown *Populus tremula* (Secchi and Zwieniecki, 2013) and comparable V_{cmax} to that of *Populus alba* (Coleman et al., 2008) and field-grown *Populus fremontii* (Sage and Sharkey, 1987). The similarity of light compensation point across genotype and condition is not surprising given that tree species were shown to display similar light compensation points regardless of the level of light available to them (Groninger, et al., 1996). Both groups of plants at control water conditions showed significantly higher maximum assimilations and J_{max} than

plants under drought conditions. Given these data, the transgenic poplars seem to reduce photosynthetic activity compared to wild types, although it has been shown previously that these transgenic poplars have increased growth rates (Kirby et al., 2006). Furthermore, leaf area production can be a better indicator of growth than photosynthetic capacity per unit area or net assimilation rate (Rowan et al., 1987).

It has been shown in other species (*Acer spicatum*) that a decrease in soil moisture is correlated with a decrease in TPU (Danyagri and Dang, 2013) which is also shown in this study (Table 1). This suggests that the expression of the GS transgene does not have an effect on triose phosphate utilization. In the first trial, the transgenic poplars displayed higher rates of dark respiration when compared to wild types, whereas in the second trial this is reversed: the transgenic poplars displayed significantly less respiration in the absence of light when compared to wild types. In both trials, the transgenic poplars under both water conditions displayed higher quantum yields when compared to wild types, suggesting that they are better able to utilize the light that is available to them. The quantum yield values were comparable to other poplars (**Su et al., 2011; Brinker et al., 2010**) and also to other transgenic glutamine synthetase-expressing species, such as *Oryza sativa* (**Hoshida et al., 2000**).

Water use efficiency can be described as the ratio of assimilation to amount of water lost through the stomata (Blum, 2005). Optimization theory of transpiration and assimilation has been developed for stomatal responses, and it states that stomatal behavior is optimal if minimal loss of water is sustained for a

given amount of carbon assimilated over time (Eamus, 1991). Farquhar et al. (1980) have presented data that suggests that stomatal behavior is optimal with respect to assimilation and water loss. Therefore, if a plant is able to achieve higher rates of assimilation via increased nitrogen use efficiency (NUE), its stomata may cause it to have increased water loss, which is what our data suggests. Although the GS poplars display decreased water use efficiency (WUE) compared to controls, they also display some enhanced photosynthetic capabilities as well as enhanced nitrogen use efficiency. Other studies using various different species of poplar have reported slightly less intrinsic WUE (Su et al., 2011), and significantly less instantaneous WUE calculated via conductance as well as transpiration (Hamanishi et al., 2012; Coleman et al., 2008). The transgenic plants also show less nitrogen mass per unit area (N_{area}) in the leaf, which has been shown to be correlated with more efficient growth rates (Rowan et al., 1987). Since NUE is calculated by the amount of assimilation per unit nitrogen, and WUE is calculated by amount of assimilation per rate of transpiration or rate of conductance, it is possible that a decrease in WUE may be followed by an increase in NUE. The transgenic poplar display increased growth rates (Kirby et al., 2005), suggesting that potentially a tradeoff is occurring and that they are sacrificing WUE in favor of NUE; which has been observed in other species as well (Field et al., 1983). The data suggest that the wild type plants are not able to assimilate carbon as efficiently with the nutrients available to them, and are not able to fix more carbon per unit of water lost. While the transgenic trees are losing water more rapidly, which is reflected in their smaller

WUEs, they are able to display signs of increased photosynthetic activity, such as quantum yield and lower C_i/C_a ratio. Thus, they are able to better withstand the effects of drought conditions via their more efficient use of nitrogen. This balance between nitrogen and water use efficiency, which has been implicated in other studies (Renninger, et al., 2013), may be the reason why these transgenic poplars showed decreased WUE in this study.

The transgenic poplars display slightly higher photosynthetic activity and achieve a higher growth rate than the wild type, especially when water is not readily available. Although the hypothesis was not supported, the decreased WUE of the GS poplars can be explained by their enhanced NUE. This effect of the expression of *GS1a* may have significant implications in the study of forests that are subjected drought conditions due to climate change, or the engineering of drought-tolerant food crops. However, in order to fully appreciate the tradeoff in nitrogen use efficiency and water use efficiency in these transgenic poplars, additional studies with a larger dataset may be required.

