

## Research regarding the influence of culture conditions upon the main physiological indices at *Paulownia shan tong*

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**Abstract** *Paulownia shan tong* is a fast-growing tree species with a considerable economic potential because of its value for wood as well as its high biomass production, and elevated stress tolerance. The objective of the present study was to evaluate the influence of different culture conditions upon the main physiological indices at *Paulownia shan tong*. The observations were made using three different variant of culture conditions starting from internodes taken from elite plants cultivated in the field, which were separated in three study variants. The variant 0 (witness variant) – in vitro culture made in the green house in normal conditions; variant 1 – in vitro culture inoculated on Murashige and Skoog tissue culture medium supplemented with 3% (w/v) sucrose and 0.6% (w/v) agar; variant 2 – in vitro culture inoculated on Murashige and Skoog tissue culture medium supplemented with 3% (w/v) sucrose and 0.6% (w/v) agar, 6-Benzylaminopurine and Gibberellic acid. The physiological indices used to determine the differences between culture conditions are: the chlorophyll, detected with the chlorophyll meter, the dry matter (%) detected with the thermo balance and the photosynthesis detected with the photosynthetic apparatus by gas changing method (Qubit Systems, 2010).

In what regards the chlorophyll, the results are situated between the values 34.12 – 48.16; the minimum value represents variant 0 and the maximum value represents the variant 1. The dry substance is found between the values 22.13 – 23.81; the minimum value being represented by variant 2 and the maximum value by variant 0. The photosynthesis is found between the values 1.7 – 2.42 with the minimum value obtained by variant 0 and the maximum value obtained by variant 2.

### Key words

*Paulownia shan tong*, in vitro culture, photosynthesis, chlorophyll, dry matter

*Paulownia* timber has many uses, including timber for construction, doors, furniture, kitchens etc. The deep rooting system of *Paulownia* in combination with the rapid rate of growth enables it to take up more nutrients than other species and may therefore offer potential for bioremediation purposes. *Paulownia* leaves can be used for animal feed and honey is made from the bright colorful flowers in China. Because of the high cellulose content of *Paulownia*, cellulosic ethanol can be produced as a renewable energy fuel. Cellulosic ethanol is reported to have much reduced greenhouse gas emissions when compared to ethanol produced via a sugar/starch-based fermentation. Interest in *Paulownia* is gaining momentum around the world, due to its fast growing nature, the ability to take up nutrients and the potential for intercropping. (AFBI Hillborough, 2008)

*Paulownia* wood is used in house construction, for paper pulp, furniture making, farm implements and musical instruments (Ayan et al., 2003). The various end uses for *Paulownia* for furniture making are

comprehensively described by Ayan et al. (2003). These authors stated that the wood is about 40% lighter than ordinary wood and is very promising for pulp and paper. Lyons (1993) reported that *Paulownia* timber air dries readily and has excellent thermal and electrical insulation characteristics. Japanese researchers described some of the properties of particle board made from low quality *P. tomentosa* and concluded that low quality *Paulownia* trees offer potential as a raw material for particle board manufacture (Kalaycioglu et al., 2005). Lyons (1993) reported that the branches of the tree can be used for household energy and a 10 year old tree has been reported to produce 350-400 kg branches for fuel (Zhaohua, 1987).

*Paulownia* is said to require minimal management and little investment (El-Showk and El-Showk, 2003) and has been receiving greater attention as a short-rotation woody crop in recent years (Bergmann et al., 1997).

*In vitro* propagation has ensured that the growing demand for superior planting material, biomass and forest products is met. A number of factors including

explant selection, macro- and micro nutrient composition, and incorporation of plant growth regulators, antioxidants, additives and adsorbents during *in vitro* culture have been optimized to develop success- full regeneration protocols for several *Paulownia* species.(Niraj,2013)

## Material and Methods

Plant material and culture conditions: From a young plantation of *Paulownia* Shan tong was selected a number of vigorous plants from which were taken internodes that were sub cultivated in three variants of culture. In the first variant, Variant 0 or variant witness the internodes were sub cultivated in soil substrate in growth conditions of 14 °C -18°C temperature and the photoperiod of 12h. In the second variant, Variant 1, the internodes were cleaned by washing the residuals and then they were sterilized in the laminar air flow hood using ethanol in a concentration of 70%, mercuric chloride 2% and the repeated wash with sterile distillate water. After the sterilizing the internodes were inoculated in culture medium Murashige and Skoog supplemented with 3% (w/v) sucrose and 0.6% (w/v) agar, where they were kept four weeks, in this period they developed leaves, stem and radicular system.