Conclusions

Isotope analysis and measurement of photosynthetic parameters on transgenic poplar plants ectopically expressing *GS1a* has revealed that they do not possess significantly enhanced water use efficiency, especially when water availability is limited. The GS transgenic poplars display higher quantum yield, dark respiration rate, marginally higher nitrogen use efficiency, and lower nitrogen mass per unit area. However, the transgenic poplars display decreased

water use efficiencies, and do not support the hypothesis put forth here. These findings suggest that there is a tradeoff between nitrogen and water use efficiency in the transgenic poplars. Although the poplars show decreased water use efficiency, they still exhibit drought resistance via this tradeoff. Thus, our work suggests that the expression of the GS1a gene in poplar does not increase their water use efficiency, but still confers drought resistance through an increase in nitrogen use efficiency. These findings may prove useful in the study of forests where water availability is limited, and in the research of drought resistant crops.

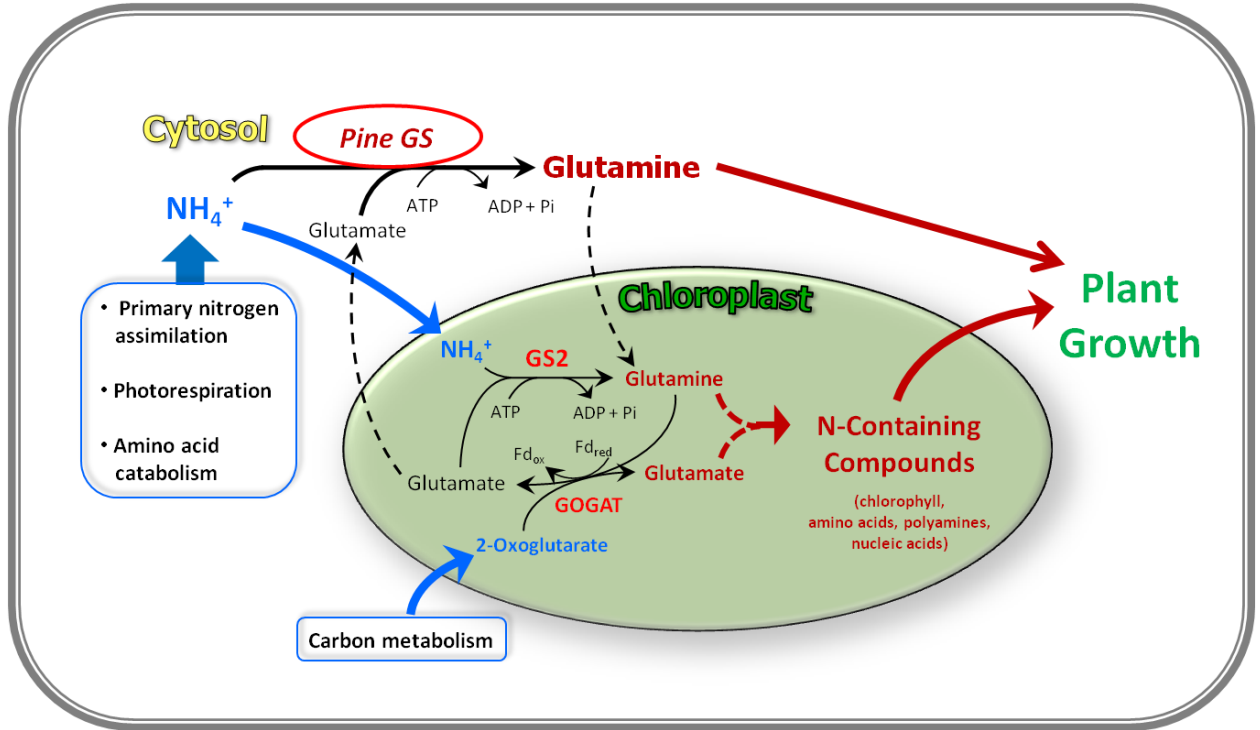


Figure 1 - Glutamine synthetase (GS) plays the central role in assimilation of ammonium into amino acids and other reduced nitrogen compounds in plants. Hybrid poplar (*Populus tremula* X *P. alba*, INRA 717-1-B4) expressing ectopically the pine glutamine synthetase (GS1) gene displays pleiotropic phenotypes, including increased growth, increased nitrogen use efficiency, altered wood chemistry, and enhanced tolerance to drought. Courtesy of J. J. Molina.



Figure 2 -- Experimental set-up for data collection using the LICor-6400XT.

Sample A/Ci Curves

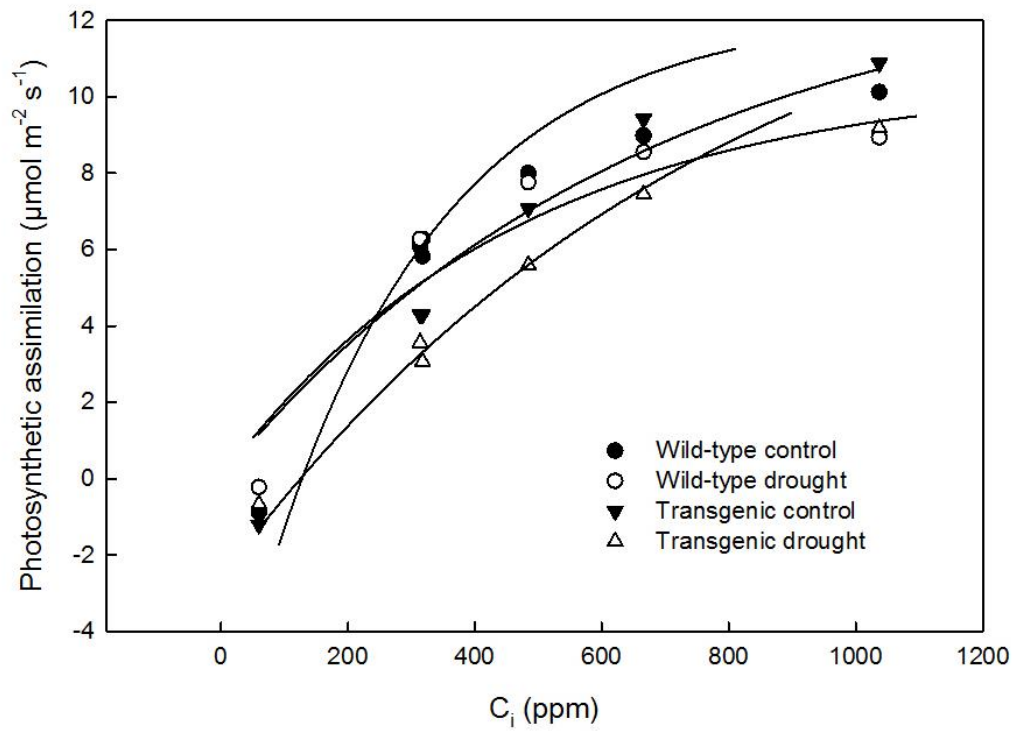


Figure 3- Typical A/Ci curves for transgenic and wild type plants under well-watered and drought conditions