Variant 2, the internodes passed the same method of sterilization as the previous variant, but the culture medium used was additional with phytohormones specialized in the growth of the internodes, mostly in inducing sprouts - 6-Benzylaminopurine and Gibberellic acid and kept four weeks.

For the evaluation of the physiological indices were used three probes for each variant and the following apparatus: chlorophyll meter (Konika Minolata) – to determine the chlorophyll content (SPAD units), four determinations were made/probe; the thermo balance (Kern) – to determine the dry matter (%),and photosynthesis apparatus (Qubit Systems, 2010) – to determine the photosynthesis (ppm CO<sub>2</sub>/cm<sup>2</sup>/h). The phenophase evaluation was made by decimal unit code BBCH (Sumalan, 2007, Camen 2014).

## Results and Discussions

By analyzing the experimental data obtained it can be observed the fact that regarding the total content of chlorophyll expressed in SPAD units there is variability between the experimental variants that have been studied.

Table 1

Experimental results regarding the dry substance and the chlorophyle

Nr Crt.	Experimental Variant	Chlorophyll (SPAD)	Dry matter (%)
1.	V0	34.12 ± 6.61	23.81 ± 1.18
2.	V1	48.16 ± 6.17	23.16 ± 1.49
3.	V2	40.74 ± 10.58	22.13 ± 3.90

Therefore in the case of variant 1, cultivated in vitro conditions on MS 0 medium without phytohormons it has been registered a total amount of chlorophyll higher in comparison with the witness variant, represented by the plants cultivated in vivo conditions. The determinations have been carried out in phenophase 1.5 BBCH (5 unfolded leaves). S. Alikamanoğlu and Collaborators, 2007 in an experiment which studied the effects of gamma radiations upon the regenerative capacity of the species *Paulownia* concluded the fact that the total amount of chlorophyll varies with culture conditions. The differences obtained regarding the total amount of chlorophyll were also observed by Gloria Irma Ayala-Astorga and Collaborators, 2010 in an experiment which tested the influence of saline stress upon the chlorophyll content on *Paulownia*. A higher percentage

of chlorophyll observed in the case of the variants cultivated in vitro conditions can be explained through the photoperiod and the temperature at which the plants were cultivated, therefore in the conditions of 16h photoperiod of light, the chlorophyll pigments synthesis is being stimulated. In this case the reduction of the total amount of chlorophyll pigments from the leaves has been directly proportional with a higher level of saline stress at which the plants were exposed. Regarding the dry substance quantity (expressed in percentages), it can be observed the fact that there are no significant differences between the experimental variants studied, although a higher percentage has been registered in the case of the witness variant (in vivo cultivation conditions). M. J. Immel and Collaborators in a study regarding the influence of photoperiod upon the growth at *Paulownia* concludes that in what

concerns the dry substance quantity registered has been directly influenced by photoperiod. The highest amount of dry substance was obtained in 24h of light (18 g.m),

but the difference registered in comparison with a 16h photoperiod (16.92 g.m) is much lower than the one registered between 8 – 16h of light (7.27 – 16.92 g.m).

Table 2

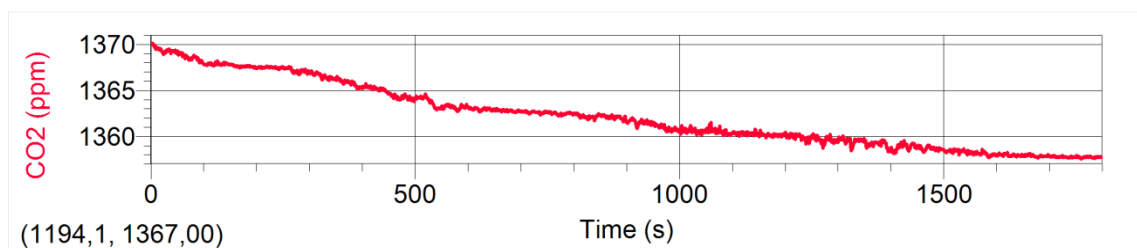
Experimental results regarding the photosynthesis intensity

Nr Crt	Experimental Variant	Photosynthesis Intensity (ppm CO <sub>2</sub> )
1.	Vo	1,7 ± 1
2.	V1	1,84 ± 1,21
3.	V2	2,42 ± 0,58