Sample Light Response Curves

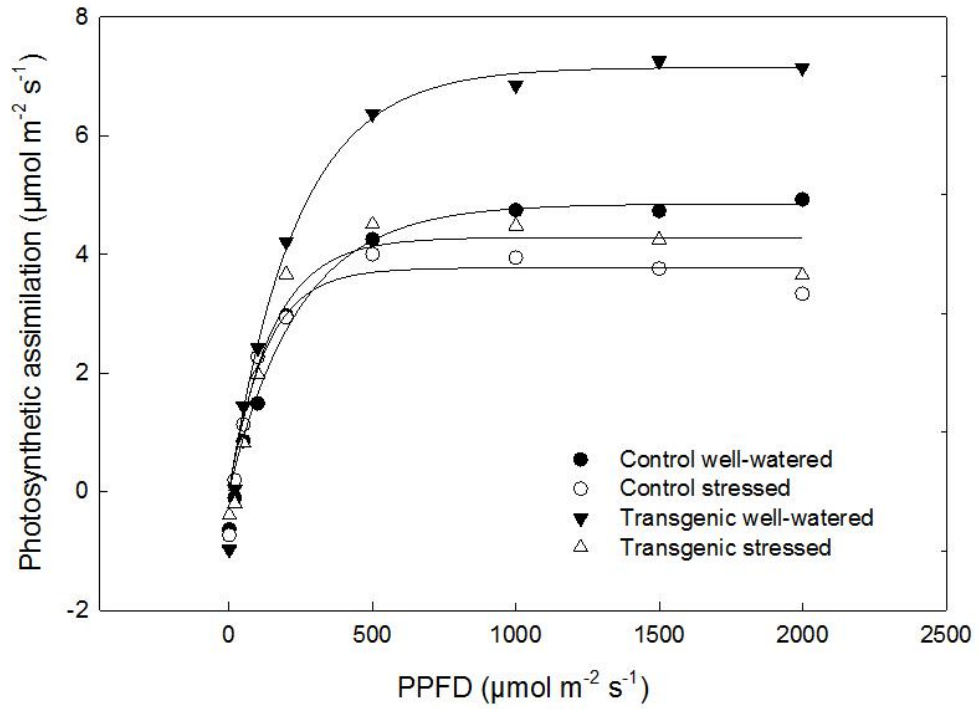


Figure 4 - Typical light response curves for transgenic and wild type plants under well-watered and drought conditions.

		Wild type drought	Wild type control	Transgenic drought	Transgenic control
Trial1	Max. assimilation rate (Amax; $\mu\text{mol m}^{-2} \text{s}^{-1}$)	1.9 (1) a	6.7 (1) b	2.3 (1) a	7.8 (1) b
	Light comp. pt. ($\mu\text{mol}_{\text{photon}} \text{m}^{-2} \text{s}^{-1}$)	25 (2) a	21 (2) a	30 (2) a	28 (2) a
	Quantum yield ($\mu\text{mol Co}_2 \mu\text{mol}_{\text{photon}} \text{S}^{-1}$)	0.030 (0.002) a	0.032 (0.002) b	0.030 (0.002) c	0.039 (0.002) d
	Dark resp. rate ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$)	-0.73 (0.09) a	-0.67 (0.08) b	-0.91 (0.08) c	-1.04 (0.08) d
	Max. carboxylation rate (Vcmax; $\mu\text{mol m}^{-2} \text{s}^{-1}$)	24 (3) a	21 (2) a	19 (2) a	23 (2) a
	Max. electron transport rate (Jmax; $\mu\text{mol m}^{-2} \text{s}^{-1}$)	92 (8) a	81 (7) ab	59 (7) b	91 (7) ab
	Triose phosphate utilization (TPU; $\mu\text{mol m}^{-2} \text{s}^{-1}$)	9.3 (0.7) a	7.5 (0.6) a	6.8 (0.6) b	8.7 (0.6) b
Trial2	Max. assimilation rate (Amax; $\mu\text{mol m}^{-2} \text{s}^{-1}$)	6.4 (1) a	15 (1) b	10 (1) a	16 (1) b
	Light comp. pt. ($\mu\text{mol}_{\text{photon}} \text{m}^{-2} \text{s}^{-1}$)	14 (2) a	13 (2) a	9.7 (2) a	8.0 (3) a
	Quantum yield ($\mu\text{mol Co}_2 \mu\text{mol}_{\text{photon}} \text{S}^{-1}$)	0.034 (0.002) a	0.039 (0.002) b	0.036 (0.002) c	0.041 (0.002) d
	Dark resp. rate ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$)	-0.50 (0.08) a	-0.52 (0.08) b	-0.35 (0.08) c	-0.34 (0.09) d
	Max. carboxylation rate (Vcmax; $\mu\text{mol m}^{-2} \text{s}^{-1}$)	16 (2) a	24 (3) a	18 (3) a	21 (3) a
	Max. electron transport rate (Jmax; $\mu\text{mol m}^{-2} \text{s}^{-1}$)	67 (7) a	94 (7) ab	73 (8) b	104 (7) ab
	Triose phosphate utilization (TPU; $\mu\text{mol m}^{-2} \text{s}^{-1}$)	8.8 (0.6) a	8.2 (0.6) a	7.1 (0.5) b	8.7 (0.7) b

Table 1– Means and standard errors (in parentheses) of photosynthetic parameters derived from light response and A/Ci curves (Fig. 1). P-values for differences between the transgenic and wild type plants under control and drought conditions are based on ANOVAs for each parameter. Bold letters denote significant differences at $p < 0.05$.