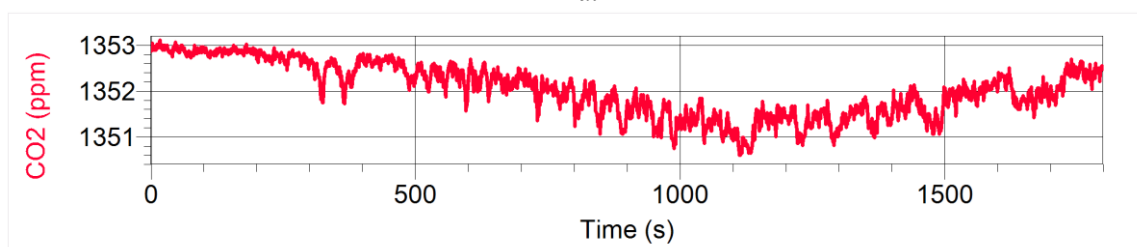
In the case of the photosynthesis intensity determined through the exchange gas method it can be observed that there are differences in CO<sub>2</sub> assimilation depending on the experimental variant. Therefore in the case of variant 2 cultivated in vitro conditions on culture medium with additional phytohormons it can be observed a total amount which is higher in the assimilation of CO<sub>2</sub> in comparison with the witness variant. The determinations have been made in 1.5 BBCH phenophase (5 leaves unfolded). Sadegh Mohajer and Collaborators, 2014 have conducted an experiment regarding the micro propagation of bio encapsulation and ultra-structural features of Sainfoin grown In vivo and in vitro, where they have also identified the variation of the photosynthesis process which is dependent of the culture type. The higher percentage in the assimilation of carbon dioxide, in the case of “in vitro” cultivated variants can be explained by photoperiod and the temperature at which the plants

were cultivated, therefore in controlled culture conditions with a photoperiod of 16h light and temperature of 24°C, as in the case of the in vitro plants it can be observed a growth of the photosynthesis process. This results are determined influenced by external factors, but in the same conditions of light and temperature in the case of in vitro cultivated plants, the photosynthetic process register a reduced intensity because of the nutrition method – half heterotrophic, as it resulted from a previous experiment made by Murashige T. 1973 The in vivo plants subjected to similar conditions as in the field with a temperature between 14 - 18°C and a photoperiod of 12h of light show a slight reduction in the CO<sub>2</sub> assimilation process compared with in vitro cultivated plants.

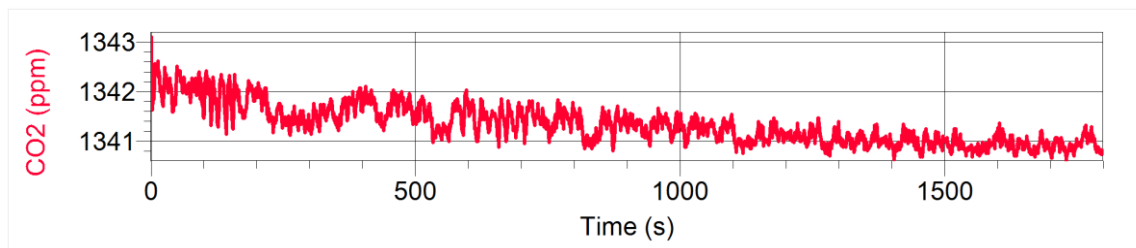
For the highlighting of the photosynthetic process a representative graphic was selected for each variant: Figure 1 – Variant 0, Figure 2 – Variant 1, Figure 3 – Variant 2.



a.



b.



c.

**Figure 3.** CO<sub>2</sub> assimilation

a. Experimental Variant V0; b. Experimental Variant V1; c. Experimental Variant V3

## Conclusions

The physiological indices used in this study: chlorophyll, dry substance and photosynthesis process recorded different results depending on the culture method chosen. In the case of chlorophyll the experimental variant with maxim results was Variant 1 (in vitro – MS0), getting  $34.12 \pm 6.61$  SPAD while the experimental variant with minimum results was Variant 0 (in vivo) getting  $34.12 \pm 6.61$  SPAD. This values were obtained because of the constant culture conditions that exist in the growth room: photoperiod of 16h light and 24°C. In what regards the dry substance the experimental variant with a maximum results was Variant 0 (witness), obtained  $23.81 \pm 1.18$  %, while the experimental variant with minimum results was Variant 2 (in vitro – MS H), obtained  $22.13 \pm 3.90$ . The photosynthesis intensity has maximum value at Variant 2 (in vitro – MS H), obtained  $1.7 \pm 1$  ppm CO<sub>2</sub>. This difference being realized on the strength of different culture conditions.

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