		Wild type drought	Wild type control	Trans drought	Trans control
Trial 1	Conductance (g _s ; mol H ₂ O m ⁻² s ⁻¹)	0.016 (0.03) a	0.12 (0.03) b	0.023 (0.03) a	0.09 (0.03) b
	C _i /C _a (isotope)	0.76 (0.03) a	0.80 (0.02) a	0.80 (0.03) b	0.85 (0.02) b
	C _i /C _a (instantaneous)	0.60 (0.04) a	0.74 (0.03) b	0.65 (0.03) c	0.67 (0.03) d
	iWUE (A/E; μmol CO ₂ mol ⁻¹ H ₂ O)	4.3 (2) a	3.2 (2) a	3.7 (2) a	4.0 (2) a
	iWUE (A/g _s ; μmol CO ₂ mol ⁻¹ H ₂ O)	90 (11) a	50 (11) b	77 (11) c	69 (11) d
	WUE _{isotope}	72 (7) a	56 (5) a	39 (7) b	45 (5) b
	δ ¹³ C (‰)	-33 (0.6) a	-34 (0.5) a	-33 (0.6) b	-35 (0.5) b
	Δ (‰)	21 (0.7) a	22 (0.5) a	22 (0.7) b	24 (0.5) b
	N conc (%)	3.8 (0.2) a	3.9 (0.2) a	3.9 (0.2) a	3.8 (0.2) a
	C conc. (%)	45 (1) a	43 (0.9) a	45 (1) a	46 (0.9) a
	C/N	11.7 (1.0) a	11.4 (0.7) b	11.9 (1) a	11.8 (0.7) b
	LMA (g m ⁻²)	34 (3) a	27 (2) b	29 (3) c	21 (2) d
	N _{area} (g m ⁻²)	1.3 (0.10) a	1.0 (0.06) b	1.1 (0.10) c	0.80 (0.07) d
	NUE (μmol CO ₂ g ⁻¹ N s ⁻¹)	2.9 (5) a	5.3 (4) b	2.9 (5) a	9.7 (3) b
Trial 2	Conductance (g _s ; mol H ₂ O m ⁻² s ⁻¹)	0.061 (0.03) a	0.21 (0.03) b	0.051 (0.03) a	0.13 (0.02) b
	C _i /C _a (isotope)	0.81 (0.03) a	0.80 (0.02) a	0.86 (0.04) b	0.83 (0.02) b
	C _i /C _a (instantaneous)	0.60 (0.03) a	0.59 (0.03) a	0.65 (0.04) b	0.43 (0.03) b
	iWUE (A/E; μmol CO ₂ mol ⁻¹ H ₂ O)	7.3 (2) a	7.2 (2) a	7.6 (2) a	15 (2) a
	iWUE (A/g _s ; μmol CO ₂ mol ⁻¹ H ₂ O)	95 (11) a	95 (11) b	91 (12) c	160 (11) d
	WUE _{isotope}	48 (7) a	50 (5) a	35 (7) b	42 (5) b
	δ ¹³ C (‰)	-34 (0.6) a	-34 (0.5) a	-35 (0.8) b	-35 (0.4) b
	Δ (‰)	23 (0.7) a	22 (0.5) a	24 (0.9) b	23 (0.5) b
	N conc (%)	2.8 (0.2) a	3.0 (0.2) a	2.6 (0.3) a	2.9 (0.2) a
	C conc. (%)	48 (1) a	46 (1) a	47 (2) a	46 (0.9) a
	C/N	17 (0.9) a	15 (0.7) b	19 (1.13) a	16 (0.62) b
	LMA (g m ⁻²)	22 (2) a	25 (3) a	18 (3) a	21 (2) a
	N _{area} (g m ⁻²)	0.60 (0.1) a	0.79 (0.1) a	0.47 (0.1) a	0.62 (0.1) a
	NUE (μmol CO ₂ g ⁻¹ N s ⁻¹)	6.8 (4) a	19.8 (5) b	19.5 (5) a	22.9 (3) b

Table 2 - Means and standard errors (in parentheses) of gas exchange, carbon isotopes and leaf nutrient compositions for transgenic and wild type plants under control and water-limiting conditions. δ¹³C, N and C concentrations, iWUE_{isotope} were derived from analysis of leaf tissue (n = 10 for control condition, n = 5 for drought). P-values for differences between the test groups are based on ANOVAs for each parameter. Bold letters denote significant differences at p < 0.05.

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